International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 158-163 www.ijprajournal.com ISSN: 2249-7781

Formulation and Evaluation of Anti-Bacterial Activity of Herbal Handwash by Using Tinospora Cardifolia

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Submitted: 01-03-2023 Accepted: 12-03-2023

ABSTRACT: The main aim of the present research work was to Formulate and Evaluate the Anti-Bacterial activity of herbal handwash by using ethanolic extraction method by using Tinospora cardifolia. Herbal remedies are widely used in health care around the world. Herbal medicines have been widely used as effective preventive & treatment options for various ailments. There are a variety of hand washes available in the market, many of them have some adverse effects such as itching, dermatitis, irritation etc. For the preparation of herbal hand wash, the hydroalcoholic leaves extract of Tinospora cardifolia were used. Three handwash formulations were prepared, & the formulations were also tested for physical attributes such as pH, viscosity & appearance. The Anti-Bacterial activity of formulated herbal handwash was tested against some bacteria such as Escherichia coli&Staphylococcus aureus by the Agar disk-diffusion method. The results obtained were compared with a standard antibiotic drug[Amoxicillin].The findings revealed that prepared herbal handwash formulation showed a significant zone if inhibition compared with a standard antibiotic.

KEYWORDS: Herbal handwash, Tinospora cardifolia, Anti-Bacterial activity, Guduchi, Satva.

I. INTRODUCTION

Hand hygiene is one of the most important measures to prevent harmful bacterial infections and to prevent infection [1]. Hand washing is the act of germless hands to remove soil, dirt, pathogenic microorganisms, and avoid transmitting of transient microorganisms [2].

Hand washing is essential for protecting the skin layer from pathogenic microorganisms and preventing the spreading of communicable diseases. Hand Washing removes visible dirt from hands and reduces the number of harmful microorganisms such as Escherichia. Coli and Staphylococcus aureus may be carried by people, animals, or equipment & transmitted to food [3]. To defend the skin from harmful microorganisms and to avoid spreading of various contagious diseases, hand washing is extremely important precaution [4].

WHO has recommended all people should wash hands before during and after preparing food, before eating food, before and after caring for someone who is sick, before and after treating a cut or wound, after using the toilet and changing diapers or cleaning up a child who has used the toilet. After blowing your nose, coughing, or sneezing, after touching an animal or animal waste, after handling pet food or pet treats and after touching garbage [5].

The World Health Organization has “Five Moments” for washing hands:

- Before patient care
- After environmental contact
- After exposure to blood/body fluids
- Before an aseptic task
- After patient care

The skin is one of the most uncovered part of the body and it must be protected from pathogenic microorganisms like bacteria, viruses, fungus etc. Nasocomial infection has emerged as a critical issue in hospital care outcome, resulting in extended hospitalization and substantial morbidity and mortality [6].The hands of Health Care workers (HCWs) are the primary routes of transmission of multidrug resistant pathogens and infection to the patients.

Hand washing with soap consistently at critical moments during the day prevents the spread of diseases like diarrhea and cholera which are

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transmitted through fecal-oral routes. Hand washing also protects against impetigo which is transmitted through direct physical contact.

II. DRUG PROFILE

TINOSPORA CARDIFOLIA

Biological source: It is a large, deciduous, extensively-spreading, climbing vine with several elongated twining branches.

Synonym: gurjo, heart-leaved moonseed, Guduchi or giloy.

Kingdom: Plantae

Class: Magnoliopsida

Order: Ranunculales

Family: Menispermaceae

Genus: Tinospora

Species: cordifolia

Molecular formula: $C_{20}H_{18}NO_4$

Molecular weight: 336.36122g/mol

Chemical constituents: One of the most important constituent present in stem of T. cordifolia is berberine, an isoqunoline alkaloid.

Description:

Leaves are simple, alternate, and exstipulate with long petioles up to 15 cm (6 in) long which are roundish and pulvinate, both at the base and apex with the basal one longer and twisted partially and half way around. It gets its name heart-leaved moonseed by its heart-shaped leaves and its reddish fruit. Lamina are broadly ovate or ovate cordate, 10–20 cm (4–8 in) long or 8–15 cm (3–6 in) broad, seven nerved and deeply cordate at base, membranous, pubescent above, whitish tomentose with a prominent reticulum beneath.

