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Development and Evaluation of Topical Polyherbal Formulations for their Antimicrobial Potential

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ABSTRACT

The different types of skin diseases caused due to microorganisms. In recent years the use of the traditional medicinal system was increased because of more minor side effects and cost effective. The single herbal drugs were found to be less potent, which can be improved by utilizing more than one herb in the single formulation, known as polyherbal formulation. The present work involved the development and evaluation of the different polyherbal formulations (cream, gel, and emulgel) using natural ingredients. The aim of the present work is to produce a formulation with improved antimicrobial potency and stability of formulations when compared with the individual extracts of herbal drugs. All the prepared formulations were tested against various microbial strains and concluded that the polyherbal formulations (C25, G1, EG1) were found potent against most selected strains. The prepared formulations can be used as a multipurpose formulation.

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INTRODUCTION

Microorganisms are the causative agents of almost all acute and chronic diseases. Dermatologic diseases are the fourth most common cause of all human illnesses. Skin disorders cause higher year loss due to disability than other diseases such as diabetes mellitus. There are around 3000 different types of skin disorders. Intensity and symptoms of infections that are self-limiting and benign tumors, chronic inflammatory disorders, and malignant neoplasms are all examples of diseases that cause considerable morbidity and have a negative impact on quality of life. Antibiotics (which kill microorganisms or stop the growth of microorganisms) are the medicines used to treat different infections. Still, they may build resistance due to longer use and becomes less effective. It may produce a severe allergic reaction. It causes diarrhea, abdominal pain, vomiting, and nausea. It also kills the healthy bacteria in the body. To minimize all these drawbacks, herbal medicine has been commonly used for the treatment and prevention of diseases and health promotion, as well as for enhancement of the span and quality of life.

In many developing societies, traditional medicine, of which herbal medicine is a core part, is the only system of health care available or affordable. However, there is a lack of a systematic approach to assessing their safety and effectiveness. The holistic approach to health care makes herbal medicine very attractive to many people, but it also makes scientific evaluation challenging because many factors must be considered. Herbal medicines are in widespread use and although many believe herbal

medicines are safe, they are often used in combination. They are drawn from plant sources with their variability in species, growing conditions, and biologically active constituents. A major hypothetical advantage of botanicals over conventional single-component drugs is the presence of multiple active compounds that can provide a potentiating effect that may not be achievable by any single compound.

The herbal plants such as Manjistha possesses antioxidant as well as antibacterial action (Leyden *et al.*, 1998), neem is used to cure bacterial and fungal skin infections, Guduchi and moringa have the potential to treat inflammatory disease condition [3-5] and aloe vera possesses antibacterial and antifungal action, also protect the skin [6-8]. The polyherbal formulation is medicinal preparation, prepared using more than one herb. Historically, the Ayurvedic literature "Sarangdhar Samhita", dated centuries ago in 1300 A. D., has highlighted the concept of polyherbalism in this ancient medicinal system. Because synergism action of polyherbal formulation enhances the action not found in the individual herbal plants. The polyherbal formulation also reduces the concentrations of single herbs, thus reducing side effects (Hoque *et al.*, 2007).

Therefore, the present work focused on developing topical polyherbal formulations such as cream, gel and emulgel, which completely cure skin disorders because herbal and oil components were combined with increased efficacy, stability and low dose compared with extracts. The prepared formulation is complete as it prevents oxidation and growth of bacteria, also have nutritional values and keeps the skin hydrated (Patel *et al.*, 2009). The products were evaluated for various parameters, including appearance, determination of the pH, spreadability, extrudability, viscosity, after feel, type of smear, irritancy, thermal stability, and removal. The study also evaluated the antimicrobial action of prepared formulations.

METHODOLOGY

Material

The authentic plant extracts and oils, including Manjistha, neem extract, Guduchi, moringa oil, and aloe vera, were procured from Amsar Pvt. Ltd. Colvale Goa.

All the chemicals and reagents used were of AR grade.

Microorganisms

Various strains such as E. coli (MTCC No. 40), E. auregenes (MTCC No. 2822), P. auregenes (MTCC No. 424), S. aureus (MTCC No. 87), B. Subtilis (MTCC No. 121), K. Pneumoniae (MTCC No. 432), A. niger (MTCC No. 281), C. Albicans (MTCC No. 183) were used for the antimicrobial study.

