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Formulation and Evaluation of Herbal Hand Wash Using Ginger Rhizomes

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Keywords: Herbal hand wash, Formulation, Evaluation, Chamomile Extracts, Skin pathogens, *Staphylococcus aureus, Pseudomonas aeruginosa*

ABSTRACT

The numerous of antiseptic hand wash available in the market are alcohol based sanitizers which have some adverse effects. To avoid these adverse effects like itching, drying, irritation, dermatitis etc., of the synthetic handwash formulations an attempt has been made to formulate a polyherbal hand wash using extracts of ginger rhizomes extracts. The anti-microbial activity of prepared poly-herbal hand wash was tested against skin pathogens collected from volunteers, and its efficiency was verified using Cup Plate Method. The results from Cup Plate Method showed that hand wash prepared from alcoholic extract of ginger rhizomes and aqueous extract of ginger rhizomes have effective activity due to combined activity of phytoconstituents present in extracts. The results from the present work support the incorporation and utilization of herbs in formulations to give a better effect. Herbal hand wash evaluated by tested parameters like physical parameters like colour, fragrance and chemical parameters like pH, Viscosity, Foam height, Foam retention, Anti-Microbial Activity, Skin irritation test etc. and obtained results were in the acceptable limits with less or no side effects. Two formulations of handwash were prepared and the formulations were evaluated for physical properties like appearance, pH and viscosity. The antimicrobial activity of prepared formulations of hand wash was checked against skin pathogens *Bacilus subtilus, Staphylococcus, Pseudomonas aeruginosa* and *Escherichia coli* by agar diffusion method. The aim of present work was to prepare formulations of poyherbal handwash from the ethanolic extracts of ginger rhizomes.

INTRODUCTION

1.1.Background

The importance of handwashing for human health - particularly for people in vulnerable circumstances like mothers who had just given birth or wounded soldiers sin hospitals - was first recognized in mid 19th century by two pioneers of hand hygiene. At that time most people still believed that infections were caused by foul odors called miasmas.

In the 1980s, foodborne outbreaks and healthcare-associated infections led United States Centers for Disease Control and Prevention to more actively promote hand hygiene as an important way to prevent the spread of infection. The outbreak of swine flu in 2009 led to increased awareness in many countries of importance of washing hands with soap to protect oneself from such infectious diseases. For example, posters with "correct hand washing techniques" were hung up next to hand washing sinks in public toilets and in toilets of office buildings and airports in Germany[4].

Industrial and hand cleansing formulations typically contain a surfactant that solubilizes or emulsifies the oils, debris, and soil present on a substrate. These formulations inherently have oil-cleansing limitations when oil-emulsifiability or solvency alone is used as a cleaning mechanism. When only surfactants in combination with non-aggressive solvent cleansers are used in the cleansing compositions, the cleaning power of the composition may be inadequate when stubborn or embedded oils are present. If the chemical formula is too aggressive in terms of its solubilizing or emulsifying power, skin can be harmed due to defatting of the dermal oils thereof, particularly when the cleanser is used repeatedly[8,9].

1.2 Scope

The perfume of the *herbal hand wash* keeps the skin fresh and lithe. The mild foaming action does not cause any irritation while using *herbal hand wash*. It also helps to remove the dirt and oil effectively from the skin. It also helps to clear antiseptic and fungal problems faced by the skin.

Removing germs through handwashing therefore helps prevent diarrhea and respiratory infections and may even help prevent skin and eye infections.

Handmade herbal soaps have therapeutic benefits in addition. Those with sensitive skin or conditions such as psoriasis or eczema can take aid from natural handmade soaps. Glycerin soap also protects the skin which is sensitive and delicate, and when herbal soaps are used, you can be sure that the glycerin content is high absorbing water in the air and ensuring the skin remains soft and healthy[5,8].

1.3 Research Objective

To achieve sustained improvements in hand hygiene compliance rates.

Accurately measure rates of staphylococcal disease a key outcome measure of the program.

Reduce the rates of healthcare associated infections.

Develop an effective education and credentialing system to improve knowledge about hand hygiene and infection control.

To kill Germs and microorganisms that can harm our body.

It will help to learn their illnesses are often caused by germs which travel from their hands to their mouths, eyes, noses, etc.

The hands of health care workers are the primary routes of transmission of infection to patients. Hence, it brings up the use of antiseptic for handwashing purposes. Evaluation of the antimicrobial activity against skin pathogens of the prepared herbal hand wash was performed using disc diffusion method.

