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IP Indian Journal of Immunology and Respiratory Medicine

Journal homepage: <https://www.ijirm.org/>

Original Research Article

Development and evaluation of herbal handwash gel containing *Andrographis paniculata* alcoholic extractKoushlesh Kumar Mishra^{1,*}, Anand Kumar², Aakash Gupta³, Sunil Singh¹, Anil Kumar Sahu⁴, Pankaj Kashyap⁴, Saraswati Prasad Mishra⁵¹Dept. of Pharmacy, Shri Sai College of Pharmacy, Prayagraj, Uttar Pradesh, India²University Teaching, Sant Gahira Guru University, Ambikapur, Chhattisgarh, India³Raigarh College of Pharmacy, Raigarh, Chhattisgarh, India⁴Royal College of Pharmacy, Raipur, Chhattisgarh, India⁵Rungta College of Pharmaceutical Sciences and Research, Raipur, Chhattisgarh, India

ARTICLE INFO

Article history:

Received 23-05-2022

Accepted 22-06-2022

Available online 09-07-2022

Keywords:

Andrographis paniculata

B subtilis

Handwash gel

Antibacterial activity

ABSTRACT

Nowadays, herbs used worldwide due to its medicinal and pharmaceutical potentials. Herbs are also used to prevent human beings from variety of diseases. In the current study, alcoholic extract of *Andrographis paniculata* which is commonly known as green chiretta used in the development of handwash (herbal). Traditionally, green chiretta was used and known for their different pharmacological action. Alcoholic extract of *Andrographis paniculata* loaded herbal hand wash prepared by using Carbopol-934 as gelling agent, TEA (triethanolamine) as neutralizer and sodium lauryl sulfoacetate (SLSA) as surfactant. Antibacterial activity of herbal handwash gel evaluated by cup plate method. *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* are used to assess the potential of gel against microorganisms. It shows good potential action against *Bacillus subtilis*. Physicochemical characterization of herbal handwash gel was performed by morphological study such as odor, color, pH, foam height, consistency and retention as per chemical standards. The developed herbal hand wash formulation showed no side effects and outcomes are in the limits.

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1. Introduction

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. Hand washing is essential for protecting the skin layer from pathogenic microorganisms and preventing the spreading of communicable diseases. Hands are generally primary mode of transmission of microbes and infections. So, hand cleanliness is maintained by using hand wash.¹

Andrographis paniculata which is also popularly known as green chiretta and it belongs from the family

Acanthaceae. As andrographolide is chief active constituent of the plant, it could be considered as excellent candidate of antimicrobial drug development. It is one of the highly used potential medicinal plants in the world, currently. Traditionally, it has been believed to be a treatment for bacterial infections and some diseases. It exhibits antibacterial, antioxidant, anti-inflammatory, anticancer, and immune stimulating properties.²

Nowadays, herbal plant bio actives are preferred over chemicals for formulation due to the fewer side effects. There are large numbers of medicinal plants available which are extensively used in different treatments of skin infections and possess pharmacological activity. The

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therapeutic activity of any plant depends upon their active constituents. Secondary metabolites found in plants include glycosides, alkaloids, tannins, saponins, terpenoids and flavonoids among others which are responsible for various pharmacological activity. According to the literature study, *Andrographis paniculata* leaves extract possess antimicrobial and antibacterial activity. Thus, we aimed to formulate and evaluate herbal handwash gel using most effective excipients in the preparation of herbal handwash.³

2. Materials and Methods

2.1. Preparation of ethanolic extract of Kalmegh

The leaves of *Andrographis paniculata* (Green chirretta) were collected from Raigarh, Chhattisgarh, India during August-September 2021 and its botanical identity was confirmed by Dr. Saeargaonkar SL, Department of Plant Breeding, College of Agriculture & Research Station Raigarh, Chhattisgarh, India. Using the soxhlet apparatus, one kg shade dried powdered leaves were extracted with ethanol. A concentrate was prepared by distilling off the solvent from the total extract and the concentrate was placed on a water bath and converted to asyrupymass and then completely dried by evaporating (31gram).⁴

2.2. Qualitative analysis

The preliminary phytochemical screening was carried out for testing the different phytoconstituents present in alcoholic extract of *Andrographis paniculata* (Kalmegh). The phytoconstituents tests were carried out and the results are represented in following-

2.3. Identification of phytoconstituents by chemical tests⁵⁻¹⁰

2.3.1. Test for alkaloid

Wagner's test: 1 ml of concentrate added in 2 ml of Wagner's reagent. The presence of alkaloids indicated by the precipitates reddish brown color.

2.3.2. Test for glycosides

Fehling's test: A small amount of extract is hydrolyzed with 5 ml of hydrochloric acid for several hours in a water bath and the hydrolyzate is subjected to Fehling's test. Add 2 ml of Fehling's solution (1 ml of Fehling's solution A and 1 ml of Fehling's solution B), add 2 ml of the extract, mix and bring to a boil. The presence of reducing sugars indicated by the precipitates yellow or red color.

