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Formulation and Evaluation of Poly Herbal Hand Wash by the Extract of Azardirachta Indica and Mimosa Pudica with Antibacterial Activity

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ABSTRACT

Hand hygiene is vital principle and exercise in the prevention, control and reduction of health care acquired infections. To avoid the adverse effects like itching, irritation, dermatitis etc.,of the synthetic hand wash formulations an attempt has been made to formulate a polyherbal hand wash by using herbs which have antimicrobial property.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 hand washing 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 . Mimosa pudica(touch me not) and Azadirachta indica(neem) is one of the most widely used and well-documented medicinal plant. Present study aimed to formulate effective, safe and nontoxic polyherbal hand wash using leaves of Mimosa pudica(touch me not) and Azadirachta indica(neem) on safety and efficacy and to avoid the risk posed by synthetic antimicrobials. .The antimicrobial activity of prepared hand wash formulations was checked against skin pathogens Bacillus subtilis, Escherichia coli by agar plate technique. Result revealed that zone of inhibition showed that the hand wash prepared from methanol extract of the combined plant materials shown significant antimicrobial activity.

keywords:- Poly Herbal Handwash, Formulation, Evaluation, Mimosa pudica(touch me not), Azadirachta indica(neem), Bacillus subtilis, Esecheria coli.

I. INTRODUCTION

Over the last decades the treatment of illness has been accomplished by administrating drugs to human body via various routes namely oral, sublingual, rectal, parental, topical, inhalation etc. Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders (e.g. acne) or the cutaneous manifestations of a general disease (e.g. psoriasis) with the intent of containing the pharmacological or other effect of the drug to the surface of the skin or within the skin. Semi-solid formulation in all their diversity dominate the system for topical delivery, but foams, spray, medicated powders, solution, and even medicated adhesive systems are in use.(1)

Topical preparations are used for the localized effects at the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes. The main advantage of topical delivery system is to bypass first pass metabolism. Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes, gastric emptying time is other advantage of topical preparations.

The topical drug delivery system is generally used where the others system of drug administration fails or it is mainly used in pain management, contraception, and urinaryincontinence. Iontophoresis, Electroporation, Sonophoresis and Phonophoresis,

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Vesicular concept and Microfabricated microneedles technology are some advanced technique which is widely being used to increase delivery through skin.[2]

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The topical drug delivery system is generally used where the others system of drug administration fails or it is mainly used in pain management, contraception, and urinary incontinence. This review describes the various formulation aspects, various excipients, evaluation tests, challenges and drugs explored in the field of topical drug delivery.[3]

Topical delivery includes two basic types of product:

- External topical that are spread, sprayed, or otherwise dispersed on to cutaneous tissues to cover the affected area.
- Internal topicals that are applied to the mucous membrane orally, vaginally or on anorectal tissues for local activity.[4]

Advantages of topical drug delivery systems:

- Avoidance of first pass metabolism
- Convenient and easy to apply
- Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes, gastric emptying time etc.
- Achievement of efficacy with lower total daily dosage of drug by continuous drug input.
- Avoids fluctuation in drug levels, inter- and intra patient variations.
- Ability to easily terminate the medications, when needed
- A relatively large area of application in comparison with buccal or nasal cavity
- Ability to deliver drug more selectively to a specific site
- Avoidance of gastro-intestinal in compatibility
- Providing utilization of drugs with short biological half-life,narrow therapeutic window
- Improving physiological and pharmacological response
- Improve patient compliance.
- Provide suitability for self-medication.[5]

II. MATERIALS AND METHOD

Selection and collection of Plants

Plant and plant parts was selected on the basis of ethno-botanical survey. Pharmacological investigations report and recent investigations were considered in respect of selected Plant.

