



## FORMULATION AND EVALUATION BY PHYTOCHEMICAL ANALYSIS OF HERBAL HANDWASH

Rohit Jaysing Bhor\*, Shubhangi Dnyaneshwar Bhadange, Rohini Jagannath Gaikwad, C.J. Bhangale

Department of Pharmaceutical Chemistry, PRES's College of Pharmacy Chincholi, Tal-Sinner, Dist-Nasik, Maharashtra, India

\*Corresponding Author's E mail: [rohit.bhor69@gmail.com](mailto:rohit.bhor69@gmail.com)

Received 1 Jan. 2018; Revised 9 Jan. 2018; Accepted 11 Jan. 2018, Available online 200 Jan. 2018

### ABSTRACT

The main objective of the work was to analyze the phytochemical constituents from herbal hand-wash prepared by using extract of Neem leaves, Tulsi leaves, Lemon leaves, Tantani leaves, Black pepper, Babool leaves and Clove. Herbal hand-wash extract was prepared by leaves of Neem leaves, Tulsi leaves, Lemon leaves, and Tantani leaves, Black pepper, Babool leaves and Clove by solvent extraction. Herbal hand-wash extract was analyzed by phytochemicals test like Tests for carbohydrates, Tests for alkaloids, Tests for steroids and sterols Tests for glycosides, Test for proteins and amino acids, Tests for Saponins and Test for flavonoids. Methanol extracts of leaf and flower buds showed presences of carbohydrates, alkaloids glycosides proteins and amino acids Saponins flavonoids

**Keywords:** Herbal hand-wash; Neem leaves; Tulsi leaves; Lemon leaves; Phytochemicals; Antibacterial activity.

### INTRODUCTION

Herbal medication is also known as Botanical treatment or Phyto-medicine. Herbal medication refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. An herbal drug treatment gives healthy life. It was general used to furnish first-line and common health provider.<sup>1</sup> Since ancient time in India, herbal medicines have been the basis of treatment and cure for various diseases. Physiological conditions in traditional methods were practiced such as Ayurveda, Unani and Siddha.<sup>2</sup> Herbal medicines having various therapeutic uses like healing wounds, treating inflammations due to infection, skin lesions, leprosy, diarrhea, scabies, venereal diseases, snake bite and ulcers etc. Many infectious agents such as virus, fungi, and parasites may harm the plants. The basic herbs have the answer with no side effects and effective medication. When two or more herbs are used in the formulation they are known as poly herbal formulations. Numerous studies have been conducted with the extracts of Neem leaves (*Azadirachta indica* Family-Meliaceae) and extract of Tulsi leaves with the combination of many other herbal drugs like Tulsi leaves, Lemon leaves, Tantani leaves, Black pepper, Babool leaves and Clove.<sup>3</sup> Along with other dosage forms herbal drugs are also available in the form of

herbal hand-wash which is semisolid preparation used to washing of hands and for several purposes. Hand washing is also known as hand hygiene.<sup>4</sup> Hand washing is the act of cleaning hands for the purpose of removing soil, dirt and microorganism. The main medical purpose of washing hands is to protect hand from infection like bacteria or virus and chemicals which can cause harm or disease.<sup>5</sup>

### **Plant Profile:**

#### **1. Neem leaves:**



*Azadirachta indica* (*A. indica*) belongs to the botanic family meliaceae. It is commonly known as Neem. It is used in traditional medicine as a source of many therapeutic agents. Neem leaves are known to contain antimicrobial & antifungal activities against different pathogenic microorganisms like *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. Neem is multipurpose tree with multiple health benefits. Different parts of the tree were shown to exhibit antimicrobial effects against a wide variety of microorganisms. Furthermore, Neem leaves may be used for the treatment of various diseases including eczema, ringworm, acne, inflammation, chronic wound infection, hyperglycemia, diabetic foot & gas gangrene.<sup>6</sup>

#### **2. Tulsi leaves (Basil leaves):**



Basil (*Ocimum basilicum* L) is a member of the Lamiaceae family. It is an annual herb which grows in several regions around the world. It is commonly known as Tulsi. It has characteristic odor. Basil is one of the medicinal plants. It gives antibacterial & antioxidant properties. Leaf extract of basil shows

antibacterial activity against some human pathogenic bacteria (*Staphylococcus aureus* & *Escherichia coli*).<sup>7</sup>