2.3.3. Test for saponins

In a water bath, 2 g of powdered sample was boiled in 20 ml of distilled water and filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and vigorously shaken to form a stable persistent froth emulsion.

2.3.4. Test for phytosterol

Salkowski test: Phytosterols were detected using the Salkowski technique. 1 ml concentrated sulphuric acid was applied to 1g plant extract and left to sit for 5 minutes in this test. The presence of phytosterols is shown by the creation of a golden yellow tint in the lower layer after shaking.

2.3.5. Test for flavonoids

Concentrated sulphuric acid was used to treat the extract. The presence of anthocyanins is indicated by a yellowish orange appearance. Flavones are found in yellow to orange colors, while flavonones are found in orange to crimson colors.

2.3.6. Test for terpenoids

Salkowski test: 5 g of each extract was dissolved with 2 ml chloroform, then a layer of concentrated H₂SO₄ (3 ml) was carefully added. To illustrate favorable findings for the presence of terpenoids, a reddish-brown coloration of the interface was generated.

2.3.7. Test for tannins

In a test tube, 0.5 g of dried powdered material was cooked in 20 ml of water and then filtered. A few drops of 0.1 percent ferric chloride were added, and the coloration was checked for brownish green or blue-black.

2.4. Preparation of herbal hand-wash

Herbal handwash gel was prepared with the help of Carbopol-934 as a gel forming agent in the concentration of 1% w/w for a period of one day. Then the gel was agitated with the help of a mechanical agitator to confirm even distribution of the gel. Then adjust the pH to 7.0 by adding small quantities of TEA with nonstop stirring. Then, the base was used to integrate extract of *Andrographis paniculata* to develop a formulation of herbal handwash. To this Sodium lauryl sulfoacetate (0.4g) as surfactant and Rosewater (15ml) was also assimilated.^{11,12}

2.5. Antibacterial activity

The antibacterial activity of herbal handwash was investigated using the agar well diffusion method. The test organisms were dispersed with bacterial suspension of optical density of 0.8 at 800 nanometers. Wells of 8-millimeter diameter were then made on plate. Each well was filled with 100ml of extract and subjected to incubation at 37°C for the duration of 24 hours. After 24 hours of incubation antagonist action is determined using zone of inhibition (centimeter). The antibacterial activity of Herbal handwash was tested against microorganism in triplicates. Ciprofloxacin is used as standard. The organism used are *S. aureus*, *E. coli*, *S. auroginosa* and *B. subtilis*.¹³

Table 1: The phytochemical evaluation of *Andrographis paniculata* leaves extract.

S.No.	Phytochemical Test	Observation	Result
1	Test for alkaloids	A little sample of the extract was filtered after being agitated with 1 ml of dilute HCl. Dragandroff's reagent was used to treat the filtrate.	The presence of alkaloids is indicated by the appearance of organic precipitate.
2	Test for saponins	In a water bath, 2 g of powdered material was cooked in 20 ml of distilled water and filtered. 10 mL filtrate was mixed with 5 mL distilled water and aggressively shaken to obtain a stable, persistent foam. The foam was combined with three drops of olive oil and vigorously shaken.	Emulsion formation occurs
3	Test for glycosides	A small amount of the extract was hydrolyzed for a few hours on a water bath with 5ml hydrochloric acid, and the hydrolysate was exposed to Fehling's test. 2ml extract was added to 2ml Fehling's solution (1ml Fehling's A and 1ml Fehling's B solution), mixed thoroughly, and boiled.	The presence of reducing sugars is indicated by the appearance of yellow or red precipitate.
4	Test for phytosterol	Phytosterols were detected using the Salkowski technique. 1 ml concentrated Sulphuric acid was applied to 1g plant extract and left to sit for 5 minutes in this test.	The presence of phytosterols is indicated by the formation of a golden yellow tint in the lower layer.
5	Test for flavonoids	Concentrated H ₂ SO ₄ was used to treat the extract. The presence of anthocyanins is indicated by a yellowish orange appearance.	Flavones are found in yellow to orange colors, while flavonones are found in orange to crimson colors.
6	Test for terpenoids	5 g of each extract was added with 2 ml chloroform, then a layer of concentrated H ₂ SO ₄ (3 ml) was carefully added.	To illustrate favorable findings for the presence of terpenoids, a reddish-brown coloration of the interface was generated.
7	Test for tannins	In a test tube, 0.5 g of dried powdered material was cooked in 20 ml of water and then filtered. A few drops of 0.1 percent ferric chloride were applied, and the results were recorded.	The coloration can found be brownish green or blue-black.