1.4 Plan of work

Literature survey.

Collection of plant material.

Drying and size reduction.

Preparation of extracts.

Formulation of herbal handwash.

Evaluation of anti-microbial activity.

Preparation of Thesis.

2.1 REVIEW OF LITERATURE

2.1.1 Wongworawat et al. (2007) carried out a randomized controlled study looking at the influence of rings on the efficacy of hand sanitization and residual bacterial contamination. They compared the impact of finger rings on the effectiveness of scrub less and water-aided alcohol-based hand sanitization methods with that of povidone-iodine scrub. The subjects were a pool of perioperative staff and medical students who knew how to carry out a pre-op surgical scrub. There was no significant difference in the number of bacteria between hands with and hands without rings (1 ring, >90% smooth) for the groups that used alcohol wash or alcohol chlorhexidine lotion. In the povidone-iodine group, the number of bacteria on hands with rings was greater than the group without rings. The alcohol-chlorhexidine group had the lowest bacterial count, regardless of the presence of rings. The presence of 1 ring did not appear to negatively impact on the effectiveness of alcohol-based hand sanitizers in a group of HCWs who were familiar with surgical scrubbing procedures.

2.1.2 Wilson (2001)

Underlined several factors, including poor staffing levels, inadequate sinks and poor water temperature controls, as reasons which discourage handwashing. Skin irritation is also perceived to be a significant barrier to compliance with hand hygiene (Larson, 1995). However, further studies are required to determine the effect of individual factors on compliance as the majority of those conducted to date have been multi modal in nature and as a result have produced data which are difficult to interpret. A number of recent initiatives have been launched to address some of these issues and to improve compliance amongst healthcare staff, including the National Patient Safety Association's 'Clean Your Hands Campaign', the English Bulletin recommending hand gels at each bed, the NHS Scotland HAI Task Force Mandatory Training Programme, NHS Education Scotland's Cleanliness Champions Programme and the RCN and ICNA initiatives.

2.1.3 Joshi (2008)

The herbal handwash was prepared using extracts of rhizomes of zingiber Officinale Roscoe, Couroupita guianensis Aubl and rinds of Garcinia indica Choisy. The antibiotic sensitivity test of the prepared herbal handwash against skin pathogens was checked using Disc

diffusion method and results were compared with the commercially available antiseptic soap. The results showed that the herbal handwash gave larger inhibition zone than the commercial antiseptic soap against Staphylococcus aureus and Pseudomonas aeruginosa. The efficacy of the herbal handwash was evaluated using Glove Juice method which revealed that the herbal handwash is efficient in reducing higher number of organisms from the hands than the commercial antiseptic soap. Hence, due to the higher antimicrobial activity these plant materials can be used in the preparation of herbal handwash on commercial scale.

2.1.4 Sharma, et al (2015)

Microbial infection has emerged as a critical issue in children and hospital care outcome, which can leads to substantial morbidity and mortality. Unhygienic hands of health care workers are the primary routes of transmission of infection directly to patients and in children, it can lead to several serious health issues. So that, it brings up the use of antiseptic for handwashing purposes. There are several commercial antiseptic available in market having chemical sanitizers as a base which has some disadvantages, adverse and side effects. Their frequent and long time use can lead to some side effects and skin irritation. Madhuca indica is one of the most widely used and well-documented medicinal plants in the world.

Present study aimed to formulate effective, safe and nontoxic herbal hand wash using barks of Madhuca indica emphasis on safety and efficacy and to avoid the risk posed by synthetic antimicrobials. Disc diffusion method was utilized for evaluation of the antimicrobial activity against skin pathogens of the prepared herbal hand. Its efficacy was checked and compared with the standard commercial hand wash. Results revealed that Madhuca indica based formulation was more efficient in reducing the number of organisms from hands than the commercial antiseptic soaps thus it can be used as an antiseptic soap based handwash with less or no side effects.

Nasocomial infection has emerged as a critical issue in hospital care outcome, resulting in substantial morbidity and mortality. The hands of health care workers are the primary routes of transmission of infection to patients. Hence, it brings up the use of antiseptic for handwashing purposes. Many of the antiseptic available in market are alcohol based sanitizers which have some shortcomings or adverse effects. Their frequent use can lead to skin irritation. Chamomile is one of the most widely used and well-documented medicinal plants in the world. This study aimed to formulate effective herbal hand wash using

Matricaria chamomilla (German chamomile) flowers with emphasis on safety and efficacy and to avoid the risk posed by synthetic antimicrobials.