### 3. Lemon leaves:



*Citrus Limon* belongs to the family Rutaceae. Lemon leaves shows antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus Subtillis*, *Pseudomonas aeruginosa* & *Salmonella species*. Leaves extracts are active against both Gram-positive and Gram-negative bacteria. Lemon leaves have great potential as antimicrobial compounds against microorganisms.<sup>8</sup>

### 4. Tantani leaves:



*Lantana Camara* (L) is one of the species of genus *lantana* commonly known as "Tantani" or "Ghaneri" Shrub. The leaves are green in color & ovate in shape & plant bears white flower. Tantani leaves extract shows highest antimicrobial activity. Tantani leaves effectively inhibit the growth of both gram negative and gram positive bacteria.<sup>9</sup> Tantani leaves gives antibacterial action against *E.coli*, *S.typhi*, *s.aureus*.

### 5. Black pepper:



Black pepper (*Piper Nigrum*) is a flowering vine in the family Piperaceae, cultivated for its fruit which is usually dried and used as a spice known as peppercorn. Medicinal plant is very important in human health; it will act as an antibacterial activity against the bacterial pathogen.<sup>10</sup>

#### 6. Babool leaves:



*Acacia nilotica* L. is the member of the family Mimosaceae & is known as Babool. Babool leaves extract shows antibacterial activity against *Escherichia coli* & *Bacillus subtilis*. Babool is multipurpose nitrogen fixing tree legume. Babool leaves are very digestible & have high levels of protein. Babool has been used as source of medicine. Methanol extract of Babool shows good antibacterial activity.<sup>11</sup>

#### 7. Clove:



Clove is the aromatic flower buds of a tree in the family Myrtaceae, *Syzygium aromaticum*. Clove possesses antimicrobial activity against bacteria and yeast. It's essential oil extracts kills many gram positive and Gram negative organisms including fungi. The antimicrobial activity of Clove is attributable to eugenol, oleic acids and lipids found in its essential oils. Clove extracts inhibit the growth of *Pseudomonas aeruginosa*, *Candida albicans*, and *Staphylococcus aureus* & *salmonella typhi*.<sup>12</sup>

## **MATERIALS AND METHODS:**

### **Collection of leaves/ flower buds and Extraction:**

All leaves of Neem leaves, Tulsi leaves, Lemon leaves, Tantani leaves and Babool leaves was collected in the month of October 2016 from Nashik region. Clove flower buds and Black pepper flower buds were purchased from local market. The sample was washed thoroughly with fresh water to remove sand particles. The plant materials were collected and separated and are then dried under shade drying for 4-5 days. Then the dried plant materials were grinded, sieved to get nearly fine amorphous powder. Then the dried plant materials were grinded, sieved to get nearly fine amorphous powder. Powdered material was extracted with suitable solvent or mixture of solvents.

### **Materials of hand wash formulation:**

1. Extract of Neem leaves
2. Extract of Tulsi leaves
3. Extract of Lemon leaves
4. Extract of Tantani leaves
5. Extract of Black pepper
6. Extract of Babool leaves
7. Extract of clove
8. Almond oil
9. Glycerine
10. Sodium Laurial Sulphate
11. Methyl Paraben
12. Water

### **Preparation of extract**

The hand wash was prepared from the methanolic extracts of each plant material; 20 gm of the powdered material were extracted with 80 ml of methanol solution for 48 hrs. The content was filtered through Whatman filter paper in order to get particle free extract.

### **Preparation of hand-wash**

The hand wash was prepared by adding methanolic extracts of each plant material in glycerin and distilled water. Finally sodium lauryl sulphate, methyl paraben and flavoring agents were added as per the requirement of standard procedure for preparation of hand wash. The solution was made homogenous under room temperature and stored for the further analysis.<sup>13</sup>

**TABLE 1: DIFFERENT FORMULATION OF EXTRACT**

Sr. No.	Ingredients	F1	F2	F3
1.	Extract of Neem leaves(ml)	4	4	4
2.	Extract of Tulsi leaves (ml)	3	4	3
3.	Extract of Lemon leaves (ml)	1	1	0.5
4.	Extract of Tantani leaves (ml)	1	1	2
5.	Extract of Black pepper (ml)	1	1	1
6.	Extract of Babool leaves (ml)	1	1	0.5
7.	Extract of Clove(ml)	0.75	1	1
8.	Almond oil (ml)	3	3	3
9.	Glycerine (ml)	10	10	10
10.	Sodium lauryl sulphate (gm)	3	3.2	3
11.	Methyl paraben (gm)	q.s	q.s	q.s
12.	Distilled water (to produce 50ml)	q.s	q.s	q.s

**Qualitative Phytochemical Analysis and Evaluation of Herbal wash:**

The hand wash preparation was subjected to the phytochemical analysis by using various chemical tests.

