

Formulation and Evaluation of Polyherbal Hand Sanitizer Gel

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ABSTRACT: Objective: This piece of work explores the formulation of an anti-microbial hand sanitizer gel using neem and orange peel extract having a good anti-microbial action to serve the purpose of maintaining personal hygiene in a biocompatible and eco-friendly way.

Method and Materials: Hand sanitizer is mainly produced using 60% of alcohol as an active substance. Therefore, many hand sanitizers' products are highly dominant with the alcohol smell. This research was aimed to formulate a sweet-scented hand sanitizer using a combination of orange essential oil as the addition of fragrant substance. Physical properties (i.e. pH, density) and microbiological tests (i.e. total plate count and area of bacterial inhibition) were performed, as well as sensory tests to determine consumer response. The sweet scene of the hand sanitizer product was clearly shown, as much as 77% of respondents favoured its aroma. The current study clearly shows that orange peels were potential as the additional ingredients in giving the sweet smell to the hand sanitizer.

Results: Herbal hand sanitizer gel was prepared and evaluated using plant extracts, having potent anti-microbial action.

Conclusion: Formulation of plant based hand sanitizer products by replacing the synthetic surfactants with natural saponins and antimicrobial extracts can be useful for regular use on skin, while reducing the toxic load on environment as well.

Keywords:-Pharmacognosy,Covid-19 ,Sanitizer gel, Antimicrobial ,Alkaloids,

I. INTRODUCTION

The Covid-19 pandemic has increased awareness regarding the significance of hand hygiene in restricting infections. The way society has adopted the appropriate use of hand wash and hand sanitizers, it not only played an important role in reducing the Covid-19 infection but the transmission of other contagious diseases as well. The marketed formulations of hand sanitizers are synthetic components based. These formulations

disturb the epidermal barrier and make the skin dry and sensitive.¹ Moreover, these formulations along with other synthetic perfumes, preservatives, and artificial colours when entering the water bodies and soil, makes the environment toxic. It necessitates the formulation development of such hand sanitizer formulations, having biocompatible properties to the human skin and the environment without compromising the cleansing action and anti-microbial properties. The use of traditional herbal products among south-east Asian countries has been looked upon by the west and a tremendous amount of work has been done to characterize the therapeutic potential of various herbs. In this study, the antimicrobial properties of orange peel extract and neem oil have been explored and incorporated to formulate a gel-based hand sanitizer. Hand sanitizing is the most efficient way to prevent the transmission of bacteria that cause diarrhoea, influenza, and the common cold. It is the easiest, most significant, and least expensive technique to promote hand cleanliness in health care and aid in the prevention of infectious disease. The WHO standard requires people to wash their hands with no antibacterial soap and water. The time duration ranged on average as short as 15 to 30 seconds, including rubbing the backs of hands, wrists, between fingers, and under fingernails. Hand sanitizing with Poly herbal hand sanitizers and water is 25% more effective than washing with water alone in removing bacteria. According to studies, only about 40% of healthcare workers, homeowners, and officers follow hand-washing procedures. Microbial infections impact the skin, which is one of the most vital areas of the body. Hand sanitizing is essential for protecting the skin from harmful germs and preventing the spread of communicable diseases.²

NEEM (*Azadirachta indica*)

Azadirachta indica is also known as Neem, margosa tree, and Indian lilac. Various neem tree parts have been used in traditional ayurvedic medicine around the world. Terpenoids,

alkaloids, tannin, saponins, flavonoids, and amino acids are found in neem leaves. It has broad antimicrobial activity against both gram-negative and gram-positive bacteria. It is also used to treat skin diseases, healthy pairs, liver function, blood

detoxification, pest and disease control, fever reduction, dental treatments, cough, asthma, ulcers, and intestinal worms. Thus, neem oil was used as an antimicrobial agent in the hand sanitizer formulation.³



Natural aroma can be obtained using essential oil, which is mainly consists of volatile compounds. One of the most abundant resources for essential oil is orange peels. Essential oil extracted from orange peels has been used in food and pharmaceutical industries for its anti-inflammatory and anti-bacterial effects. In addition, substantial quantity of this substance has been used for home care products such as toilet soaps, perfumes, and cosmetics. Several studies were reported that the essential oil from orange peels contained biological activity effect, such as anti-fungal, anti-oxidant, anti-inflammatory, and antimicrobial activities. There are also inhibitory effects to the microbial contaminant such as Salmonella and Streptococcus from the orange peels. Therefore, the present study was aimed to formulate a hand sanitizer with a sweet scent and antimicrobial activity using essential oil from orange peels.⁴