Preparation of plant Extract

The collected plants Mimosa pudica and Azadirachta indica leaves are taken and coarsely powdered. 100 grams of coarsely powdered leaves of both plants were soaked in 500 ml of methanol and kept for maceration for about 3-4 days. After maceration the extract is filtered and the filtrate was collected and used for making hand wash.[6]

Formulation of poly herbal Hand wash

S. No.	Ingredients (% weight)	Formulation code					
		F1	F2	F3	F4	F5	F6
1	Methonolic extract of Mimosa pudica and Azadirachta indica(ml)	20	20	20	20	20	20
2	Sodium lauryl sulphate (SLS) (grms)	6	6	6	6	6	6

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 1199-1205 www.ijprajournal.com ISSN: 2249-7781

3	Glycerin (ml)	40	40	40	40	40	40
4	Methyl paraben (gms)	0.3	0.3	0.3	0.3	0.3	0.3
5	Rosemerry oil (ml)	5	5	5	5	5	5
6	Purified water q.s (ml)	30	30	30	30	30	30
7	Total	100	100	100	100	100	100

Evaluationofpolyherbal handwash

Physical evaluation (color, dour) was done by sensory and visual inspection and compared with the marketed hand wash. [13]

pH

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

Viscosity

The viscosity of hand wash was determined by using digital Brook filed viscometer. Measured quantity of hand wash was taken into a beaker and the tip of viscometer was immersed into the hand wash and viscosity was measured in triplicate.[7]

Spread ability

A sample of 0.5 g of each formula was pressed between two slides and left for about 5 minutes where no more spreading was expected Diameters of spreader circles were measured in cm and were taken as comparative values for spreadability. The Spread ability A sample of 0.5 g of each formula was pressed between two slides and left for about 5 minutes where no more spreading was expected Diameters of spreader circles were measured in cm and were taken as comparative values for spreadability. The results obtained are average of three determinations.[8]

Spreadability was calculated using the following formula:

S= M* L/T

Where,

S = Spreadability,

M = Weight in the pan (tied to the upper slide),

L = Length moved by the glass slide and

T = Time (in sec.) taken to separate the slide completely each other.

Foam Height

One gram of sample of hand wash 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.

Foam Retention

25ml of the 1% hand wash was taken into 100ml graduated cylinder. The cylinder was covered with hand and shaken 10 times. The volume of foam at 1 minute interval was recorded for 4 minutes.[9]

Evaluation of antimicrobial activity

Preparation of the Nutrient Medium

Nutrient agar medium (for Bacteria) was prepared by taking required amount and was weighed, according to the standard composition. The solution was sterilized in an autoclave at 121°C at 15 lbs pressure for 15 min. The suspension was cooled and poured into sterile Petri-dishes and allow it solidify.

Preparation Cultures and Inoculation:

- **Bacterial strain:**

Gram positive bacteria- Bacillus subtilis

Gram negative bacteria- Escherichia coli

- **Inoculum preparation:**

Well isolated colonies of the same morphological type were selected from a culture Media. The top of the colony was touched with a sterile inoculating loop. It was then transferred aseptically into a culture tube containing 15ml broth medium (Nutrient broth for bacteria). The broth culture was then incubated at 37°C until it achieves or exceeds the required growth.[10]

Inoculation of Test Plates

- 50µl of the inoculum from the broth (containing the desired growth of specific organisms) was taken with a micropipette.
- It was then transferred to fresh and sterile solidified Agar Media Plate.

International Journal of Pharmaceutical Research and Applications
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- The agar plate was inoculated by spreading the inoculum with a sterile spreader, over the entire sterile agar surface.
- It was uniformly spread by rotating the plate approximately 60° each time. After an even distribution of inoculum, the test plate was covered with lid.
- Each test plate was inoculated and spread with different microorganism.

Preparation of Wells

- After the inoculation and spreading done on sterile solidified Media plate, wells of 10mm diameter were created with sterile cork borer.
- In a plate, four wells were made at equal distance to each other.
- The Wells are then filled to about three-quarters full (50µl with micropipette) with different concentration of the extract which were labeled accordingly.
- The plates were than pre-incubated for 1 hour for adequate diffusions of the extracts and incubated at 37°C for 24-48 hours in upright condition.[11]

Reading Well and Interpreting Results:

- After 24-48 hours of incubation, each plate was examined. If the inoculum was spread satisfactorily and evenly over the plate, the resulting zones of inhibition were uniformly circular.
- The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well.
- Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background.
- The zone margin was taken as the as the area showing no obvious, visible growth that can be detected only with a magnifying lens at the edge of the zone of inhibited growth, was ignored.
- Diameter of zone of inhibition (including well diameter) were measured at four different lengths. Mean of four values were considered as zone of inhibition in well diffusion assay.