**Tests for carbohydrates:**

- **Molish test:** Take 1 ml of hand wash extract in the test tube and added with 1 mL of  $\alpha$ -naphthol solution and few drops of concentrated sulphuric acid, it gives purple or reddish violet color gives positive result.
- **Fehling's test:** Take 1 ml of hand wash extract in the test tube and to this added the equal quantities of Fehling's solution A and B, it gives a brick precipitate indicates the presence of carbohydrate.

#### Tests for alkaloids:

- **Mayer's test:** Take 1 ml of hand wash extract in the test tube and add 2-3 drops of conc. Nitric Acid solvent, it gives a dull white precipitate indicate the presence of alkaloids.
- **Wagner's test:** Take 1 ml of hand wash extract in the test tube and 2 to 3 drops of Nitric Acid solvent and sulphuric acid is added, it gives presence of reddish brown precipitate indicates the presence of alkaloids.

#### Tests for steroids and sterols:

- **Salkowski test:** Take 1 ml of hand wash extract and dissolved in the chloroform, the same amount of concentrated sulphuric acid is added. Cherry red color is observed in the chloroform layer.

#### Tests for glycosides:

- **Baljet's test:** Take 1 ml of hand wash extract in the test tube and added with sodium picrate solution, yellow to orange colour indicates the presence of glycosides.
- **Keller-Kiliani test:** The hand wash extract was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. Reddish brown color is formed which gradually becomes blue indicating the presence of glycosides.

#### Tests for Saponins;

- **Foam test:** Take 1 mL of hand wash extract and added with the distilled water. The sample was shaking and the foam was observed.

#### Test for flavonoids:

- **Shinoda test:** Take 1 ml of the hand wash extract in the test tube and added with the ferric chloride, followed by the addition of concentrated hydrochloric acid. A red color indicates the presence of flavonoids.

#### Tests for tannins:

- **Ferric chloride test:** Take 1 ml hand wash extract, ferric chloride was added. Dark blue or greenish black color indicates the presence of tannins.
- **Potassium dichromate test:** To the hand wash extract, potassium dichromate solution was added. A precipitate indicates the presence of tannins.

### Test for proteins and amino acids:

- **Biuret test:** To the hand wash extract, 1 mL of 40% sodium hydroxide and 2 drops of 1% copper sulphate solution were added. A violet color indicates the presence of proteins.

### Evaluation of Herbal wash:<sup>14</sup>

Organoleptic evaluation (color, odor) was done by sensory and visual inspection and compared to the marketed hand wash. The evaluations were carried out on the hand wash by using the following parameters;

**Color and odor:** Color and odor of prepared ointment was examined by visual examination.

**pH :** The pH of ointment was determined by digital pH meter. 1 g of ointment was dissolved in 50 ml of distilled water and the pH was measured.

**Stability study:** The stability study was carried out for the prepared ointment at temperature of 37°C for 15 days.

### Fragrance test

It was based on individual 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.

### RESULT AND DISSCUSSION:

The preliminary phytochemical screening of methanolic extract of 3 hand wash formulation was tested and it gives result which was shown below. It gives presence of carbohydrates, steroids, glycoside, proteins, amino acids and absence of flavonoids and Saponins (Table 2).

**Table 2: Phytochemical analysis of leaves**

Sr.no.	Sample	Solvent extraction Methanol		
		F <sub>1</sub>	F <sub>2</sub>	F <sub>3</sub>
	Phytochemical analysis Tests	Phytochemical analysis Tests result	Phytochemical analysis Tests	Phytochemical analysis Tests
1	Tests for carbohydrates	+	+	+
2	Tests for alkaloids	+	+	+
3	Tests for steroids and sterols	+	+	+
4	Tests for glycosides	+	+	+
5	Tests for Saponins	-	-	-
6	Test for flavonoids	-	-	-
7	Tests for tannins	+	+	+
8	Test for proteins and amino acids	+	+	+

The Quality control evaluation of formulation of methanolic extract of 3 hand wash was tested and it gives result which was shown below. Three different hand washes were formulated and the physicochemical parameters such as color, odor and pH were evaluated. All Three different hand washes have different colors and characteristic odor. The pH of the five ointments was in the range of 6 – 7.

