



Formulation and Antimicrobial Analysis of Polyherbal Wash in Ambikapur Surguja Chhattisgarh

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ABSTRACTS

Many medicinal plants have been found effective in the cure of bacterial disease and have diverse medicinal properties. Due to increasing antibiotic resistance in microorganisms and side effects of synthetic antibiotics, medicinal plants are now gaining popularity in the treatment of bacterial and fungal infections. To prevent the bacterial infection, it is important to protect skin especially hands from bacterial pathogens as they are the most exposed part of the body. Therefore, herbal hand wash was formulated, which has no side effect and with potential antibiotic activity.

Keywords: Tulsi, Retha, Neem, Turmeric, Cinnamon, Tea.

Introduction

Skin is one of the most exposed parts of the body requires protection from the pathogens. To protect the skin from harmful microorganisms and to prevent spreading of many contagious diseases hand washing is absolutely an important precaution (M. Joy et al., 2012). Hands are considered to be the primary root for the transmitting microbes and infections to the individuals. Personal as well as hand hygiene is important to prevent many communicable diseases. The word "hygiene" is derived from the ancient Greek goddess "Hygeia" that means "goddess of healing". The importance of hygiene is universal recognized and it is well known that hand hygiene is crucial to prevent and minimize health care -associated infections. The centers for disease control and prevention, the world health organizations (WHO), and many others health expert promote hand hygiene as the single most important measure in the prevention of hospital acquire infections (M. Jain et al., 2016). Normal human skin harbor's bacteria (between 10² and 10⁶ CFU/cm² and during daily activity health care workers progressively accumulate microorganisms on their hands from direct patient contact or contact with contaminated environmental surfaces and devices (Mondal et al., 2004). The of the present work is to prepare and physically evaluate a poly herbal wash from commonly available plants, instead of adopting synthetic preparation (Grace et al., 2015).

Many of the chemical antiseptic, hand wash now available in market are alcohol based synthetic chemical sanitizers and these solutions help to reduce health care associated transmission of contagious disease more effectively but they have many side effects (Joshi et al., 2008). And have some adverse effect as follows: -1. Dryness, 2. Their frequent use can lead to skin irritation, 3. Also resistance among pathogen.

Therefore, research has been increased tremendously towards making natural products with improved quality yet less in cost and no side effect over chemical agents (Jayant, et al., 2015).

Although good and simple hygiene technique is single most important, easy and less expensive means of preventing health care associated infections and the spread of antimicrobial multidrug resistance but unfortunately poor hand hygiene practice are still observed due to lack of scientific awareness, lack of knowledge of risk and unavailability of hand hygiene facilities (Thombare et al., 2015). We therefore, prepared herbal hand wash using plants like Ocimum, tenuiflorum (Tulsi), Aloe vera, Sapindus mukorossi (Ritha), Azardichta indica (Neem), Cinnamon, Citrus limon (lemon) Camellia sinensis (Tea), Curcuma longa (Turmeric).

Hand washing has the following health benefits:

Helps minimize the spread of influenza. Diarrhea prevention, Avoiding respiratory infections, A preventive measure for infant death at their home birth deliveries, Improving hand washing practices have been shown to lead to small improvements in the length growth in children under five year of age.

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 (Londhe et al, 2015).

Plants are the oldest resource of pharmacologically active compounds and have provided human kind with many medicinally valuable compounds from enturies (Powar P. V et al., 2015). The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (Wadood et al., 2013).

Phytochemicals are bio-active chemicals of plant origin. They regarded as secondary metabolites because the plant that manufactures them may have little need for them. They are naturally synthesized in all parts of the plant body bark, leaves stem, root, flower, fruits, seeds, etc. i.e. any part of the

plant body may contain active components (Jyothiprabha et al., 2016).

Thus, we aimed to formulate and evaluate herbal hand sanitizer comprising of alcoholic extracts combination of these astonishing herbs using other suitable excipients which can be used as ready to use herbal hand sanitizer (Thombere et al., 2015).

Material and Method

In order to formulate our herbal drug (herbal hand wash) we perform so important tests like, phytochemicals tests of taken plants leaves and their antimicrobial assay.