Stability Studies

The stability study was performed as per ICH guidelines. The formulated Poly Herbal Based hand wash were filled in the collapsible tubes and stored at different temperatures and humidity conditions, for a period of three months and studied for appearance, pH, viscosity, foam height, foam retention and Spreadability.[12]

III. RESULT AND DISCUSSION

Percentage Yield

Plant Extraction

The plant material of Mimosa pudica and Azadirachta indica were extracted by maceration extraction method and the percentage yield calculated by the following formula:

$$\% \text{ yield} = \frac{\text{Weight of extract} \times 100}{\text{Weight of plant material used}}$$

Table 6: Percentage Yield of Mimosa pudica

S. No.	Solvent	Colour of extract	Weight of Plant material (gms)	Weight of extract (gms)	% yield
1.	Petroleum ether	Dark Green	100	0.827	0.827
2.	Methanol	Dark Green	94.87	3.795	4.00

Table 7: Percentage Yield of Azadirachta indica

S. No.	Solvent	Colour of extract	Weight of Plant material (gms)	Weight of extract (gms)	% yield
1.	Petroleum ether	Dark Green	100	0.827	0.827
2.	Methanol	Dark Green	93.60	2.535	2.51

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Physicochemical evaluation of formulated ointment

Formulation Code	Physicochemical parameter				
	pH	**Viscosity**	**Spreadability**	**Foam Height**	**Foam Retention**
F1	6.29	52.2	11	130	12
F2	6.29	44.9	10.4	150	22
F3	6.28	215	10	110	10
F4	6.28	218	9.4	150	11
F5	6.31	224	8.72	200	17
F6	6.31	341	8.72	200	24

Antimicrobial activity

The Anti-microbial efficacy of the formulations of Polyhedral Hand Wash was tested on Bacillus subtillis and Esecheria coli by agar plate technique. The results of zone of inhibition showed that the hand wash prepared from methanol extract of the combined plant materials shown significant antimicrobial activity. The hand wash prepared from Azadirachta indica formulation (f5)showed little higher activity than Mimosa pudica formulation (f6).

The data of zone of inhibition of formulation F6 is shown in below table

Microorganisms	Different concentration			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Bacillus subtilis	3.2mm	4.2mm	6mm	10mm
Esecheria coli	3.6mm	4 mm	9mm	10.2mm

The antibacterial assay was performed using the well diffusion method of F6 formulation with concentration 100µg/mlagainstBacillus subtilis which best showed the 10mm zones of inhibition in diameters.

The antibacterial assay was performed using the well diffusion method of F6 formulation with concentration 100 µg/ml against Esecheria coli which best showed the 10.2 mm zones of inhibition in diameters.

International Journal of Pharmaceutical Research and Applications
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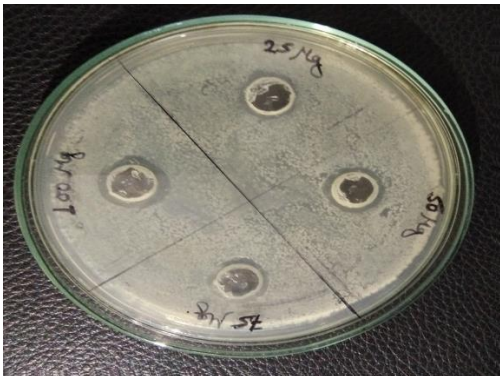

Figure: Different concentration of formulation

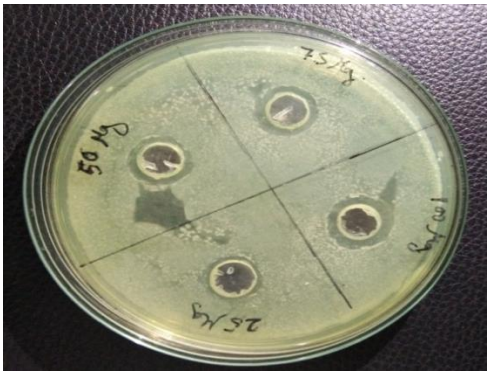

Figure : Different concentration of formulation

F6 with Bacillus subtilisF6 with Esecheria coli.