Evaluation of the antimicrobial activity against skin pathogens of the prepared herbal hand wash was performed using disc diffusion method. Its efficacy was checked and compared with the commercial ones. Results revealed that chamomile soap formulation was more efficient in reducing the number of organisms from hands than the commercial antiseptic soaps thus it can be used as an antiseptic soap with less or no side effects. Routes of transmission of infection to patients.

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Keywords: Herbal hand wash, Formulation, Evaluation, Chamomile Extracts, Skin pathogens, *Staphylococcus aureus, Pseudomonas aeruginosa*.

2.1.5 Arvind et al (2015)

One of the primary modes of transmission of micro-organisms are our hands. Hand-washing is important in food production, food service and daycare preparations. Hence it brings us to the use of antiseptic for handwashing purposes. Many of the antiseptic available in market are alcohol based sanitizers which have some shortcomings or adverse effects. Their frequent use can lead to skin irritation. The present research was aimed to evaluate the anti-microbial efficacy of herb and plant material such as Lemongrass and Bermuda grass by agar-cup method of these plant extracts that were obtained by soxhlet extraction. Also, the research was carried out to formulate and evaluate the herbal hand wash liquid containing the above extracts. The anti-fungal activity of the formulated hand wash gel was tested against *Aspergillus niger* by Agar Cup Method. Thus this work suggests and supports the incorporation and utilization of herbs and traditional plant materials in the formulation to give better antifungal effect. The further development should be performed to achieve broad antifungal activity with different extracts.

2.1.6 Londhe et al

Plants has medicinal, pharmaceuticals and cosmetic potential, using it many innovative products can be prepared useful for humans. In present study, herbal hand wash was formulated. These plants are traditionally known to possess different medicinal properties. Methods: Hand wash were prepared by three different methods viz, using SDS (Sodium dodecyl sulphate) as a base and plant extracts, saponified Cummins seed oil and plant extracts (II) and reetha fruits extracts and plant extracts. The antibacterial activity of formulated herbal hand wash and individual plant extracts were examined using agar well diffusion method.

2.1.7 Sharma et al (2015)

Herbal medicines are significant part of healthcare throughout the world. Herbal medicines have been extensively utilized as effectual remedies for the prevention and management of multiple health conditions. Hands are a prime mode of transmission of microbes and nosocomial infections. Hand-washing is extremely imperative in healthcare and domestic sector. Numerous of the antiseptic hand wash available in the market are alcohol based sanitizers which have some adverse effects. To avoid these adverse effects like itching, drying, irritation, dermatitis etc., of the synthetic handwash formulations an attempt has been made to formulate a polyherbal hand wash using extracts of zingiber Officinale Roscoe. The anti-microbial activity of the prepared poly-herbal hand wash was tested against the skin pathogens collected from volunteers, and its efficiency was verified using Cup Plate Method.

2.1.8 Shukla et al (2011)

This study was carried out with an objective to investigate the antimicrobial potentials of rhizomes of ginger. The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on some bacterial and fungal strains. In the present study, the microbial activity of hydroalcoholic extracts of rhizomes of ginger was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar disc diffusion method. The antibacterial and antifungal activities of extracts of *Cassia fistula* were tested against two Gram-positive *Staphylococcus aureus, Streptococcus pyogenes*; two Gram-negative *Escherichia coli, Pseudomonas aeruginosa* human pathogenic bacteria; and three fungal strains *Aspergillus niger, Aspergillus clavatus, Candida albicans*. Zone of inhibition

of extracts were compared with that of different standards like ampicillin, ciprofloxacin, norfloxacin, and chloramphenicol for antibacterial activity and nystatin and griseofulvin for antifungal activity.

2.2 PLANT OVERVIEW

2.2.1 Botanical Information

Zingiber officinale Roscoe rhizomes.

2.2.2 Taxonomical classification

Table No. 1: Taxonomical classification of Ginger.