Herbal extracts preparation

The leaves of the selected plants were removed from the plants and then washed under running tap water to remove dust. The plant samples were then air dried for few days and the leaves were crushed into powder and stored in polythene bags for use. Plant materials were weighed and mixed with 100ml of methanol, in conical flasks and kept in rotatory shaker at 150rpm for 24 hours. After 24 hours it was filtered with Whatman No.1 filter paper. And these were used to examine antibacterial activity and to formulate herbal hand wash. But Aloe-Vera extract was prepared by collecting fresh branch of Aloe Vera, it was then washed with tap water and rinsed with distilled water and pulp was extracted from the leaf by cutting horizontally, the pulp was then filtered with muslin cloth.

Media preparation

Nutrient agar media

1. Peptone 5.0gm
2. Beef extract 3.0gm
3. NaCl 5.0gm
4. Agar 20.0gm
5. Distilled water 1000ml

Muller Hinton agar

1. Beef extract 2.0gm
2. Acid hydrolysate of casein 17.50gm
3. Starch 1.50gm
4. Agar 17.00gm
5. Distilled water 1000ml

Phytochemical tests

A. Test for tannins and phenolic group:

B. Evaluation parameters: 1. Color: It was determined visually, 2. Odor: It was determined manually, 3. pH: It was determined by using pH meter.

C. Antimicrobial testing:

1. Well diffusion assay
2. Disc diffusion assay

D. Preparation of herbal hand wash

1. Preparation of sample 1 polyherbal hand wash without SDS

Neem 23ml, Cinnamon 23ml, Ritha 30ml, Distilled 54ml, Glycerin 10ml, Aloe vera 10ml

2. Preparation of sample 2 polyherbal hand wash with SDS

Turmeric 23ml, Tea 23ml, Ritha 30ml, Distilled water 54ml, glycerin 10ml, Aloe vera 10ml

Result

After preparing two different samples using some common and some different plant extract, we parted each sample into 4 different samples. And then we mix SDS (6g) into two samples. This formulation was further used to examine its antibacterial activity at different concentration.

Phytochemical test:

Before formulation of herbal hand wash, we have to test the plant material that which kinds of biochemical's and bio-molecule they have. And this is confirmed by phytochemicals testing of each plant materials. While testing the plant extracts, we found that Ritha, Tea, Turmeric, tulsi, Cinnamon, and neem contained the following bioactive chemical compound.

S.NO.	Phytochemical test	Phytochemical Compounds Present			
		Turmeric	Ritha	Tea	Tulsi
1.	Ferric acid test	-	-	+	+
2.	Gelatin test	-	+	+	+
3.	Flavonoid test	-	+	+	-
4.	Protein test	-	-	+	-
5.	Sterol test	+	+	+	-
6.	Triterpenoid test	+	+	+	-
7.	CBH test	+	-	-	-
8.	Cow metric test	+	+	-	+
9.	Quinine test	+	-	-	-
10.	Starch test	+	-	-	-

Phytochemicals compounds of Turmeric, Ritha, Tea, Tulsi.

S.NO.	Phytochemical test	Phytochemical Compounds Present			
		Cinnamon	Neem	Lemon	Aloe Vera
1.	Ferric acid test	+	+	-	-
2.	Gelatin test	+	-	-	-
3.	Flavonoid test	-	+	+	+
4.	Protein test	-	-	-	-
5.	Sterol test	+	-	+	-
6.	Triterpenoid test	+	-	-	-
7.	CBH test	-	+	+	-

8.	Cow metric test	-	+	-	+
9.	Quinine test	-	-	-	-
10.	Starch test	-	+	-	-

Phytochemicals compounds of Cinnamon, Neem, Lemon, Aloe Vera.

