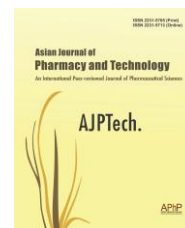


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RESEARCH ARTICLE

To Study *In-Vitro* Antimicrobial Activity of Polyherbal Handwash Formulation

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ABSTRACT:

The COVID-19 pandemic provides an important reminder that one of the most effective ways to stop the spread of germs and stay healthy is also one of the simplest — handwashing. Numerous side effects and increased antimicrobial drugs resistance encouraged us to discover cost effective, secure formulation of herbal plants. In the present study, research work is focused on evaluating the potential of different formulation incorporated with different herbal extracts against antimicrobe. This Polyherbal formulated handwash presents the efficacious study of 3 herbs consisting *Andrographis peniculata*, *Zingiber officinalis* and *Citrus limon* reflecting antimicrobial activity. The aim of present work is to avoid the adverse effects of synthetic formulation. An attempt has been made to formulate this polyherbal handwash by Maceration method of extraction. Followed by formulating then testing. The antimicrobial activity of prepared handwashes was checked against skin pathogens by diffusion wall method. Different combination of ingredients with plant extracts were tested to evaluate the antimicrobial activity by proper zone of inhibition. Thus, these extracts, showed maximum activity with strains of *E. coli* i.e., gram negative bacteria and *Staphylococcus aureus* i.e., gram positive bacteria and were found more potent than marketed preparation. Different evaluation parameter i.e., physical testing, pH, foaming, viscosity etc. were carried out on the formulation of different grades. This study “result” divulges that development of Polyherbal Handwash could have great potential for superficial hygiene of hands in turn thus trigger the good health. The usefulness of polyherbal handwash consisting of *Andrographis peniculata*, *Zingiber officinalis*, *Citrus limoni* against most common pathogens in dermatology, suggesting possible role in treating multidrug-resistant mixed infections. This action may be attributed to the synergistic effects for its use. Thus, proved to be most effective product over Healthy Human Hygiene.

KEYWORDS: Polyherbal Formulation, Kalmegh extract, Ginger extract, lemon oil, antimicrobial activity, Human hygiene, Handwash etc.

INTRODUCTION:

Hand hygiene is one of the most important types of hygiene and due to which one can reduce the spread of pathogens and prevent infections including the COVID-19 virus. Infectious diseases are the major problem in developing countries. During various daily activities hands come in contact with variety of pathogens which are responsible for auto-infection. Our hands have about

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5,000 germs (hand washing for life, 2014). Hospital acquired infections are mostly transmitted by healthcare workers hands. So, they must perform hand hygiene practices. Hand washing compliance rate of healthy personnel is about 40% (why Does Low Hand Hygiene Compliance Still Plague, 2013).¹

Using soap or hand wash is more effective than use of water alone. Surfactants in soap remove soil and microbes from skin like Cryptosporidium, Norovirus and Clostridium difficile.² Many chemical antiseptics are used for hand washing such as Chlorhexidine and alcohol, but they have some adverse effects. They can cause skin irritation and sometime becomes resistant to certain microorganisms due to its frequent use. Various Indian medicinal plants have antimicrobial property and inhibitory activity against various bacteria, virus and pathogens such as Staphylococcus aureus, Pseudomonas spp, Klebsiella pneumonia and Proteus vulgaris which are skin pathogens³. Main advantage of herbal products is that they are cheap, synergistic in effect and harmless as compared to chemical products.⁴

Andrographis paniculate (Acanthaceae) also referred as Kalmegh and popularly known as King of bitters. Various parts of Kalmegh plant have been used as traditional Ayurvedic medicine in India as specially for worms and improving digestive system.⁵ *Andrographis paniculata* has proven antibacterial, antioxidant, anti-inflammatory, antidiarrheal, anticancer, antifungal, antiviral and antiprotozoal activity.⁶ Diterpenoids and flavonoids are the main antimicrobial constituents of *Andrographis paniculata*.⁷