Kingdom	**Plantae**
Order	Zingiberales
Family	Zingiberaceae
Clade	Angiosperms
Genus	Zingiber
Species	Z. officinale

2.2.3 Description

Ginger are aromatic herb with an underground rhizome and an erect stem, up to 75 cm. in height. Leaves of ginger are simple, alternate, linear-lanceolate, sheathing at the base, sessile, acuminate at apex, glabrosus, up to 15 cm long. Inflorescence a spike on a distinct scape flowers densely arranged, bisexual, irregular, each subtended by a persistent scarious bract. Calyx tubular shortly 3-lobed; corolla bilabiate, tubular below, yellow with purplish spots stamens 3 in one whorl, one of which is perfect the other 2 united to form a labellum; filament of perfect stamen short, anther cells contiguous, connective produced into a beak, ovary of 3 carpels, syncarpous, 3-celled, inferior; ovules many on axile placentas; style filiform, lying in a groove of the anther; stigma subglobose. Fruit an oblong capsule, many seeded; seeds arillate, globose, with a small embryo and copious endosperm.

2.2.4 Geographical Distribution

Ginger is indigenous to Indo-Malayan region and has been in cultivation in India since prehistoric times. It is now cultivated over a greater part of the tropical and temperate

regions. The chief ginger growing countries of the world are India, China, Australia, East Indies, West Indies, Mexico, Jamaica, North Africa and West Africa.

2.2.5 Chemical Constituents

Ginger contains 1-2% of essential oil. The ginger oil contains a mixture of constituents such as monoterpenes, namely phellandrene, camphene, cineole, linalool, limonene, citral, geraniol, citronellol, borneol and sesquiterpenes, namely α-zingiberene, ar-curcumene, β-bisabolene, β-sesquiphellandrene, zingiberol and zingiberenol along with some aliphatic aldehydes.

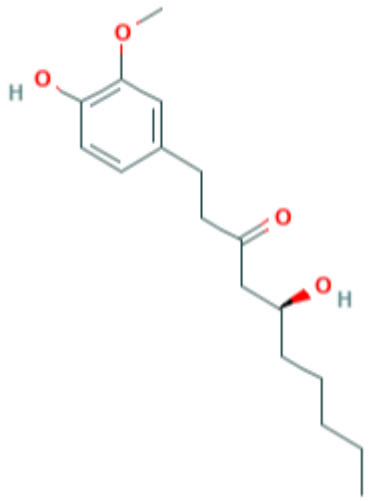

Fig no. 1. Chemical structure of gingerol.

2.2.6 Pharmacological activies

Antimicrobial Activities

Isolated from ginger rhizome, demonstrated antibacterial activity against periodontal bacteria.

Anti-Inflammatory Effects

Ginger has a long history of use as an antiinflammatory and many of its constituents have been identified as having anti-inflammatory properties. Ginger has been shown to share pharmacological properties with non-steroidal anti-inflammatory drugs (NSAIDs) because it suppresses prostaglandin synthesis through the inhibition of cyclooxygenase-1 and cyclooxygenase-2. However, ginger can be distinguished from NSAIDs based on its ability to

suppress leukotriene biosynthesis by inhibiting 5-lipoxygenase. This discovery preceded the observation that dual inhibitors of cyclooxygenase and 5-lipoxygenase may have a better therapeutic profile and have fewer side effects than NSAIDs. It was also discovered that a ginger extract derived from Zingiber officinale (and Alpina galanga) inhibits the induction of several genes involved in the inflammatory response, including genes encoding cytokines, chemokines, and the inducible enzyme cyclooxygenase-2. This discovery provided the first evidence that ginger modulates biochemical pathways activated in chronic inflammation. Identification of the molecular targets of individual ginger constituents provides an opportunity to optimize and standardize ginger products with respect to their effects on specific biomarkers of inflammation.

Antiemetic Effects

The mechanism of action of ginger's effect on nausea and vomiting remains uncertain. However, there are several proposed mechanisms. The components in ginger that are responsible for the antiemetic effect are thought to be the gingerols, shogaols, and galanolactone, a diterpenoid of ginger.

Antioxidant Effects

In vitro, ginger has been shown to exhibit antioxidant effects. (6)-gingerol appears to be the antioxidant constituent present in ginger, as it was shown to protect HL-60 cells from oxidative stress.7 Ginger oil has dominative protective effects on DNA damage induced by H2O2. Ginger oil might act as a scavenger of oxygen radical and might be used as an antioxidant.

Antigenotoxic Activity

Norethandrolone and oxandrolone were investigated for their genotoxic effect on human lymphocyte chromosomes using chromosomal aberrations and sister chromatid exchanges as parameters and subsequently, Genistein and [6]-gingerol were used as antigenotoxic agents to ameliorate the genotoxicity induced by the steroids. Norethandrolone and oxandrolone were studied at 5, 10, 20, 30 and 40 μM, respectively and were found to be significantly genotoxic at 30 and 40 μM. Genistein and gingerol proved to be equally effective in reducing genotoxic damage at appropriate doses.