Orange peel extract :

Natural aroma can be obtained using essential oil, which is mainly consists of volatile compounds. One of the most abundant resources for essential oil is orange peels. Essential oil extracted from orange peels has been used in food and pharmaceutical industries for its anti-inflammatory and anti-bacterial effects. In addition, substantial quantity of this substance has been used for home care products such as toilet soaps, perfumes, and cosmetics. Several studies were reported that the essential oil from orange peels contained biological activity effect, such as anti-fungal, anti-oxidant, anti-inflammatory, and antimicrobial activities. There are also inhibitory effects to the microbial contaminant such as Salmonella and Streptococcus from the orange peels. Therefore, the present study was aimed to formulate a hand sanitizer with a sweet scent and antimicrobial activity using essential oil from orange peels.⁵



II. BACKGROUND:

Zeeshan Afsaret et al. (2016) formulation and evaluation of poly herbal soap and hand sanitizer. The objective seen in this research project was to prepare hand sanitizer and soap formulations using the extracts of Cassia fistula, Millettia pinnata and Ficus religiosa and to

investigate the antimicrobial activity of the extracts against the common organisms which cause nasocomial infections. Furthermore to evaluate testability and phytochemical parameters of the prepared formulations so that they can be further standardized and used commercially. Zeeshan Afsaret et al (2016) firstly collect the plants Cassia

fistula, *Milletia pinnata* and *Ficus religiosa* from Mysore district, the specimen were authenticated at RRL, Bangalore. For extract preparation Zeeshan Afsaret et al used leaves and bark of *Cassia fistula*, *Milletia pinnata* and *Ficus religiosa* were dried in hot air oven at 35°C for three days, powdered to a mesh size of # 40 and stored in air tight for extraction. Zeeshan Afsaret et al (2016) used solvents such as Petroleum ether, Chloroform, Ethyl acetate, Methanol, and 40% methanol for extraction. In this scenario, preliminary antimicrobial screening by agar well diffusion method against the organisms *E. coli* (MTCC - 1698), *S. aureus* (MTCC - 1143) and *P. aeruginosa* (MTCC - 2453). The extracts which exhibited maximum activity were selected for the formulation. Zeeshan Afsaret et al (2016) prepared combination in two different concentrations i. e. 250 mg each (750 mg) and 500 mg each (1500 mg) and these concentration were further used in the formulation and evaluation parameter was checked, the result revealed in the project of Zeeshan Afsaret et al (2016) was most of the extracts exhibited good antimicrobial effect among which the ethyl acetate bark extracts of *Cassia fistula* and *Ficus religiosa* and methanolic bark extracts of *Milletia pinnata* and *Cassia fistula* exhibited maximum activity with zones of inhibition ranging from 14 to 18 mm. Also The prepared formulations when tested for antimicrobial activity exhibited zones of inhibition ranging from 18 to 26 mm which was far better than the zones of inhibition of individual extract.⁶

Rina maskare et al. (2019) formulation and evaluation of poly herbal hand sanitizer. The objective of this project was to prepare herbal hand sanitizer and to investigate whether the formulation show an antimicrobial activity against the common organisms which cause nasocomial infections. Rina maskare et al. (2019) used leaves of *Azadirachta indica* and *Eucalyptus globules* collected from Gondia city in 2019. Later the leaves was dried in shed, coarsely powdered and then used for further work. For the formulation of hand sanitizer, the methodology used by Rina maskare et al. (2019) is as follows ; firstly extraction of *Azadirachta indica* and *Eucalyptus globulus* was done in MIBP Gondia, for extraction solvents like Ethanol, methanol was used which was obtained from S D FINE - CHEMLIMITED, Mumbai, India. Also Bacterial Strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* were procured from D. B. Science College, Gondia. Was used in this project

to observe inhibition of bacterial growth. The result revealed in this study was the % yield was found to be 14% and 16% for *Azadirachta indica* and *Eucalyptus globulus* respectively. The methanolic and ethanolic extract showed good antibacterial activity against *E. coli*, *S. aureus*, *B. subtilis* with 1.56 mg/ml, 3.12mg/ml, 3.12mg/ml and 1.56mg/ml, 3.12mg/ml, 3.12mg/ml and mild to moderate antibacterial activity against *P. aeruginosa* with 6.25mg/ml and 12.5mg/ml respectively.⁷