All the bacterial strains were grown and maintained on nutrient agar slants for 24 hours and Candida albicans was grown on Sabouraud's Dextrose Agar slants for 48-72 hours. All the microorganisms were confirmed by the staining technique.

Method of preparation and composition of Cream, gel and emulgel

Method of preparation of cream

Different formulations of creams C1- C32 were formulated by using different combinations of oil waxes and emulsifiers. The oil phase was prepared by melting the waxes at 75°C and mixing the ingredients such as emulsifiers and BHT. The aqueous phase was prepared by dissolving the water-soluble ingredients such as sodium benzoate, glycerin, and propylene glycol in deionized water (Patel and Mishra, 2011). The water phase was warmed to 75–80°C until all ingredients were dissolved. When the water and oil phases were at the same temperature, the aqueous phase was slowly added to the oil phase with moderate agitation and stirred until the temperature dropped to 40°C. The emulsion was cooled to room temperature to form a semisolid cream base. The water soluble actives were dissolved in warmed deionized water, and the solutions were added to the cream base using an overhead stirrer. The mixture was stirred until the formulation became uniform (Table 1, Table 4).

Method of preparation of gel

Different formulations of gels G1- G9 were formulated using gelling agents such as carbopol, HPMC, and HEC. Weighed all the actives and added one by one in the vortex in the beaker containing $\frac{3}{4}^{th}$ ml of water and kept on a magnetic stirrer rotated at 300 rpm. Stir the mixture for 10 mins. Then add a weighed amount of carbopol in it (Mohammed and Manan, 2015). Continue the stirring for 20 mins until the mixture is free from carbopol lumps resulting in the gel. The mixture of triethanolamine (1 ml) and water (2.5 ml) was added dropwise to the gel to neutralize and thicken the gel (Table 2, Table 5).

Method of preparation of emulgel

Different formulations of emulgels EG1- EG9 were formulated by using different combinations of oils and gelling agents. Weighed all the actives (powdered form) and added one by one in the vortex in the beaker containing $\frac{3}{4}$ th ml of water and kept on a magnetic stirrer rotated at 300 rpm. Stir the mixture for 10 mins. They dissolved the stated amount of oils in the 5 ml of ethanol (Shamim *et al.*, 2004).

Table 1: Formula for preparation of Cream

Sl. No.	Category	Ingredients	C1% w/w	C9 % w/w	C25 % w/w
1.	Actives		8	8	8
2.	Oil component	Coconut oil	10	-	-
		Shea butter	-	10	-
		Emulsifying wax	-	-	10
3.	Emulsifier	Glycerol monostearate	8	8	8
		Cetosteryl alcohol	-	-	-
		Cetomacrogol 1000	-	-	-
		Stearic acid	-	-	-
		Cetosteryl alcohol	2	2	2
		PEG 400	-	-	-
4.	Antioxidant	BHT	0.1	0.1	0.1
5.	Preservative	Sodium benzoate	0.3	0.3	0.3
6.	Humectant	Propylene glycol	5	5	5
7.	For better skin penetration	Glycerin	1	1	1
8.	Vehicle	Water	100 q.s	100 q.s	100 q.s

The oil- ethanol mixture was added to the above mixture (powder and water). A weighed amount of carbopol and then added in it. Continue the stirring for 20 mins until the mixture is free from carbopol lumps and resulting in the emulgel. The mixture of triethanolamine (1 ml) and water (2.5 ml) was added dropwise to the gel to neutralize and thicken the emulgel (Table 3, Table 6).

Table 2: Formula for preparation of gel

Sl. No.	Ingredients	G1%w/w
1.	Actives	
2.	Carbopol	2
3.	Triethanolamine	q.s.
4.	Water	100 q.s.

Table 3: Formula for preparation of emulgel

Sl. No.	Ingredients	EG1%w/w
1.	Actives	
2.	Carbopol	2
3.	Ethanol	5
4.	Triethanolamine	q.s.
5.	Water	100 q.s.

Table 4: Amount of actives in the preparation of cream

Sl. No	Name of actives	Quantity of actives for 30 gm of cream
1.	Manjishta extract	0.96g
2.	Guduchi extract	0.48g
3.	Aloe extract	0.48g
4.	Neem oil	0.24g
5.	Moringa oil	0.24g

Evaluation of formulations

Determination of surface pH

The pH of the formulation was determined using a pH paper by referring to the standard pH scale.