Gastrointestinal Effects

There is evidence that ginger rhizome (root) increases stomach acid production. If so, it may interfere with antacids, sucralfate (Carafate), H2 antagonists, or proton pump inhibitors. In contrast, other in vitro and animal studies have revealed gastroprotective properties.16, 17 in addition, (6) shogaol, generally more potent than (6)-gingerol, has inhibited intestinal motility in intravenous preparations and facilitated gastrointestinal motility in oral preparations. Ginger extract has also been reported to inhibit the growth of Helicobacter pylori in vitro. 5 However, Desai et al. observed a significant increase in the exfoliation of gastric surface epithelial cells following the consumption of 6g or more of ginger (after examining gastric aspirates in 10 healthy volunteers).

2.2.7 Traditional Uses

Ginger is stimulant, carminative and diaphoretic. It is used in cold and cough and as a febrifuge. It is used to flavour foodstuff, beer and other drinks. It is used as a condiment in curries.

2.2.8 Contraindications

Burning feeling in mouth/throat.

Abdominal pain.

Diarrhea.

Heartburn.

Bleeding/bruising.

Rash.

Itching/swelling.

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Material

The fresh form of *Zingiber officinale* (ginger Rhizome) and dry the ginger rhizome also make the powder ginger rhizome.

3.1.2 Glasswares and equipments

Table No. 2:

Sr. No.	Glasswares and equipments.
1.	Beaker
2.	Conical flask
3.	Test tube
4.	Petri dish
5.	Heating Mantle
6.	Water bath
7.	Incubation chamber.

3.1.3 Chemicals

Table No. 3:

Sr. No.	**Ingredients and chemicals**	**Brand Name**
1.	Carbapol-940	(Repol)
2.	Triethanolamine	(Trolamina Polypeptide)
3.	Glycerine	(Glycerine Suppositories)
4.	Polysorbate-20	(Kafan)
5.	Perfume	(Giorgio Armani)
6.	Chloroform	(Dichloromethane)
7.	Ethanol	(Alcohol ethyl)
8.	Distilled water	(Aquafina)
9.	Sodium lauryl sulphate	(Carsonol SLS).

3.2 Methods

1. Samples of ginger rhizome were collected during process.

2. Dry the ginger rhizome make the powder.

3. The 10 g of powder were extracted with 100 ml of ethanol solution (9 parts of ethanol and 1 part of distilled water) by means of extraction.

4. This mixture was heated on water bath at 60C for 1 hour. The content was filtered through Whatman filter paper in order to get particle free extract. Filtrate was used as ethanol extract.

5. Take the 10ml of extract solution.

6. The contents were filtered and the filtrate was used as methanol extract.

7. The herbal hand wash was prepared by adding 40 ml of the alcohol extract.

8. 1gm of Triethanoplamine (sodium lauryl sulphate SLS) was added as per the requirement of standard procedure for preparation of hand wash.

9. And 1gm of add the Carbapol-940 it is an easy to **use** water soluble polymer, used as an emulsifying, stabilizing, suspending, thickening agent.

10. 5ml add glycerine and 1ml use for perfume agent.

11. To the final add the preservative and add the 20ml of distilled water.

12. The solution was made homogenous under room temperature and used for the room temperature.

Preparation of agar medium

1. Prepare MHA from the dehydrated medium according to the manufacturer's instructions. Media should be prepared using distilled water or deionized water.

2. Heat with frequent agitation and boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 min.

3. Check the pH of each preparation after it is sterilized, which should be at room temperature. This is done by macerating a small amount of medium in a little distilled water or by allowing a little amount of medium to gel around a pH meter electrode.

4. Cool the agar medium to 40-50°C. Pour the agar into sterile glass or plastic petri dish on a flat surface to a uniform depth of 4 mm.

5. Allow to solidify.

6. Prior to use, dry plates at 30-37°C in an incubator, with lids partly ajar, for not more than 30 minutes or until excess surface moisture has evaporated. Media must be moist but free of water droplets on the surface. Presence of water droplets may result to swarming bacterial growth, which could give inaccurate results. They are also easily contaminated.

Storage

If plates are not to be immediately used, they may be stored in the refrigerator inside airtight plastic bags at 2-8°C for up to 4 weeks.

Unpoured media may be stored in airtight screw-capped bottles under the conditions specified by the manufacturer.