Flowers are unisexual, small on separate plants and appearing when the plant is leafless, greenish-yellow on axillary and terminal racemes. Male flowers are clustered, but female flowers are usually solitary. It has six sepals in two series of three each. The outer ones are smaller than the inner. It has six petals which are smaller than sepals, obovate, and membranous. Fruits aggregate in clusters of one to three. They are ovoid smooth drupelets on thick stalks with sub terminal style scars, scarlet or orange colored.

Phytochemicals:Tinosporacordifolia contains diverse phytochemicals,
including alkaloids, phytosterols, glycosides [7], tinosporide, and mixed other chemical compounds.

Part used: The stem and leaves of Giloy is of maximum utility, but the root can also be used.

Benefits:

- Helps to minimize the spread of influenza.
- Avoiding respiratory infections.
- A preventive measure for infant deaths at home birth deliveries.

III. MATERIALS AND METHODS

Chemicals: Sodium lauryl sulphate, Sodium benzoate, Glycerin, Acacia, Agar, Amaranth, Essential oil.

Plants Collection and Authentication: The leaves of Tinospora cardifoliawere collected from the garden of Nalanda Institute of Pharmaceutical Sciences, Kantepudi, Guntur, Andhra Pradesh, India.

Preparation of Leaf Extracts: The leaves of collected plant Tinospora cardifolia are taken and

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coarsely powdered. 50gms of coarsely powdered leaves were soaked in 400ml of 70%(V/V) methanol and water mixture and kept for percolation (size reduction, packing, imbibition, maceration, percolation) for about 1 week. After percolation, the extract is filtered, and the filtrate was collected and used for making hand wash.

Preparation of Herbal Handwash:

1. All the water-soluble ingredients such as acacia, sodium benzoate, sodium lauryl sulphate were triturated finely.
2. To this mixture glycerin is added while grinding.
3. Methanolic extract of Tinospora was added as per formulation concentration.
4. Then make up with purified water up to required volume.

Three formulations of herbal handwash: Formulation 1 (F-1), Formulation 2 (F-2) & Formulation 3 (F-3) were prepared.

S.No	Ingredients	Formulation 1(F-1)	Formulation 2 (F-2)	Formulation3(F-3)
1	Tinospora cardifolia (methanol extract)	5ml (5%)	10ml (10%)	20ml (20%)
2	Acacia	2.4gm	2.4gm	2.4gm
3	Sodium benzoate	0.5gm	0.5gm	0.5gm
4	Sodium lauryl sulphate	0.5gm	0.5gm	0.5gm
5	Glycerin	20ml	20ml	20ml
6	Amaranth	Q.S.	Q.S.	Q.S.
7	Essential oil	Q.S.	Q.S.	Q.S.

Evaluation of Anti-bacterial activity of herbal hand wash using agar- disk diffusion method:

The agar diffusion test (Kirby-Bauer antibiotic testing or disc diffusion antibiotic sensitivity testing) is a test of antibiotic sensitivity of bacterial. It uses antibiotic impregnated wafers to test the extract to which bacteria are effected by those bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called zone of inhibition.

The size of this zone depends on how effective the antibiotic is at stopping the growth of the bacterium. A stronger antibiotic will create a larger zone, because a lower concentration of antibiotic is enough to stop growth.

The bacteria in question are swabbed uniformly across a culture plate. A filter paper disc impregnated with compound to be tested is then placed on the surface of the agar. The concentration of the compound will be highest next to the disc, and will decrease as distance from disc increases. If the compound is effective against bacteria at certain concentration in the agar neither is greater than nor equal to effective concentration, no colonies will grow where the concentration in the agar is greater than or equal to effective concentration. This is the zone of inhibition. This along with the rate of antibiotic is used to estimate the bacteria sensitivity to that particular antibiotic. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic for those bacteria. Inhibition produced by the test is compared with that produced by known concentration of a reference compound. This information can be used to choose appropriate antibiotics to combat a particular infection.