Zingiber officinale (Zingiberaceae) commonly known as ginger which is the most common ingredient of our daily diet commonly in Tea and mostly preferred on indigestion as an household remedy. Its rich in secondary metabolites (gingerol, paradol, shagoal), volatile sesquiterpenes (zingiberene and bisabolene) and monoterpenes (zingiberene and bisabolene) and monoterpenoids (curcumin and citral).⁸ It is used as antimicrobial in the treatment of bacterial infections due to its direct antimicrobial activity.⁹ It also possesses strong antibacterial, antifungal, anti-inflammatory and anticancer activity.¹⁰

Lemon is the plant from family Rutaceae and having antibacterial, antifungal, antidiabetic, antiviral and anticancer activity.¹¹ It is rich in flavonoids, glycosides, coumarins and volatile oil.¹² Essential oils, protopine and corydaline alkaloids, lactones, polyacetylene, hypericin, sesquiterpene, hypericin, sesquiterpenes are effective towards various micro-organisms. Terpenes, alcohols, aldehydes and esters contribute to antimicrobial effects of essential oil.¹³

The aim of this study was to evaluate the potential of polyherbal handwash formulation on standard microorganism strains by using routine antibacterial assay method.

MATERIAL AND METHODS:

MATERIALS:

Chemicals:

All the chemicals were of analytical grade quality, issued from Institute's Central Chemical Lab.

Microorganisms:

The two species of microorganisms *E. coli*, *S. aureus* were obtained from Microbiology Laboratory of Kankavli College Kankavli, Dist.- Sindhudurg (South Konkan).

Collection of medicinal plant:

Plant material i.e., species of *Andrographis paniculate* (Kalmegh) were collected from green-deciduous forest area of Konkan region i.e., Math Vengurla, Sindhudurg. *Zingiber officinale* and Citrus limon were obtained from vegetable market.

METHODS:

Preparation of herbal extracts:

The acquired plants were harvested and subjected to shade drying for 2-3 days and powdered using mixer grinder. Later the 10g of each drug was soaked in 100ml of ethanol each and stirred for 6-8hrs. at 970rpm continuously using the mechanical stirrer and subjected to Cold Maceration for about 72hrs. in refrigerator. Later the macerated product was filtered and the filtrate was stored for its further usage.

Formulation of polyherbal handwash:

1g of Carbopol 934 was dispersed in 10ml of glycerin with little agitation until a homogenous mix is formed. To another cylinder 3g of SLS was dissolved in 10ml of glycerin with slower stirring and form another dispersion. Preservatives consisting with 2:1 ratio of Methyl paraben and propyl paraben were added, to it 1g of HPMC i.e., viscosity modifier were dissolved in little quantity of water and added to 2nd dispersion. The required quantities of Kalmegh extract, Ginger extract, Lemon juice were added to SLS dispersion prepared. Now both the dispersion was mixed thoroughly to avoid bubbles and lumps formation and 3ml Alovera was added to give its moisturizing effect and Roseberry perfume to mask the bitter smell and provide better fragrance. Then volume was made up to 50ml. Mixture was stirred and mixed properly to obtain clear yellowish-brown dispersion.

Formulation of handwash of Individual Handwash:

The polyherbal handwash formulated using all three individually extracts in the similar way as that by using the optimized formula mentioned in Table no.1.

Table No. 1. Optimized Formulae of Polyherbal Handwash

Sr. No	Ingredients	F ₁	F ₂	F ₃	F ₄
1.	Kalmegh extract	----	5ml	5ml	5ml
2.	Ginger extract	5ml	---	5ml	5ml
3	Lemon juice	7.5ml	7.5ml	---	7.5ml
4.	Alovera pulp	3g	3g	3g	3g
5.	Sodium Lawry Sulphate	3g	3g	3g	3g
6.	Glycerin	20ml	20ml	20ml	20ml
7.	Methyl paraben	0.15g	0.15g	0.15g	0.15g
8.	Propyl paraben	0.075g	0.075g	0.075g	0.075g
9.	Rosebery syrup	4ml	4ml	4ml	4ml
10.	HPMC	1g	1g	1g	1g
11.	Carbopol 934	1g	1g	1g	1g
12.	Water	q. s	q. s	q. s	q. s