Determination of the pH of 10% solution of the formulation

1g of the formulation was weighed and dissolved 10ml of water to produce 10% solution. The pH of the formulation was determined using a pH meter.

Spreadability

It is an unofficial test. In this, two glass slides of

Table 5: Amount of activesin the preparation of gel

Sl. No.	Name of actives	Quantity of actives for 30 gm of gel
1.	Manjishta extract	0.4g
2.	Guduchi extract	0.2g
3.	Aloe extract	0.2g
4.	Neem extract	0.1g

Table 6: Amount of actives in the preparation of emulgel

Sl. No.	Name of actives	Quantity of actives for 30 gm of emulgel
1.	Manjishta extract	0.4
2.	Guduchi extract	0.2g
3.	Aloe extract	0.2g
4.	Neem oil	0.1g
5.	Moringa oil	0.1g

dimensions 25cm*7.5cm were taken. 0.1g of the formulation was weighed and placed on one slide, and then the approximate diameter of the formulation applied was measured in mm and then the second slide was placed on top of that slide and then 100g of weight was placed on it (Roberts and Travis, 1995). The weight was kept for 1 minute and after 1min again, the diameter of the formulation was measured. The increase in diameter of the formulation tells about its spreadability.

Extrudability

The formulation was filled in the 5g tubes. The tubes were filled and crimped. The tube was placed on the table at a height. The weighing machine was placed on the floor, holding a Petri plate. The tube was pierced and the specific weight was placed on it and the amount of the formulation that falls in the Petri plate in 1min was recorded by recording its weight (Sato and Ohta, 1990).

Viscosity

The viscosity of approx. 50g of the formulation was measured using a Brookfield viscometer with an LV spindle having low viscosity and a 64 spindle number. The evaluation of formulations is given in Table 7.

Antimicrobial study

The disc diffusion method was used for testing antimicrobial activity. The media (25ml) inoculated with a suspension of experimental organisms was poured into sterilized petri dishes and left to gel at room temperature . Whatman's No.1 filter paper discs (7mm) were soaked in 0.2 ml manjistha, neem, guduchi, moringa oil, aloe vera extracts at a concentration of μg and a C1, C9, C25, G1, EG1 polyherbal formulations at a concentration of 1000μg. The filter paper discs were placed equidistantly on inoculated media and diffusion of the solution was allowed for 30 minutes at room temperature. Plates were incubated at 37°C for 24 hours. The average zone of inhibition was recorded (Mohanty *et al.*, 2010). The bacteria E. coli, E. auregenes, P. auregenes, S. aureus, B. Subtilis, K. Pneumoniae, A. niger, and C. Albicans have been studied.

RESULTS AND DISCUSSION

Evaluation of formulations

The prepared formulations such as cream (C1- C32), gel (G1- G9), and emulgel (EG1- EG9) were evaluated for various parameters, including colour, appearance, texture, pH, surface pH 10% pH solution, spreadability (diameter), extrudability (weight in a min), viscosity. The present work involved the preparation of 32 creams, 9 gel and 9 emulgel. Creams were formulated using different bases such as coconut oil, shea butter, bees wax and emulsifying wax. Gels were prepared by using various gelling agents like carbopol, HPMC, HEC and emulgels were prepared by using different gelling agents (HPMC, carbopol, HEC) and oils in combinations (psoralen and coconut oil, psoralen and jojoba oil, psoralen and sesame oil) The evaluation concluded that formulations such as C1, C9, C25, G1 and EG1 were found to be better and selected for further antimicrobial studies.

Evaluation data for the selected formulation was given in Table 8.

Antimicrobial study

The antimicrobial evaluation of polyherbal formulation and herbal extracts was given in Table 9.

01-06 mm Zone of Inhibition = Low level of Antimicrobial Activity.

07-10 mm Zone of Inhibition = Moderate Level of Antimicrobial Activity.