COMPOSITION OF NUTRIENT AGAR MEDIUM

S. No	Ingredient name	Quality taken in (%)
1	Peptone	0.5%
2	Beef Extract	0.3%
3	Agar	1.5%
4	Sodium Chloride	0.5%

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5	Distilled water	Q.S.

METHOD:

The prepared nutrient agar medium was taken in 2 Petri plates. The medium was solidified in each Petri plate. After solidification selected organisms were inoculated into each Petri plate. The selected organisms include E.coli and Staphylococcus aureus. After inoculation 3 discs (Whattman filter paper discs) are taken and the discs are dipped into formulated sample, commercial herbal hand wash sample. These are inserted into each Petri plate. Finally, the Petri plates are placed in incubator for 24hrs to observe the zone of inhibition.

Evaluation Parameters:

1. **Physical Evaluation:** Physical evaluation (color, odour) was done by sensory and visual inspection and compared with the marketed hand wash.
2. **pH:** For the pH evaluation, the end of the pH strip was dipped into the hand wash formulations. After a couple of seconds, remove the paper and compare the color of the pH strip to the color chart provided with the pH paper kit.
3. **Viscosity:** The viscosity of hand wash was determined by using a digital Brook field viscometer DV-II. Measured quantity of hand wash was taken into a beaker, and the tip of the viscometer was immersed into the hand wash, and viscosity was measured in triplicate.
4. **Fragrance test:** It was based on observation for its acceptability. 5 people were asked for acceptability of fragrance and their opinion was taken. And fragrance was evaluated based on the below-described criteria;

A. Fragrance was good, as good as the fragrance of reference hand wash.
B. Fragrance was not so good but comparable to the reference hand wash.
C. Fragrance of the toothpaste was poor than the reference hand wash.

IV. RESULTS & DISCUSSION:

Hands are major source of transmission of microbes and infections. Contaminating hands can serves as vectors for transmission of micro-organisms. Hand wash is mandatory in food production health care setting and day preparation. The present investigation in food production health care setting and day preparation. The present investigation was targeted to evaluate the anti-bacterial activity. The anti-bacterial activity of formulated herbal hand wash was tested against E.coli and Staphylococcus aureus by Agar-disc diffusion method and results were compared with the commercially available anti-bacterial hand wash.

Anti-Bacterial Activity:

Preliminary anti-bacterial activity screening tests observations were shown in Table 1. Tinospora leaf extract formulation proved to be beneficial with excellent activity against all the tested microorganisms. A significant ($p<0.05$) value was found against S. aureus. Hence it was encouraging to be used as a potent antiseptic in the preparation of herbal hand wash. All the observation data for in vitro anti-bacterial activity evaluation of Tinospora herbal hand wash is presented in Table 2. The formulation had significant anti-bacterial activity against all tested organisms than the commercial hand washes. No significant values were obtained from the blank hand wash which is indicative of; anti-bacterial activity of herbal hand wash is solely due to the activity of active components. The activity of test hand wash against Staphylococcus aureus was of significant interest ($p<0.05$). Our skin contains large numbers of microorganisms, mainly Gram-positive. Staphylococcus aureus is ubiquitous and is not easily washed and eliminated by routine washing and scrubbing even with some antiseptic hand wash. Hence the activity of the Tinospora herbal hand wash against Staphylococcus aureus was very remarkable.