INOCULUM

Preparation

1**.** From a pure bacterial culture (not more than 48 hours, old except for slow growing organisms), take four or five colonies with a wire loop.

2**.** Transfer colonies to 5 ml of Trypticase soy broth or 0.9% saline.

3**.** Incubate the broth at 30°C or at an optimum growth temperature until it achieves or exceeds the turbidity of 0.5 MacFarland standard (prepared by adding 0.5 ml of 0.048 M BaCl2 to 99.5 ml of 0.36 NH2SO4; commercially available).

4**.** Compare the turbidity of the test bacterial suspension with that of 0.5 MacFarland (vigorously shaken before use) against a white background with contrasting black line under adequate light. Arrow points to tube with correct turbidity.

5**.** Reduce turbidity by adding sterile saline or broth.

Inoculation of plates

1. Dip a sterile cotton swab into the standardized bacterial suspension.

2. Remove excess inoculum by lightly pressing the swab against the tube wall at a level above that of the liquid.

3. Inoculate the agar by streaking with the swab containing the inoculum.

4. Rotate the plate by 60° and repeat the rubbing procedure. Repeat two times. This will ensure an even distribution of the inoculum.

5. Allow the surface of the medium to dry for 3-5 minutes but not longer than 15 minutes to allow for absorption of excess moisture.

3.2.1 Qualitative Investigation of Plant Material

Pharmacognostical features

The transverse section of the rhizome shows a zone of cork tissue which is differentiated on the arrangement of cells. The outer zone of cortical cells in the cork are suberized (deposited with suberin which can be stained with fluoral yellow) without division and hence are irregularly arranged.

The inner zone contains cortical cells which arrange in a radial row and produced by tangential division. The cork cambium is not differentiated.

Inner to the cork cells is the cortex composed of cortical cells. The cortical cells contain plenty of simple, ovoid (sack-shaped) starch grains (sized 5-60μm) which can be stained with iodine. Inner to the cork is a broad cortex.

The outer cortex is composed of mainly flattened parenchyma, while the inner zone is composed of mainly normal parenchyma. The cortex also holds suberized oil cells which hold a yellow-brown oleoresin.

The inner cortex contains generally three layers of closed, collateral vascular bundles which contain phloem. The larger vascular bundles are protected in a sheath of nonlignified fibres. The vascular bundles contain sieve tubes and xylem with reticulate thickened vessels (these do not stain with phloroglucinol and HCl) which are some times accompanied by secretion cells (holding dark contents).

Inner to the cortex lies a single layer of endodermis which is devoid of starch.

Going inwards further from the endodermis lies the outermost layer of the stele which is characterized by a single-layered pericycle. The vascular bundles of the stele resemble that of

the cortex with the exception of a ring of small scattered bundles within the pericycle. The stele is mainly composed of parenchyma containing starch and oil cells similar to the cortical parenchyma. The innermost vascular bundles of the stele may contain a fibrous sheath.

3.3.1 Chemical Investigation

Detection of alkaloids

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates

a) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

b) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of Proteins

i) Biuret test: To the methanolic extracts, 4% sodium hydroxide and 1% copper sulfate solution were added and formation of violet or pink color indicated the presence of proteins.

Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

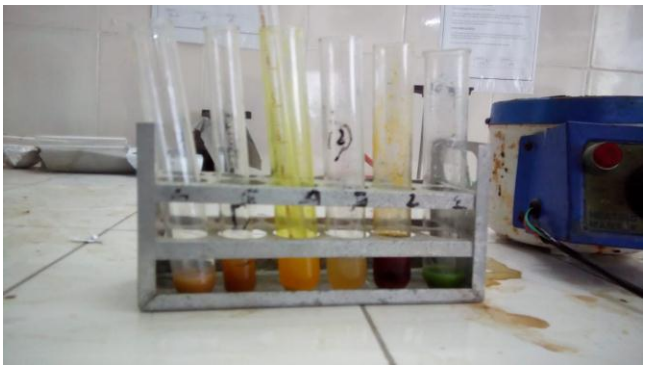

Figure No. 2: Alkaloid Test & Protein Test.

3.4.1. Qualitative Investigation

Pharmacognostical features

Macroscopic characters

Rhizomes are 7-15 cm long and 1-2.5 cm broad, irregularly branched with node and internodes. Scale leaves are present at the nodal region. The outer surface of the rhizome is smooth and light grey in colour, internally light yellow. These are hard and brittle, breaking with a short fracture, fragrant odour, aromatic, spicy and slightly bitter in taste.