Mounika et al (2017), Formulation and evaluation of poly herbal hand sanitizer gel containing essential oils. The objective seen in this research project was to evaluate the antibacterial efficiency of various herbal oils such as eucalyptus oil, cinnamon oil, geranium oil, peppermint oil, rosemary oil, clove oil and orange oil. The anti-microbial activity of the formulated herbal hand sanitizer gel was tested on common organisms by pour plate technique and the results obtained were compared with commercial antibacterial standards. A. Mounika et. al firstly collect the oils from Allin exporter Mumbai and Ooty. In this scenario preliminary anti - microbial screening by pour plate techniques against *E. coli* and *S. aureus*. A. Mounika et. al prepared two Formulation in which first formulation is prepared by using HPMC - 50 as gelling agent and other by using carbopol 940. And evaluation parameters was checked, the result revealed in the project of A. mounika et. al According to zone of inhibition the combination of cinnamon and geranium oil was equally effective against both the bacteria. It produces wider zone of inhibition against *S. Aureus* 7.5 mm, *E. coli* 8 mm.⁸

III. METHODOLOGY:

Collection of plant material: (Neem)

The plant Neem [*Azadirachta indica*] leaves were collected from Sachdeva Institute of Pharmacy, College Campus, Gharuan. To remove sand particles from sample, wash it thoroughly with fresh water. The plant material dried under sunlight for 4 to 5 days. Then the dried plant material was crushed, sieved to get nearly fine amorphous powder. Powdered material was extracted with a suitable solvent.

Extraction of plant materials: (Neem)

Step 1. Collected leaves of *Azadirachta indica* (Neem) were air-dried and powdered using a mixer grinder

Step 2. 10gm powdered material were soaked in 120 mL of ethanol solution (9 parts of ethanol, 1 part of distilled water) and kept for maceration for about 2-5 days.

Step 3. This mixture was then heated in a water bath at 65°C for 60 minutes

Step 4. The extract was then filtered via filter paper to remove any particles

Collection of plant material: (Orange peel)

The orange peels were collected from Sachdeva Institute of Pharmacy, College Campus, Gharuan. To remove sand particles from sample, wash it thoroughly with fresh water. The plant material dried under sunlight for 4 to 5 days. Then the dried plant material was crushed, sieved to get nearly fine amorphous powder. Powdered material was extracted with a suitable solvent.

Extraction of plant materials: (Orange peel)

The washed orange peels were crushed in order to reduce their size. The crushed samples were used to isolate an essential oil by hydro distillation as follows:

Step 1. Crushed pieces of orange peels (225 g) were placed in to 1000 ml round bottom flask and 720 ml of distilled water was added to it.

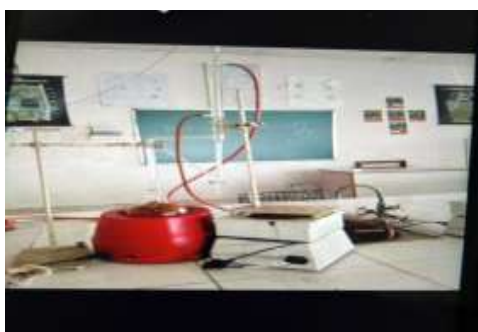
Step 2. After addition of boiling chips the flask was heated slowly for two hours.

Step 3. The distillate forms two layers in the Clevenger apparatus with organic compounds at upper and hydrosol at bottom.

Step 4. The organic phase was collected by small bottles and dried with anhydrous sodium sulphate.

Step 5. The weight of essential oil was measured and the percentage yield of oil was calculated.

Step 6. The procedure was carried out twice for orange peel extraction essential oils.



Qualitative analysis of phyto-chemical constituents:

Qualitative analysis:

The obtained peel extract were subjected to various qualitative analysis.

Test for alkaloids:

A pinch of crude powder was mixed with 1% HCl and about 6 drops of Mayor's reagents. A creamish or pale yellow precipitate indicated the presence of respective alkaloids.

Test for amino acids:

A pinch of crude extract was treated with few drops of Ninhydrin reagent. Appearance of purple color showed the presence of amino acids.

Test for tannins:

A pinch of crude extract was treated with few drops of 0.1% ferric chloride and observed for brownish green or a blue-black coloration. The result was positive for presence of tannins.