11 and above Zone of Inhibition = High Level of Antimicrobial Activity

The zone of inhibition of different polyherbal formulations and extracts against different microorganisms was carried out using the disc diffusion method in Figure 1, Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, Figures 7 and 8. From Figure 1, Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, Figures 7 and 8

Table 7: Evaluation of formulation

Formulation	Speed	Dial reading	Dial reading × factor
Cream	1.5	15	15×4K=60,000cp
Gel	1.5	10	10×4K = 40,000cp
Emulgel	1.5	10	10×4K = 40,000cp

Table 8: Evaluation parameters

Sl. No	Parameters	Formulations				
		C1	C9	C25	G1	EG1
1.	Color	Light brown	Light brown	Light brown	Reddish brown	Reddish brown
2.	Appearance	Bit thick	Light weight	Light weight	Slightly glossy	Slightly glossy
3.	Texture	Smooth	Smooth	smooth	smooth	Smooth
4.	pH	6	6	6	6	7
5.	surface pH 10% pH solution	5.55	5.43	6.94	5.81	5.14
6.	Spreadability (diameter)	Before-7mm After-19mm	Before-9mm After-20mm	Before-10mm After-27mm	Before-6mm After-23mm	Before-5mm After-26mm
7.	Extrudability (weight in a min)	1.16g	1.15g	1.17g	2.3g	1.51g
8.	Viscosity	60000cps-72000cps			36000-40000cps	40000cps

Table 9: Antimicrobial evaluation of polyherbal formulation and herbal extracts

Microorganism Strain with MTCC Number	Polyherbal Formulations					Herbal Extracts			
	C1	C9	C25	G1	EG1	MO	NM	GU	MA
E. coli – 40	14	10	14	08	10	04	06	04	06
E. auregenes – 2822	08	06	10	08	12	04	08	04	06
P. auregenes – 424	08	00	08	08	10	00	06	00	06
S. aureus – 87	04	06	06	12	08	04	04	00	06
B. Subtilis - 121	06	06	08	12	14	00	06	00	06
K. Pneumoniae – 432	06	06	06	07	09	00	04	00	04
A. niger – 281	06	10	08	14	12	00	04	00	02
C. Albicans - 183	06	06	06	06	10	04	06	02	04

C: Cream; G: Gel; EG: Emulgel; MO: Moringa; NM: Neem; GU: Guduchi; MA: Manjishta.

The diameter of the zone of inhibition of polyherbal formulations was found to be more when compared with the herbal extract of each plant. This concluded that the development of a herbal plant to the topical polyherbal formulation synergized the action of others also. The development of herbs in pharmaceutical formulations enhanced the potency of the formulation, and ultimately, the dose gets