Microscopic characters

The rhizome is circular with epidermis, cortex, endodermis and closed collateral vascular bundle. Transverse section of the rhizome showed outer single layered epidermis having rectangular and elongated cells, followed by thin walled cork cells of 6-10 layers, irregularly elongated. Cortex consists of several layers of parenchymatous cells with intercellular air spaces and contains starch. Oil cells are present in cortex. Central cylinder region contains a yellow to orange coloured oleo-resin. Endodermis consists of single layer of cells. Stele consists of a broad central zone of ordinary parenchymatous cells.

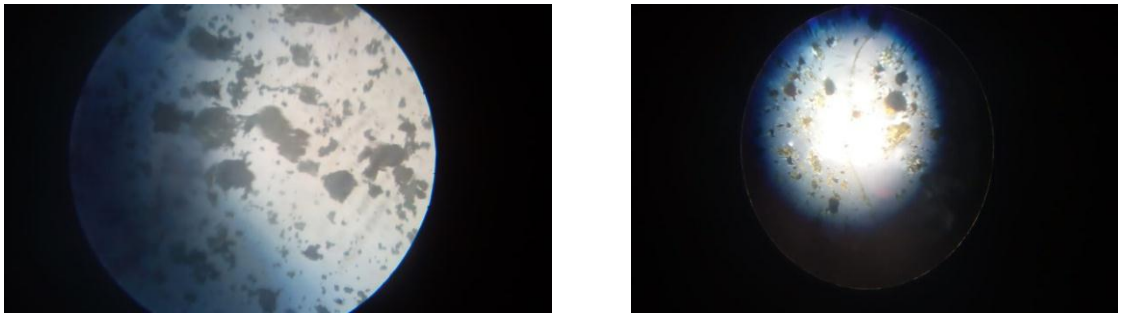

Figure No. 3: Microscopic study of ginger powder.

3.4.2. Quantitative Investigation

Determination of Total Ash value

In the estimation of total ash value, the carbon content must be removed at low temperature 450ºC as possible because the presence of alkali chloride, which may be volatile at high temperature.

If carbon content is yet present after heating at moderate temperature, the water-soluble ash may be separated and residue again ignited.

3.4.3 Pharmacological Evaluation-

Ginger was reported to have an inhibitory action on platelet aggregation and also causes to reduce platelet thromboxane production *in vitro*. 8-Gingerol, 8-shogaol, 8-paradol, and gingerol analogues show antiplatelet actions. Although *in vivo* effects of ginger is not well studied yet. Ginger is liable for the decrease in platelet aggregation. Lumb has reported that ginger has no effect on platelet count, bleeding time, or platelet aggregation.

Ingenol and 6-shogaols are reported to have antiviral activity. Gingerol was found to be an inhibitor of *tuberculosis in vitro.* Gingerol and related compounds were shown to produce antimicrobial activities. Ginger was found the synergistic effect of ethanol extract of ginger and garlic against *Bacillus spp*. and *Staphylococcus aureus.* They also reported that antimicrobial activity of the ethanol extract of ginger, lime and garlic against a broad range of bacteria including *Escherichia coli,* and *Salmonella spp.* In another study, Ginger have carried out the antibacterial activity of ginger extracts. In their experiment, they obtained ginger extracts using solvents, n-hexane, ethyl acetate, ethanolic soxhlet and water. The

results showed that all the extracts except the water extract have antibacterial activity and that the inhibition of bacterial growth was dose-dependent.

Recently have reported the antimicrobial activity of the soybean extract of ginger different bacterial species. The antimicrobial activity of the ginger was reported to be highest against *Salmonella*. While lowest activity was found against *Escherichia coli*. *Staphylococcus aureus* exhibited lower sensitivity to ginger extract as compared to the most other Gram-negative bacteria.

3.5.1 Formulation

1. The hand wash was prepared from the ethanolic extracts of *Zingiber officinale*.

2. 10 g of the powdered root of plant were extracted with 100 ml of ethanol solution 9 parts of ethanol and 1 part of distilled water by means of extraction. This mixture was heated on water bath at 60ºC for 1 hour.

3. The content was filtered through Whatman filter paper in order to get particle free extract.

4. Filtrate was used as ethanol extract .10 g of plant materials were added separately to 100 ml of methanol solutions. This mixture was heated in water bath at 60ºC for 60 minutes.