Test for saponins:

Froth test for saponins was used. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Test for protein:

A pinch of crude powder was treated with 4% Sodium Hydroxide and few drops of 1% Copper sulphate was added. Violet or pink colour appearance confirmed the presence of protein.

Test for terpenoids (Salkowski test):

A pinch of crude powder was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Method of Preparation:

- 1) Polyherbal hand sanitizer Gel was prepared using Carbopol(940) as Gelling agent which is soaked in 15mL distilled water overnight.
- 2) Neem extracts were measured accurately and dissolved by gentle heating.
- 3) After heating, keep the solution aside for sometimes.

- 4) The required quantity of Glycerine was mixed in aqueous phase with continuous stirring.
- 5) The alcohol 60% was dissolved in remaining quantity of purified water and dispersed into the extract.
- 6) 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 orange peel essential oil was added for Fragrance.

- 7) Lastly, it was stored in well closed container and labelled suitably for further analysis.

Formulation Table:

Sr. No.	Ingredients	Proportion	Uses
1.	Carbopol 940	0.5%	Gelling agent
2.	Alcohol 70%	60%	Aroma
3.	Glycerin	1%	Softening agent
4.	Essential oil	1%	Perfume
5.	Distilled Water	Up to 100mL	Vehicle

Evaluation Parameters of Polyherbal hand sanitizer gel:

Prepared formulation of Polyherbal hand sanitizer gel was subjected to following evaluation parameters:

- 1) **Organoleptic Evaluation Parameters** like colour, odour, texture was carried out: Colour and texture were evaluated by visual and touch sensation respectively. The Odour was inspected by sensing the formulation.
- 2) **Appearance and Homogeneity:** Appearance and Homogeneity was evaluated by visual inspection.
- 3) **Grittiness:** 1ml of Gel was taken on finger tips and rubbed between two fingertips, then the formulation was evaluated.
- 4) **Skin Irritation Test:** Skin Irritation Test was evaluated by applying polyherbal hand sanitizer gel on skin and left for 30 min, after 30 minutes of washing observe any itching, rashes or redness on skin by sensory and visual inspection.
- 5) **pH:** 1gm of Sample of polyherbal hand sanitizer gel was taken and dissolved it into 100ml distilled water. The pH solution was measured by standardized digital pH meter.
- 6) **Spread ability:** 0.5gm of Sample of polyherbal hand sanitizer gel was pressed between two slides and left for about 5 minutes where no more spreading was expected. Diameter of spreaded circle was measured in cm and was taken as comparative values for spread ability.

7) **Stability:** The Stability studies were carried out for polyherbal hand sanitizer gel formulation by storing at different temperature conditions like 40°C, 25°C, and 37°C for 1 week. During the stability studies no change in color and no phase separation were observed in the formulated hand sanitizer.

8) **Antimicrobial Study of Polyherbal hand sanitizer gel:** The Screening of anti - microbial efficacy of the formulated polyherbal hand sanitizer gel was performed on soil Microbes by using agar plate method as per standard procedure. Two Sterile petri plates were taken for testing the antimicrobial activity against soil microbes. The plates were filled with nutrient agar solution and allowed for solidification. After solidification the soil extract from the subculture were poured into the nutrient agar media by Pour Plate Method and inoculated for 24 hours. After 24 hours of inoculation, two cavities were made in it by Cup Plate Method. The First cavity is filled with Marketed Herbal Hand sanitizer (Patanjali), Second one with Formulated polyherbal hand sanitizer gel. It was taken care that sample should be placed at the level of cavity. The Plates are placed in incubator at 37°C to test the activity. After 48 hours, the plates were observed for the formulation of Zone of Inhibition. From the Zone of Inhibition, the antimicrobial activity of formulation is estimated. Efficiency of polyherbal hand sanitizer gel was determined by measuring the diameter of zone of inhibition.