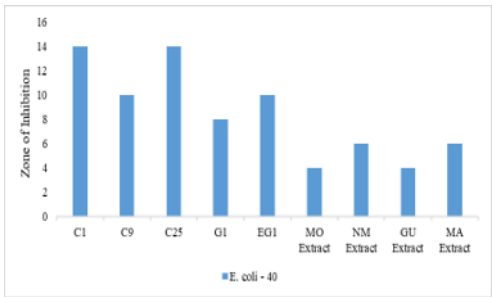

Figure 1: Zone of Inhibition against *E.coli*

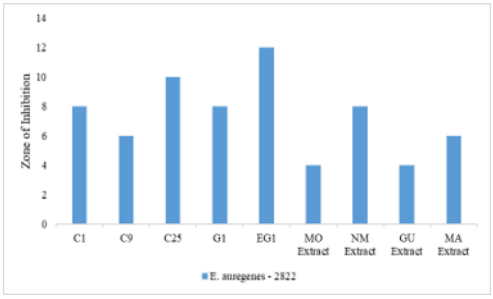

Figure 2: Zone of Inhibition against *E. auregenes*

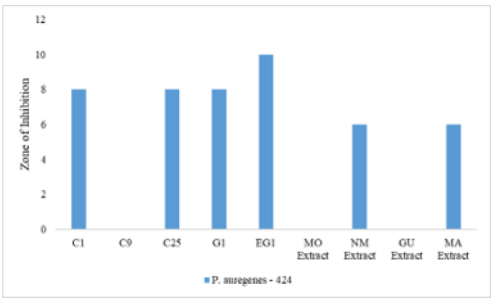

Figure 3: Zone of Inhibition against *P. auregenes*

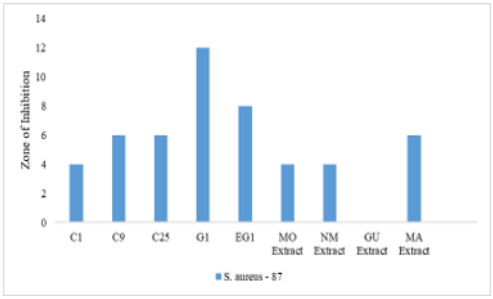

Figure 4: Zone of Inhibition against *S.aureus*

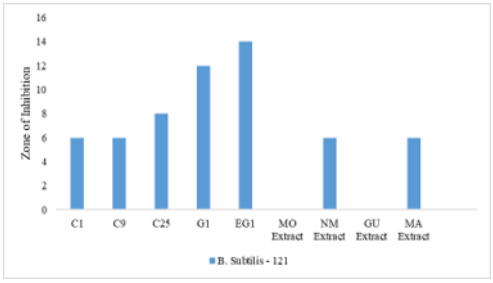

Figure 5: Zone of Inhibition against *B. subtilis*

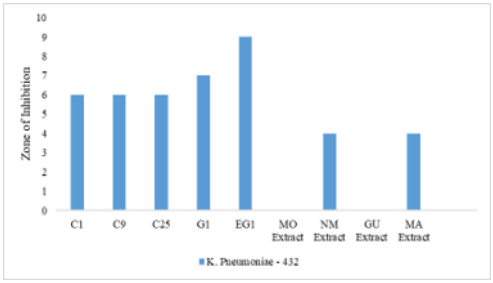

Figure 6: Zone of Inhibition against *K. pneumoniae*

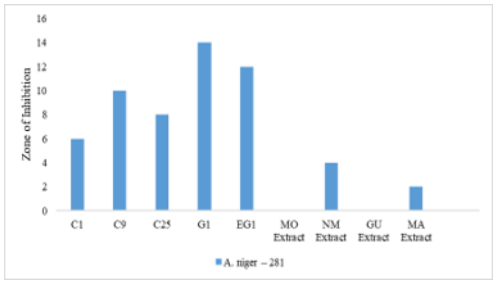

Figure 7: Zone of Inhibition against *A. niger*

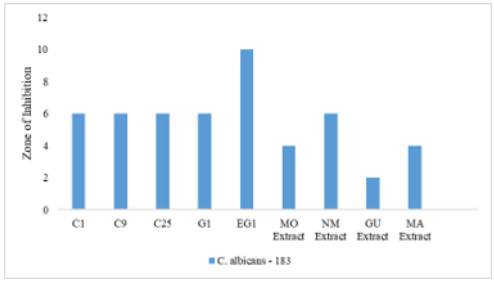

Figure 8: Zone of Inhibition against *C. albicans*

reduced. The stability of the formulation was also improved through this dosage form. While comparing all the selected formulations, C1 and C25 showed a high level of antimicrobial activity, while C9 and emulgel (EG1) showed a moderate action against the E. coli strain. The emulgel tested against the E. auregenes strain possesses high antimicrobial potential, whereas cream C25 and gel G1 moderately inhibit the growth of microorganisms. Cream C9 was inactive against P. auregenes; C1, C25, G1, and EG1 showed a moderate level of antimicrobial action. The gel was highly active against S. aurreus, whereas emulgel EG1 was moderately active compared to all creams. Gel and emulgel showed high-level antimicrobial action and other formulations showed a moderate or low action against the B. subtilis microorganism. Similar results were observed against the A. niger strain. Gel and emulgel showed a moderate level of antimicrobial action against K. pneumonia when compared with cream and herbal extracts. Emulgel showed a moderate level of action against C. Albicans, and other formulations showed a low level of activity. The conclusion revealed that C25, G1 and EG1 were better against most microbial strains. The zone of inhibition of selected gel and emulgel was found to be more effective against all the pathogens.

CONCLUSION

Microorganisms cause all types of acute and chronic skin diseases. People are focusing on the traditional medicinal system as it does not produce severe adverse reactions. However, using a single herb to

treat the infection showed less potency. This limitation can be avoided with the use of multiple herbal plants at the same time. This is termed a polyherbal formulation. The present work evaluated the different polyherbal formulations (cream, gel, and emulgel) using natural ingredients. The utilization of pharmaceutical excipients improves the antimicrobial potency and stability of formulations compared with the individual extracts of herbal drugs investigated through antimicrobial studies. The formulation reduces the dose for application which prevents toxicity and adverse effects. It was concluded that the polyherbal formulations (C25, G1, EG1) were found potent against most selected strains. The prepared formulations can be used as a multipurpose formulation as the actives provide different actions such as antioxidants, anti-inflammatory, antibacterial, antifungal, and retain moisture in the skin.

Conflict of Interest

The authors declare that they have no conflict of interest.

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