Table 2: Antibacterial activity of the herbal hand wash

Concentration (mg/mL)	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>
Zone of inhibition (mm)			
10	-	-	6.00±1.00
20	-	-	9.26±1.63
50	-	-	13.23±1.12
Ciprofloxacin (5µg)	15.34	17.16±1.41	18.12±1.42

Values are represented as mean ± Standard Deviation (n=3). P<0.05 between extract and negative control treated Cup plate.

Table 3: Physical evaluation of formulation

S.No.	Parameters	Observation
1	Appearance	Green color
2	Texture	Homogenous
3	pH	6.8
4	Viscosity (m P)	80-95

2.6. Physico-chemical evaluation of formulation:^{14–17}

Physico-chemical evaluation of the herbal hand wash gel was determined by appearance, pH, viscosity, foam height and retention.

2.7. Homogeneity and appearance

Morphological or physical evaluation of hand wash gel for color, odor and texture were carried out by visual inspection.

2.8. pH

200 ml distilled water was taken and 2 gm of herbal handwash was dissolved in it. A previously standardized digital pH meter was used to determine the pH of the solution.

2.9. Determination of viscosity

Digital Brookfield viscometer was used to determine the viscosity of herbal handwash.

2.10. Determination of foam height

Herbal gel-based hand wash sample of about 1gm was taken and it was mixed with 50 ml of water. It is after transferred to 250 ml stoppered beaker. With distilled water, the capacity was increased to 100 mL. After 25 motions, the solution was allowed to stand until the aqueous volume reached 100 ml and the foam height was measured above the solution.

2.11. Determination of foam retention of the gel

A 50 mL herbal gel-based hand wash was placed in a 100 mL measuring cylinder and shaken 10 times. For 5 minutes, the volume of foam was measured at 1-minute intervals. Foam retention should be stable for at least 5 minutes.

3. Results and Discussion

Phytochemical Screening- The qualitative analysis of alcoholic *Andrographis paniculata* leaves extract was performed for phytochemical screening. The results were shown in Table 1.

The results show that saponins, flavonoids, terpenoids, alkaloids, and glycosides are present in the alcoholic extract of *Andrographis paniculata* leaves. The extract was further formulated in gel dosage form and then evaluated for its anti-microbial activity.

3.1. Antibacterial activity-¹⁸

The antibacterial efficacy of the formulation was tested on *S. aureus*, *E. coli*, *S. auroginosa* and *B. subtilis*. Herbal hand wash has shown significant antimicrobial activity against *B. subtilis* (Table 2).

The results show that the herbal hand wash formulations are used in different concentrations was shown significant effect against *B. subtilis*. The different concentrations of 10, 20, 50 mg/ml of formulation the zone of inhibition against *B. subtilis* was found to be 6.00 ± 1.00 , 9.26 ± 1.63 and 13.23 ± 1.12 mm, respectively. On the other side, ciprofloxacin (5 µg/ml) was used as standard drug for positive control and it shows 18.12 ± 1.42 mm zone of inhibition against *B. subtilis*. In previous studies, Lai Xiaoping et al. (2014), received patent grant (CN103301049A) entitled “*Andrographis paniculata* hand sanitizer and preparation method thereof”. So, results confirmed that *Andrographis paniculata* leaves alcoholic extract containing formulation has good potential as herbal handwash.

3.2. Evaluation of formulation

Herbal handwash was also evaluated by physical parameters (Table 3) physical and chemical standards like odor, color, consistency, pH, viscosity, foam retention and foam height were performed and results were found to be in the acceptable limits.

Previously, Patel et al. (2017), formulated and evaluated herbal hand wash containing ethanolic extract of *Glycyrrhiza glabra* root extract. On comparison with that study, our study shows concordant significant antibacterial activity as well and used in future after clinical trials.

4. Conclusions

An *Andrographis paniculata* leaves extract containing herbal hand wash was developed with potential antibacterial and enhanced quality using *Andrographis paniculata* leaves extract as antibacterial agent, Carbopol-934 as gelling agent, TEA as neutralizer and Sodium lauryl sulfoacetate (SLSA) as surfactant. The antibacterial efficacy results confirmed its effectiveness of hand wash against *B. subtilis* micro-organism. The finding also revealed that herbal hand wash are promising formulation preparation which protect us from many daily encounter bacteria and beneficial to eliminate various skin disorders. As a future scope, these preclinical findings must be extrapolated to human subjects in order to confirm their clinical value. Finally, it is concluded that this herbal gel hand wash provided an effective and safe alternative to existing marketed hand wash gels.

5. Acknowledgements

Authors are thankful to Shri Sai College of Pharmacy, Prayagraj, Uttar Pradesh, India for providing necessary facility to carry out this research work.

6. Conflict of Interest

None.

7. Source of Funding

None.

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Cite this article: Mishra KK, Kumar A, Gupta A, Singh S, Sahu AK, Kashyap P, Mishra SP. Development and evaluation of herbal handwash gel containing *Andrographis paniculata* alcoholic extract. *IP Indian J Immunol Respir Med* 2022;7(2):73-77.