Evaluation of Handwash:

I. Physiological Investigation:

- A) Colour- The colour was inspected visually
- B) Odour- The odor was precepted by sensing the formulation
- C) Viscosity-
 - i) Viscosity of polyherbal hand wash was measured by using Oswald Viscometer.
 - ii) Measured Quantity of polyherbal hand wash was taken into the beaker
 - iii) The tip of Viscometer was immersed into hand wash
 - iv) Flow time was observed to Measure the viscosity
- D) pH-
 - i) PH was measured through PH meter, before using it was calibrated with buffered solution pH 4.0 and 7.0.
 - ii) After calibration electrode was rinsed and then submerged into the test solution and standard.
 - iii) Recorded the displayed PH reading.

II. Chemical Investigation:

A) Foaming Test:

In 100ml of beaker, 5gm of formulation with 40 ml Water was taken, allowed to stand for 30 mins with covered watch glass. Thereafter, Mixture was stirred with glass rod and slurry was transferred in 250 ml measuring cylinder. The remained portion was

transferred with 5-6 ml of water, volume was made up to 50ml and temperature was maintained up to 300C. Stirred continuously to obtain uniform suspension. At 30⁰, stirring was stopped with 12 complete shakes and allowed to stand for 5min. The volume of foam with water was measured as (V₁) and water portion with (V₂). Foam was calculated by (V₁-V₂).

B) Skin Irritation:

The formulation was applied on skin (Hand) and left for 30 min.

C) Testing of Antimicrobial Activity:

Test microorganisms:

The two microbe's species containing gram positive and gram-negative bacteria namely Escherichia coli and Staphylococcus aureus were used for testing of antimicrobial assay.

Antimicrobial Assay:

Cup Plate and Pour Plate Method:

Nutrient agar medium is used for the antimicrobial assay. Nutrient agar was prepared by its prescribe procedure and autoclaved at 121⁰C for 45 minutes. The sterilized media was allowed to cool at 37⁰C- 38⁰C. Plates were filled with nutrient agar solution and allowed for solidification. After solidification, the microorganisms from the subculture were inoculated into the nutrient agar medium. Sub-cultured Bacteria were inoculated by striking on the surface media of the petri plate and subjected to incubation. Later 4 wells were drawn with help of borer in each plate to receive Herbal Extracts, Individual Formulation, Polyherbal Formulation and the marketed reference standard. These were immediately poured into it and kept for incubation for 24 hours at 37⁰C for growth of microorganisms and the test and standard samples to diffuse through it. After the incubation the zone of inhibition was measured by zone meter and recorded.

RESULTS:

I. Physiological Investigation:

Physiological screening results were presented in Table 2. It reveals that, Physical parameters like colour, odour was found satisfactory.

Table No. 2. Physical Investigational Parameters

Sr No.	Evaluation Parameters	Formula 1	Formula 2	Formula 3	Formula 4	Marketed Formulation
1	Colour	Yellowish-Brown	Yellowish-Brown	Yellowish-Brown	Yellowish-Brown	Yellowish- Brown
2	Odour	Rose Water	Rose Water	Rose Water	Rose water	Fragrance smell
3	Viscosity (centipoises)	52	52	53	50	55
4	pH	6.32	5.70	5.73	5.90	6.71

- A] Colour of all the formulation was yellowish brown and odour is sense like rose water.
- B] The pH of formulated polyherbal formulation was found to be in the range of 5.7 – 6.32 and marketed formulation has pH 6.71.
- C] The viscosity of formulated polyherbal formulations was compared with marketed formulation and it was found to be in the range of 50-55 and results to be good.
- D] pH of formulation matches with the range of marketed formulation and hence can be used on skin for procuring its goal.



Fig No. 2 Results of Skin Irritation

II. Chemical Investigation.

A] Foam Testing:

Foam volume of the formulation was measured by $V_1 - V_2$.

Were,

V_1 = volume of foam with Liquid.

V_2 = volume of Liquid.