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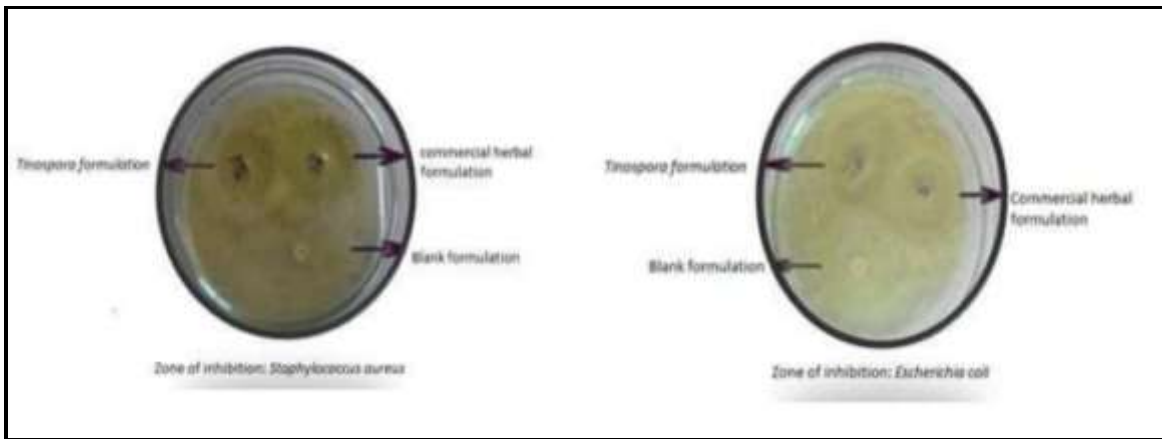

Zone of inhibition of Staphylococcus aureus&Escherichia coli

Table 1: Anti-bacterial Activity of Tinospora leaf extract Inhibition zone diameter (mm) ±SD*.

S.No	Organism	Inhibition zone diameter (mm)
1	Escherichia coli	10.8 ± 0.46
2	Staphylococcus aureus	18.6 ± 0.53

*: Values calculated as means ± SD (n=3); **: Results are significant ($p<0.05$).

Table 2: In vitro Anti-Bacterial Activity Evaluation of formulated herbal hand wash.

S.No	Test organism	Zoneof inhibition	Zone of inhibition	Zone of inhibition
		Tinospora herbal hand wash	Standard hand wash	Blank hand wash
1	Escherichia coli	+	+	No zone
2	Staphylococcus aureus	+	+	No zone

+; presence of bacterial inhibition zone. All values are means of triplicates.

Table 3: Reduction in Hands bacterial count.

S. No	Formulation	Bacterial flora reduction	Bacterial flora reduction	Bacterial flora reduction
		Control	Test	Reduction
1	Tinospora herbal hand wash	27.55×10^3	5.85×10^3	80%
2	Blank hand wash	23.86×10^3	88×10^3	67%
3	Commercial hand wash	17.88×10^3	5.38×10^3	74%

Table 3 and Fig.1 (a-d) indicates growth reduction in bacterial count obtained after the use of Tinospora herbal hand wash formulation, commercial herbal hand wash and blank hand wash. Highest reduction was observed by Tinospora herbal hand wash followed by commercial herbal hand wash and blank hand wash. The percent of reduction in the count shown by Tinospora herbal hand wash was 80%, while it was found to be least in case of control blank hand wash (67%). The commercial herbal antibacterial hand wash showed more reduction than a blank hand wash (74%). A percentage reduction in bacterial count was calculated using the formula given below.

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% Reduction=Control (cfu/ml) – Test (cfu/ml) × 100……. (eq.1).

The significant ($p<0.05$) activity of the formulated hand wash may be due to presence of tannins and phenolics in the Ethanolic extract. Tannins are reported to be bacteriostatic or bactericidal against Staphylococcus aureus due to its action on the membranes of the microorganisms. The results of the screening of the anti-bacterial activity showed that the natural anti-bacterial agents are present in the extract and hand wash prepared by them were far more active than the synthetic anti-bacterials used in commercial antiseptic hand wash.

V. CONCLUSION:

The present study of the Ethanolic extract of Tinospora cardifolia leaf shown superior inhibition against various gram +ve, -ve and fungal skin pathogens than synthetic anti-bacterial agents present in the commercially available antiseptic liquid hand washes.

Therefore, this compound should be extracted, to prepare superior antibiotic handwash with significant having less or no side effects. Its resistance of these organism and provide safer and healthier living through bacterial free hand.

Although, the renewal is not provided 100% but a significant number can be reduced with natural, economic and safe Tinospora cardifolia herbal handwash.

As per the observations of many researches, herbs are highly beneficial agents that may be used as handwash with reduced adverse effects and longer-lasting benefits.

Formulation can also be routinely used for improving hygiene of healthy children and adults [8].

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