Antimicrobial Activity:

The antimicrobial activity of methanolic extract using agar well diffusion technique, Neem and Turmeric showed maximum zone of inhibition (25 mm) against *P. aeruginosa* and followed by *S. aureus* (1.5 mm). Similarly, the methanolic extracts of Retha and Cinnamon have highest antimicrobial activity with *S. aureus* and *Pseudomonas sp.* (24 mm) respectively. On the other hand, the Tulsi and Tea, showed the minimum activity (20 mm), and no activity against *P. aeruginosa* and *S. aureus* respectively. The overall observation indicated that the methanolic extracts of Tulsi and Tea have lesser impact on the two different human pathogens than the other four species of medicinal plants extract including Ritha, Neem, Turmeric and Cinnamon.

Antimicrobial activity of plants extracts against *Pseudomonas aeruginosa* and *staphylococcus aureus*.

S.NO.	Plant extract	Sensitivity of bacteria (mm)	
		<i>S.aureus</i>	<i>P. aeruginosa</i>
1.	Tulsi	-	-
2.	Ritha	15 mm	24 mm
3.	Neem	15 mm	25 mm
4.	Turmeric	15 mm	25 mm
5.	Cinnamon	-	24 mm
6.	Tea	-	20 mm

Antimicrobial Activity of Herbal Hand Wash Sample:

Antimicrobial activity of prepared polyherbal hand wash in different aqueous and methanolic extracts of medicinal plants, were investigated using disc diffusion method. For this, some selected human pathogens such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas sp.* Sensitivity of these three pathogens were recorded in Table. All the medicinal plant extracts used against the pathogenic organism have showed varied degree of antimicrobial activity against the pathogens.

Antimicrobial Activity of formulated polyherbal hand wash sample against the *Sauers*, *P. aeruginosa*, and *E. coli*

S.NO.	Name of microbes	Sample (1) (Without SDS)	(With SDS)	Sample (2) (Without SDS)	(With SDS)
1.	<i>Staphylococcus Aureus</i>	16mm	30mm	17mm	35mm
2.	<i>Pseudomonas aeruginosa</i>	18mm	30mm	14mm	29mm
3.	<i>E. coli</i>	20mm	33mm	20mm	34mm

Ph of Formulated Herbal Hand wash Sample:

All of the formulated polyherbal hand washes, with SDS and without SDS, with SDS hand wash have higher pH (5.1) and without SDS polyherbal hand wash have low (4.6 and 4.7)

pH of formulated polyherbal hand wash.

S.N.	Sample	pH
1.	Without SDS	4.6
	With SDS	5.1
2.	Without SDS	4.7
	With SDS	5.1

COLOR:

Formulated polyherbal hand wash sample 1st color is dark green because of the presence of neem and cinnamon and the sample 2nd color is dark orange because it is made up of turmeric and tea.

ODOR:

Formulated polyherbal hand wash sample 1st smell good something like cinnamomic fragrance because of the presence of cinnamon, and sample second have turmeric and tea fragrance.

The results of dip well method showed that the hand wash prepared from methanol extract of the combined plant materials with SDS had greater activity than the activity of the herbal hand wash without SDS. The hand wash prepared with mixture of extraction of neem, tulsi, aloe-vera, turmeric etc. showed better activity than the formulation prepared without them. During the formulation of herbal hand wash different parameters were observed.

DISCUSSION

Many medicinal plants have been found effective in the cure of bacterial diseases, and have diverse medicinal properties (Raskin et al, 2012). Due to increasing antibiotic resistance in microorganisms and side effects of synthetic antibiotics; medicinal plants are now gaining popularity in the treatment of bacterial and fungal infections (Karupiah P and Rajaram S, 2012). Plants, herbs and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against pathogens (Deans and Ritchie 1987; Kumar et al., 2006). To prevent the bacterial pathogens as they are the most exposed part of the body. Therefore, herbal hand wash was formulated, which has no side effect and with potential antibiotic activity.

Formulating the hand wash; was done by two different methods (Formulation I and II). Explain in methodology section and plant extracts were added externally. Plant was selected on the basis of their reported biological activity and their traditional use in Indian medicine. Ritha traditional used as a detergent and saponin extracted from this fruit were used to formulate hand wash. *Aloe vera* used for its moisturizing activity. *Oscimum sanctum*, *Azadirachta indica* reported to have anti-bacterial activity. In addition to this *Oscimum sanctum* and Turmeric gives the unique aroma to the herbal hand wash formulation. Plants are rich in potential secondary metabolites, such as flavonoids, tannins alkaloids polyphenols etc. These compounds have diverse biological activity including antibacterial activity. Most of the polyphenols are extracted in the methanol; therefore, methanol was used as solvents for extraction.