5. The contents were filtered and the filtrate was used as methanol extract. The herbal hand wash was prepared by adding 4 ml of the ethanol extract of combined plant materials in 6 ml of distilled water.

6. To the final volume of 10 ml, 3 g of sodium lauryl sulphate was added as per the requirement of standard procedure for preparation of handwash.

3.5.2 EVALUATION

Physical Evaluation

Physical evaluation (color, dour) was done by sensory and visual inspection and compared with the marketed hand wash gel.

Foam Height

One gram of sample of hand wash gel was taken and dispersed in 50ml distilled water. Dispersion was transferred to 500ml measuring cylinder. Volume was made up to 100ml with water. 25 strokes were given and kept it aside. The foam height above the aqueous volume was noted.

Viscosity

The viscosity of hand wash gel was determined by using digital Brook filed viscometer. Measured quantity of hand wash gel was taken into a beaker and the tip of viscometer was immersed into the hand wash gel and viscosity was measured in triplicate.

Stability

The stability studies were carried out for all the gel formulation by freeze thaw cycling. Hereby subjecting the product to a temperature of 4°C for 4 days, then at 25°C for 3 months and then at 40°C for 4 days and studied for appearance, pH, viscosity and spreadability.

pH

One gram of sample of polyherbal hand wash gel was taken and dissolved it into 100ml distilled water. The pH of solution was measured by previously standardized digital pH meter.

After hand wash

One ml of pure herbal hand wash was squeezed out and applied on both hands. Hands are gently cleaned using running water. Hands are directly kept in agar media. The Petri dishes were incubated in an incubator at 37ºC for 24 to 48 h.

4.1 RESULT

The present study was carried out to formulate ginger rhizome extracts based hand wash using gel base as carriers. The formulation was prepared by using generally approved excipients that are compatible with any similar hand cleansing formulations. It was organoleptically evaluated to ensure product stability and performed in-vitro antimicrobial test to prove its efficacy to act against infectious bacteria collected from volunteers.

And disc diffusion method are showed the antimicrobial activity.

4.1.1 Evaluation of herbal hand wash

Table No. 4: Evaluation Parameters Teste

Sr. No.	Parameter	Observations
1.	Colour	Light yellow
2.	Odour	Characteristic
3.	PH	6.5
4.	Viscosity	52 c Pascal's
5.	Foam hight	350ml
6.	Foam Retention at 5min	25.7ml

4.1.2 Phytochemical Screening of the Plant Extracts

Table No. 5: Test for Alkaloid

Test	Result
Mayer's	+
Hager's	+
Wager's	-
Dragondroff's	+

Table No. 6: Test for Carbohydrate

Test	Results
Benedict's	+
Barfoed's	-
Fehling's	+

Antimicrobial activity of extract and Herbal Hand Wash

Agar disk-diffusion testing method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing.

In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at the desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of

inhibition growth zones are measured shows the growth media, temperature, period of incubation and inoculum size required by standards.

Table No. 7: Anti-microbial activity of ethanolic extract of Ginger rhizome.

Concentration(mg/ml)	Lactobacillius	Salmonella
	Zone of inhibition(mm)	
25	20.00 ± 2.65	7.00 ± 1.00
50	25.00 ± 2.00	9.30 ± 1.53
100	24.67 ± 3.51	12.00 ± 1.00
Ciprofloxacin(5 µg)	25.67 ± 2.51	24.00 ± 1.00

Table No. 8: Anti-microbial activity of herbal hand wash of ginger rhizome.

Concentration(mg/ml)	Lactobacillius	Salmonella
	Zone of inhibition(mm)	
100	9.47 ± 3.05	11.00 ± 3.00
250	7.47 ± 1.53	12.33 ± 1.53
500	11.00 ± 2.00	16.67 ± 2.09
Ciprofloxacin(5 µg)	30.33 ± 3.06	27.67 ± 3.2

DISCUSSION

The ginger rhizome of this plant are also rich various compound as Alkaloid, Tannins, Glycoside, Carbohydrates etc.it is perform the phytochemical screening of ginger extract.

The ginger extract hand wash are show the anti-microbial activity and the cleaning the harmful micro-organism.

To prevent 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 formulate, which has no side effect and with potential antibiotic activity.

Formulating the hand wash, saponification was done by three different methods, explain in methodology section and plant extracts were added externally.

Plants were selected on the basis of their reported biological activity and their traditional use in Indian medicine.

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