IV. RESULT AND DISCUSSION

Table 1:

Sr. No.	Evaluation Parameters	Formulated Polyherbal Hand sanitizer Gel	Marketed Herbal Hand sanitizer (Patanjali)
1	Colour	Light Greyish	Light Orange
2	Odour	Pleasant	Pleasant
3	Texture	Smooth	Smooth
4	Appearance and Homogeneity	Translucent	Translucent
5	Grittiness	Non - Gritty	Non - Gritty
6	Skin Irritation test	No irritation	No Irritation
7	pH	7.92	8.11
8	Stability	Stable	Stable

1.1 Spreadability

Table 2: Spreadability of formulated polyherbal hand sanitizer gel

Sr. No.	Diameter (cm)	Radius (cm)
1	3.4	1.7
2	4	2
3	4.2	2.1

Table 3: Spreadability of marketed hand sanitizer

Sr. No.	Diameter (cm)	Radius (cm)
1	3.5	1.75
2	3.5	1.75
3	4.0	2.0

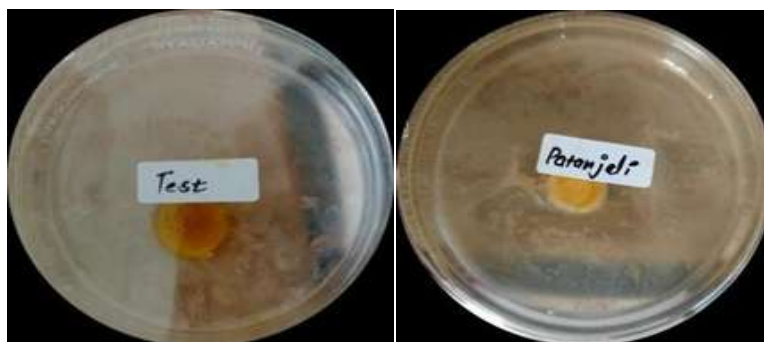
1.2 Antimicrobial Activity

Table 4: Antimicrobial activity of Formulated Polyherbalhand sanitizer gel

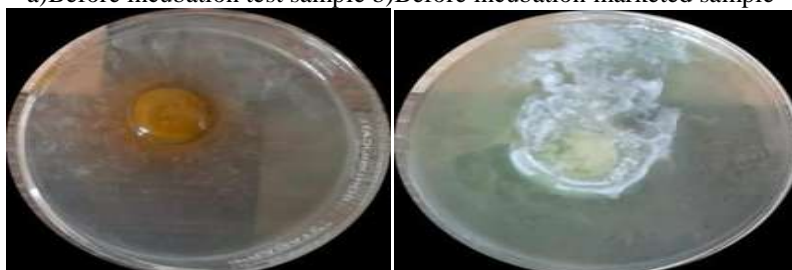
Efficacy	Diameter
Before Incubation	1.7cm
After Incubation	3.4cm

Table 5: Antimicrobial activity of marketed hand sanitizer

Efficacy	Diameter
Before Incubation	1.7cm
After Incubation	3.6cm



a) Before incubation test sample b) Before incubation marketed sample



b) After incubation test sample c) After incubation marketed sample

The results of visual inspection of the formulation are, light greyish in colour, the odour of the formulation is orange like and smooth in texture. The formulation is translucent in appearance and homogeneous. As we talk about the grittiness, the formulation was found to be non – gritty. Accurate pH of the formulation is important for minimizing irritation to the skin. The formulation has pH 7.9 which is suitable and non - irritating for the skin.

The spreading ability of the formulation was studied by taking 0.5 gram of sample and passing between the two slides and kept for about 5 minutes. The parameter set for taking readings was the reported time required by the formulation to cover the distance which was measured in centimeter by using measuring scale. The spreading ability of the formulation was found to be good.

To study the zone of inhibition the resulting formulation was studied against soil culture. The results showed that the formulation contained a broad spectrum of antibacterial agents. From the investigation it was clear that formulation produced zone of inhibition against soil culture.

Before incubation the observed diameter was noted as 1.8 centimeter and after incubation the zone of inhibition diameter spreads up to 3.4 centimeter. Hence it can be concluded that the formulation has good antibacterial activity.

V. CONCLUSION

Like Cosmetics, Cosmeceuticals (A cosmetic that has or is claimed to have medicinal properties) are topically applied but they contain ingredients that influence the biological functions of skin. The WHO estimates that 80% of the population of Asian country presently use herbal medicine for primary aspect of primary health care and for the purpose of hand hygiene includes preparation of Hand sanitizer. The present study was carried out to formulate Polyherbal hand sanitizer Gel containing herbal extract which is used not only for the purpose of cleaning hands but also for the prevention of bacterial growth. Its composition was prepared according to delicateness of skin so that it cannot cause any type of irritation. Hence, it can be concluded that the Polyherbal hand sanitizer Gel are much better than

existing marketed synthetic hand sanitizer due to their ingredients and effectiveness on our skin of hands and as well as suitable for all type of skin.

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