It was observed that the phytochemical analysis of methanolic extract of 3 hand wash formulation (table 2) showed presence of carbohydrates, reducing sugar, steroids, glycoside, proteins, amino acids and absence of flavonoids and Saponins.

**Table 3: Quality control evaluation of formulation**

Sr.no.	Parameters	Observations		
		F <sub>1</sub>	F <sub>2</sub>	F <sub>3</sub>
1	Sample Color	Yellow	Yellow	Yellow
2	Odor	Characteristic	Characteristic	Characteristic
3	Texture	Smooth	Smooth	Smooth
4	Stability	Stable	Stable	Stable
5	pH	Acidic	Acidic	Neutral
6	Solubility	Soluble in boiling water, miscible with alcohol, ether, chloroform	Soluble in boiling water, miscible with alcohol, ether, chloroform	Soluble in boiling water, miscible with alcohol, ether, chloroform
7	Wash ability	Good	Good	Good
8	Non irritancy	Non irritant	Non irritant	Non irritant

## CONCLUSION

In the present experimental study showed that it is possible to develop and evaluate preliminary phytochemical screening of methanolic extract of 3 herbal hand wash gives good result and quality control evaluation of formulation also gives better result. Formulation can also be routinely used for improving hygiene of healthy children and adults.

## ACKNOWLEDGMENT

Authors are grateful for and would like to express gratitude to all the faculty of PRES's College of Pharmacy, Chincholi; Nashik.

## REFERENCES:

1. Ravi K, Pratibha MD and Kolhapure SA. Evaluation of the antimicrobial efficacy and safety of Pure Hands as a hand sanitizer, Indian Journal of Clinical Practice. 2005; 15(10): 19-27.
2. Burke JP. Patient safety: Infection control a problem for patient safety, N Engl. J. Med. 2003; 348: 651-656.
3. Aiello AE and Elaine BL. Antibacterial cleaning and hygiene products as an emerging risk factor for antibiotic resistance in the community, The Lancet Infectious Diseases. 2003; 3(8): 501–506.

4. Snyder OP, Paul St, Safe Hand Washing. Hospitality Institute of Technology and Management. 1988; 1: 1-3.
5. Kirana H and Srinivasan BP, Aqueous Extract of Garcinia Indica Choisy Restores Glutathione in Type 2 Diabetic Rats. J Young Pharm. 2010; 2(3): 265–268.
6. Biswas K, Chattopadhyay I, Banerjee RK and Bandyopadhyay U. Biological activities and medicinal properties of neem (*Azadirachta indica*). Current Science. 2002;82(11):1336–1345.
7. Kumar A, Rohal A, Chakraborty S, Tiwari R, Latheef SK, et al. Ocimum sanctum (Tulsi): a miracle herb and boon to medical science–A Review. Int J Agron Plant Prod. 2013; 4: 589.
8. Dostalova L, Detvanova L and Kalhotka L. Antimicrobial activity of aqueous herbal extracts. Mendelnet. 2014;2:403-406.
9. Ghisalberti EL. Lantana camara L.(Verbenaceae) Fitoterapia. 2000;71:467–86.
10. Chiranjib B, Narayn VS, Variyar PS and Bandyopadhyay C. Phenolics of green pepper berries (*Piper nigrum*). J. Agri. Food. Chem. 1990; 38: 8-12.
11. Prusti A, Mishra SR, Sahoo S and Mishra SK. Antibacterial activity of some indian medicinal plants. Ethanobot. Leaflet. 2008; 12: 227-230.
12. Durairaj, S. Srinivasan, S. and Lakshmanaperumalsamy P. In vitro Antibacterial activity and stability of garlic extract at different pH and temperature. Electronic Journal of Biology. 2009; 5(1): 5-10.
13. Hera O, Kiefer M, Farell D and Kamper K. A review of twelve commonly used medicinal herbs. Arch Farm Med. 1998; 6: 523-536.
14. Hera O, Kiefer M, Farell D and Kamper K. A review of twelve commonly used medicinal herbs. Arch Farm Med. 1998; 6: 523-536.