When we form phytochemical test among the turmeric, Ritha tea and tulsi, test result shows that the tea and turmeric possess higher quantities of bio-active chemical compounds, in which tea possesses ferric acid, gelatin, flavonoid, protein sterol and Triterpenoid and Turmeric possess sterol, Triterpenoid, CBH quinine and starch. On the other hand, tulsi possesses smaller quantity of bio-active chemical compounds which are ferric acid gelatin and cowmeric. Test results shows that the turmeric and tea is more significant to use them as an ingredient of polyherbal hand wash. That's why, we use them to make polyherbal hand wash sample I (phytochemical test no.1)

Similarly, for the polyherbal hand wash sample 2 we use neem and cinnamon because test result shows that the neem and cinnamon contain higher quantities of bio-active chemical compounds. In which Neem possesses, CBH, cowmeric and starch. While the cinnamon possesses ferric acid, gelatin sterol, and Triterpenoid. On the other hand, aloe vera possess smaller quantity of bioactive chemical compound which are flavonoid and cowmeric are common phytochemical test Table 2.

The better activity of the prepared formulation may be due to the combined activity of these phyto-constituents. The formulation prepared with turmeric extract showed best antimicrobial activity than formulation without turmeric means other than 2nd sample. These better results may be due to the presence of antiseptic properties in turmeric extract. Turmeric antiseptic properties are reported to have potential effect on bacteria. In the present context the plants under study are rich in these varied compounds and hence are more effective against skin pathogens.

The antimicrobial activity of methanolic extract using agar well diffusion technique, Neem and Turmeric showed maximum zone of inhibition (25mm) against *P. aurigenosa* and followed by *S. aureus* (1.5mm). Similarly, the methanolic extracts of Ritha and Cinnamon have highest antimicrobial activity with *S. aureus* and *Pseudomonas sp.* (24mm) respectively. On the other hand the Tulsi and tea, showed the minimum activity (20mm), and no activity against *P. aurigenosa* and *S. aureus* respectively. On the other hand the tulsi and tea, showed the minimum activity (20mm), and no activity against *p.aurigenosa* and *S.aureus* respectively. The overall observation indicated that the methanolic extracts of Tulsi and tea have lesser impact on the two different human pathogens than the other four species of medicinal plants extract including Ritha, Neem Turmeric and Cinnamon (phytochemical test no.3).

Antibacterial activity of the each plant extracts, and two polyherbal solutions were tested against the skin pathogen *pseudomonas aeruginosa*, *Staphylococcus aureus*, *E.coli*. The results suggested that herbal extracts in mixture giving higher activity than the individual extracts. The combination of the antibacterial compounds from different plant extracts. The combination of the antibacterial compounds from different plant extracts may show synergistic effect enhancing their antimicrobial activity. *Sapindus mukorossi* (Ritha), which are used as surfactant and as a detergent in polyherbal hand wash sample 1. *Aloe vera* is used as moisturizing agent, while *Azadirachta indica* for antibacterial activity. When these extracts combined to formulate the polyherbal hand wash antibacterial activity enhance.

CONCLUSION

The results suggest that of the various extracts of *Azadirachta indica*, *Cinnamomum zeylanicum*, *Ocimum sanctum*, *Camellia sinensis*, *Curcuma longa*, *Sapindus mukorossi*, *aloe-vera* and their combinations are capable of giving superior inhibition without SDS. This might be rational basis for use of herbs in preparation of polyherbal hand wash and use of these compounds in making antiseptic lotions or soaps in place of chemicals. The leaves we taken to

formulate hand wash are widely used for medicinal purposes. The antimicrobial results clearly proved that the herbal hand wash shows very good antimicrobial with SDS too. Thus, these compounds can be found to come back antibiotic resistant of pathogenic organism and provide safe and healthy living through germ free hand all though the removal is not 100% but a number can be reduced.

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