Table No. 3. Recordings of Foaming Capacity

Sr. No.	Formulation	V_1	V_2	Foaming
1	Formula 1	70	50	20
2	Formula 2	65	50	15
3	Formula 3	75	50	25
4	Formula 4	75	50	25
5	Marketed Formulation	75	50	25

Foaming capacity of formulated polyherbal handwash formulation was compared with marketed handwash formulation and it was found to be in the range of 15-25. (Fig. No. 1)

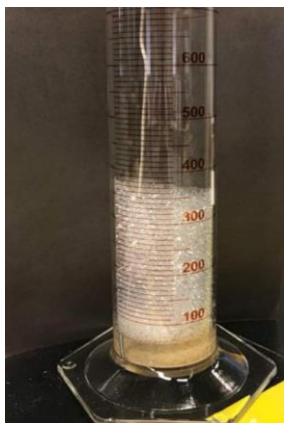


Fig No. 1 Results of Foaming Capacity

B] Skin Irritation:

No irritation was felt on skin and no redness was observed. So, the formulated polyherbal formulation do not cause any adverse effects like irritation, itching, dermatitis etc. (Fig. No. 2)

C] Determination of MIC (Minimum Inhibitory Concentration):

The results of zone of inhibition (Fig. No. 3) formed from the plant extract and formulation prepared against bacteria isolated are tabulated in Table no. 4.

Table No. 4. Results of Zone of Inhibition

Sr No.	Formulation	Zone of inhibition(mm)	
		Streptococcus aureus	Escherichia coli
1	Kalmegh Extract	4	2.8
2	Ginger Extract	3.2	4.2
3	Lemon juice	2.3	3.1
4	Formula 1	2	1.5
5	Formula 2	3.1	2.7
6	Formula 3	4	2.8
7	Formula 4	3	3.4
8	Marketed formulation	5	3

The antimicrobial activity was confirmed from zone of inhibition. The details are tabulated in table no.4. The individual extracts consisting *Andrographis paniculate* and *Zingiber officinalis* showed high anti-microbial activity against *S. aureus* as compared to *Citrus limon* and *Zingiber officinalis*, *Citrus limon* gives optimized results against *E. coli* than Kalmegh.

Activity of Formulation also reveals different results when consider in combination. Formula 3 and Formula 4 gives optimized results and gives maximum zone of inhibition as compared with the Formula 1. Reference standard showed potential activity against selected microorganisms. As per combination prepared, the results obtained predict that all the three extracts are capable of inhibiting the growth of microbes showing the different zone of inhibition and hence we can use this formulation i.e., Formula 3 and Formula 4 for better antimicrobial activity of hand wash preparation. So, the prepared formulation can be considered for further evaluation.

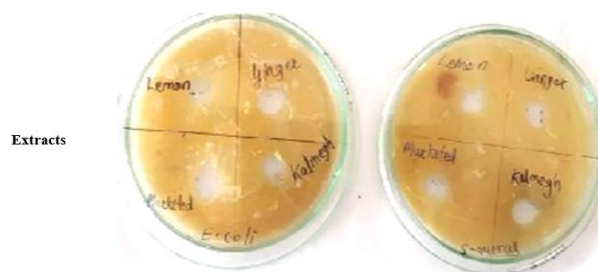


Fig No. 3. Results of Antimicrobial Activity on Extracts and Marketed Preparation

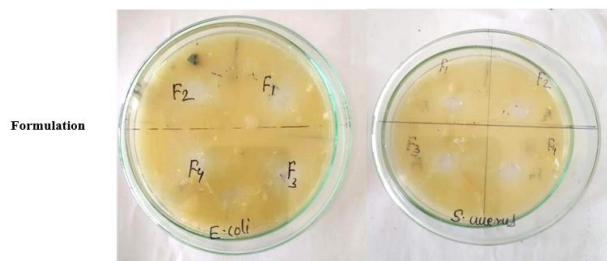


Fig No. 4. Results of Antimicrobial Activity on Different Polyherbal Preparation

DISCUSSION:

Skin infection is most leading cause of this era causing nonfatal disease for longer term with costly treatment. Various official documentation has proved the plants as boom on different dermatological treatments. Allopathic medication has high potency but a limited exposure towards the problem but in turn polyherbal medication shows widespread action with getting rid off from the root cause.