The data of zone of inhibition of formulation F5 is shown in below table

Microorganisms	**Different concentration**			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Bacillus subtilis	4.6 mm	5.2 mm	8.2 mm	10.2 mm
Esecheria coli	4.8mm	5.4mm	8.6mm	11.5mm

The antibacterial assay was performed using the well diffusion method of F5formulation with concentration 100 µg/ml against Bacillus subtilis which best showed the 10.2 mm zones of inhibition in diameters.

The antibacterial assay was performed using the well diffusion method of F5 formulation with concentration 100 µg/ml againstEsecheria coli which best showed the 11.5 mm zones of inhibition in diameters.

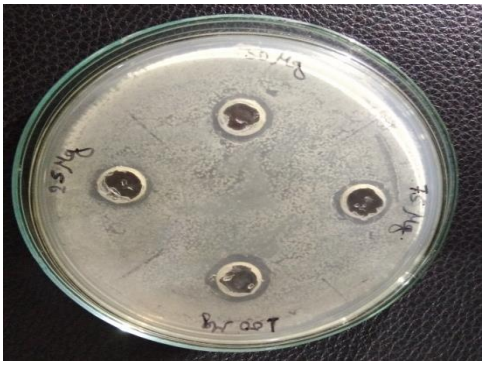

Figure: Different concentration of formulation F5 with Bacillus subtilis

Figure: Different concentration of Formulation (F5) with Esecheria . coli

Evaluation of Stability Studies

Evaluation parameters for gelling agent polyherbal hand wash for F6 formulation is given in the below table.

	Formulation Code	**Appearance and Homogeneity**	**Color**	**pH**	**Foam retention (ml)**	**Foam Height (ml)**	**Spreadability (cm)**	**Viscosity**		
								50 (rpm)	**60 (rpm)**	**100 (rpm)**
1st Month	F6	Translucent	Greenish brown	6.31	23	154	8.6	300	331	353
2nd	F6	Transluce	Greenish	6.29	22	152	8.55	298	321	34

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Volume 6, Issue 6 Nov-Dec 2021, pp: 1199-1205 www.ijprajournal.com ISSN: 2249-7781

Month		nt	brown							0
3rd Month	F16	Transluce nt	Greenish brown	6.27	22	150	8.49	249	302	25 6

Evaluation parameters for gelling agentpolyherbal hand wash for F5 formulation is given in the below table.

Months	Formulation Code	Appearance and Homogeneity	Color	pH	Foam retention (ml)	Foam Height (ml)	Spreadability (cm)	Viscosity		
								50 (rpm)	60 (rpm)	100 (rpm)
1st Month	F5	Transluce nt	Greenish yellow	6.24	24	160	9.7	191	263	27 1
2nd Month	F5	Transluce nt	Greenish yellow	6.22	25	159	9.5	186	259	28 6
3rd Month	F5	Transluce nt	Greenish yellow	6.19	22	159	9.3	179	254	28 1

IV. CONCLUSION

Natural remedies are more acceptable in the belief as they are safer with fewer side effects than the synthetic ones. Herbal formulations have emergent demand in the global market. An attempt was made to formulate the polyherbal handwash using neem and touch me not leaves extract. Formulated polyherbal handwash were transparent greenish brown color in appearance. Results on human volunteers showed considerable reduction in growth of microbial colonies after hand wash. Hence it can be concluded that this polyherbal hand wash provide an effective and safe alternative to existing marketed hand wash.

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