The study confirmed that the polyherbal extract i.e., Kalmegh extract, Ginger extract, lemon extract, show synergistic action against antimicrobial by proving its Zone of inhibition. The findings suggest possible use of polyherbal handwash and reduce the additional use of synthetic drugs damaging the skin.

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REFERENCE:

1. Mousmi D Thakur, Navin R. Sheth, Mihir K Raval, Assessment of In-vitro Anti-inflammatory activity of ginger and diclofenac sodium combination, International Journal of Pharmaceutical Sciences and Drug Research 2020; 12(5): 442-447, ISSN: 0975-248x.
2. Sameer Shafi, Giridhar Raosaheb Shendarkar; Antimicrobial activity of a polyherbal formulation containing Grimum Sanctum, Rubia Cordifolia and glycirrhiza glabra extracts against different

- microbial species; International Journal of Pharmaceutical Sciences and Drug Research 2021; 13(1): 36-41, ISSN:0975-248x.
3. Mohan Lal; Hand hygiene – effective way to prevent infections; International Journal of Current Research, March 2015, vol. 7, issue:3, pp 13448-13449.
4. Why does low Hand Hygiene Compliance Still Plague...? www.beckershospitalreview.com/.../why_does_low_hand_hygiene_compliance accessed 10 Aug 2020.
5. Hand Washing Reason To Save Many – SlideShare www.slideshare.net/doctorrao/hand_washing_reason_to_save_many accessed 10 Aug 2020.
6. Sandeep D.S et al. Formulation of Antimicrobial Polyherbal Hand wash; Research J. Pharm. and Tech. 9(7): July 2016 ISSN 0974-3618 (Print), 0974 -360X (Online).
7. V. Rajalakshmi, Dr. L. Cathrine; Phytochemical screening and antimicrobial activity of ethanolic extract of *Andrographis paniculata*; Journal of Pharmacognosy and Phytochemistry 2016; 5(2):175-177.
8. Joseph Joselin and Solomon Jeev, *Andrographis paniculata*: A Review of its Traditional Uses; Phytochemistry and Pharmacology: Medicinal and Aromatic Plants. Volume 3, Issue 4, 2004: 1-15. ISSN : 2167-0412.
9. Niranjan A, Tiwari SK, Lehri A., Biological activities of Kalmegh (*Andrographis paniculata*) and its active principles a review; International journal of Natural Products and Resources, 2010; (2): 125-135.
10. V. Santo Gracee, Antimicrobial activity of ethanolic extract of *Zingiber officinale* -an In vitro study; Journal of Pharmaceutical Science and Research, al J.Pharma.Sci and Res. vol 9 (9), 2017, 1417 - 1419, ISSN: 0975 – 1459.
11. Tan Bkh and Vanitha J (2004), Immunomodulatory and antibacterial effects of some Traditional Chinese Medicinal Herbs: A Review; Curr. Med. Chem., 11(11); 1423-1430.
12. Habib S. H. M., Makpol S. et al, Ginger extract (*zingiber officinale*) has anticancer and anti-inflammatory effects on ethionine induced hepatoma rats- clinics 63; 807-813.
13. Burt, S. A. 2004, Essential oils: Their antibacterial properties and potential applications in foods: A Review. Inter. J. Food Microbial. 94: 223-253.
14. Shahnah, S. M., S. Ali et al, New sesquiterpene derivative from fruit peel of Citrous limon (Linn) Burn. F. Sci. Pharm., 75: 165-170.
15. Maruti J. Dhanavale et al. Dept. of Microbiology, Shivaji University Kolhapur-416004 Maharashtra, India. Study antimicrobial activity of lemon (*Citrus Lemon*) peel extract, British Journal of Pharmacology and Toxicology 2(3): 119-122, 2011. ISSN - 2044 – 2407.