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Polyherbal Ointment Formulation and Evaluation for Acne Vulgaris

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Abstract
Aim: The aim of this study was to develop and evaluate a new polyherbal ointment, incorporating zinc and silver bhasma along with selected plant extracts, as a potential natural treatment for Acne vulgaris.
Background: Acne vulgaris is a prevalent dermatological condition, primarily affecting teenagers and young adults, often causing physical and psychological distress. Traditional acne treatments, including topical retinoids, benzoyl peroxide, isotretinoin, and antibiotics, are associated with side effects such as dryness and sensitivity. Hence, there is a growing interest in exploring alternative, Ayurvedic, and herbal treatments for acne, which are believed to not only treat the condition but also protect the skin from adverse effects.
Objective: The objective of this study was to develop a polyherbal ointment incorporating zinc and silver bhasma, known for their potential antibacterial properties, and select plant extracts with antimicrobial properties, and evaluate its physicochemical qualities, in vitro drug release, stability, microbiological purity, and efficacy in treating Acne vulgaris.
Method: The study involved the development of a polyherbal ointment using extracts of forest burr, kali musli, and chaff flower in combination with zinc and silver bhasma. The ointment was subjected to various tests and evaluations, including physicochemical analysis, in vitro drug release assessment, stability testing, and microbiological purity evaluation. Additionally, the efficacy of the ointment in treating Acne vulgaris was assessed.
Results: The results of the study indicated that the prepared polyherbal ointment exhibited favorable physicochemical qualities, in vitro drug release profiles, stability over time, and microbiological purity. Moreover, the ointment demonstrated promising effectiveness in the treatment of Acne vulgaris.
Conclusion: In conclusion, the polyherbal ointment developed in this study, containing extracts of forest burr, kali musli, and chaff flower in combination with zinc and silver bhasma, appears to be a potential natural alternative for the treatment of Acne vulgaris. The study's findings suggest that this ointment warrants further investigation and potential future clinical usage as a safe and effective acne treatment option with fewer side effects compared to traditional treatments.
Other: It is important to note that further research, including clinical trials, safety assessments, and long-term efficacy studies, should be conducted to validate the potential of this polyherbal ointment as a mainstream treatment for Acne vulgaris. Additionally, understanding the specific mechanisms by which the ointment acts on acne-causing bacteria and skin inflammation would be valuable for its broader acceptance and utilization in dermatology.

Key words: Acne, bhasma, herbal extract, ointment, antibacterial effect.

1. Introduction

Acne is the most frequent health issue in the present circumstances [1]. It has been stated that throughout the globe, 79-95% of reported instances of acne in the age bracket of 16-18 years and in India, 50.6% of boys and 38.13% of girls have the complication of acne out of all recorded incidents.These stated occurrence ratios and prevalence rates aid in the search for prospective targets for acne therapy [2-3].
Acne is a persistent provocative skin complication with four major sites of pathophysiology identified: hyperkeratinization and obstruction of sebaceous follicles, irregular desquamation of follicular epithelium, an androgen-stimulated increase in sebum era, and Propionibacterium acnes multiplication, which causes irritation [4]. Irritation is caused by the disruption of the preclinical precursor lesion known as the microcomedo, which leads to the pustules and papules of clinical sickness and may finally result in scarring.Anti-acne medications target Propionibacterium acnes[5-6].
Acne is described in Ayurvedic medicine as "Shalmali thorn-like eruptions on the face of adolescents caused by disturbances of Kapha, Vata, and Rakta" and is known as YuvanaPidika, Tarunya Pitika, or Mukhadushika [7]. Furthermore, three types of chikitsa (therapy) are recommended for acne in the Ayurvedic system of medicine,

including Shodhana (body cleansing) and Shamana (preservationist treatment) Chikitsa or a mixture of both [4-5].

Humans have been employing natural treatments or therapies, such as herbal medicine, for acne for thousands of years. Because most current antibacterial and antimicrobial medications have fundamental drawbacks such as drug resistance, side effects, and tolerance, recent research has focused increasingly on herbalmedicine research [5]. The purpose of this study is to assess the efficacy of herbal combinations along with the bhasama in the treatment of acne.

The plants like forest burr, chaff flower, and kali musli are said to possess the anti-microbial activity against the acne. Along with that there are many reports which suggest that the silver and zinc bhasma also possess the anti-bacterial and anti-inflammatory activity.[8-10]

Therefore, in present research we combine the herbal extracts along with the bhasma powder to formulate the anti-acne cream for the treatment of acne.

2. Material and methods

The raw herbs like forest burr, chaff flower, and kali musli was obtained from local market. The zinc and silver bhasma was formulate, the other ingredients like hard paraffin, yellow soft paraffin, lanolin, cetyl alcohol, and glycerine were obtained from central drug house private limited.

The ethanolic extracts of the herbs were formulated:

By soxhlet extraction method in which 100gm of plant material were taken and were coursed powdered and then 150ml of ethanol is added. And after that ethanolic extract of herbs were collected after drying in water bath. [11]

Physicochemical properties of herbs and its extracts

The foreign matter content, moisture content (loss on drying at 105 °C), and total ash content (at 450 °C) were all measured [12-13].The extracts were also tested for alkaloids (Mayer test, Wahner's test, Dragendroff's test, and Hager's test), carbohydrates (Molisch test, Benedict test, Fehling test), glycosides (Modified Borntrager's test, Legal's test), flavonoids (alkaline reagent test, Lead acetate test), and tannin (Ferricchloride test, Lead acetate test).

For the bhasma preparation:

- Take 20 grammes of premium pure zinc and silver.
- Cleanse the zinc and silver to remove any contaminants by washing it in distilled water or rose water.
- Using a mortar and pestle, finely ground the pure zinc and silver.
- Set up the crucible and pot as previously mentioned.
- Sprinkle some of the zinc and silver powder on top of the crucible.
- Add a layer of dried neem leaves or Giloy stems on top of the zinc and silver layer.
- Continue layering until all 20 grams of zinc and silver powder have been utilized.
- To ensure an airtight seal, cover the crucible with clay and cow dung combination.
- Gradually heat the setup, bringing the temperature up to around 800-900°C (1472-1652°F).
- The crucible should be left to cool naturally after the heating procedure.
- Remove the grayish-white residue from the crucible, which is the zinc and silver bhasma. Make sure the powder is fine.
- If any black or unburned particles remain, repeat the procedure with the leftover material until you have a pure bhasma.
- Keep the zinc and silver bhasma away from dampness and direct sunshine in an airtight glass container. The preparation date should be written on the container.[14-15]

Preparation of anti-acne ointment[16]

The ointment was formulated in the conc. of 1, 3, 5 and 7%. The method of preparation is as follows as shown in fig 1-4:-

Preparation of 1% poly herbal ointment

- To prepare the ointment base, 85gm of yellow paraffin taken in a beaker. After that 5gm of hard paraffin was added into it. Additionally 5gm of lanolin and 5gm of cetyl alcohol was also added into the same beaker (weighed by using the digital weighing balance)
- The mixture was continuous stirred.
- The content in the beaker was kept on water bath approximately for 10 min at 70 °C temp to melt the componentswith continuous stirring
- 10ml of solution (hot mixture) was measured in the measuring cylinder and poured intothe mortar. The mixture was kept cooling at room temperature

- After cooling, 0.5gm of Bhasm was weighed and transfer to the mortar
- 1 mL extract of chaft flower, forest burr and kali musli was measured andtransferred to the motar
- The formulation was transferred into the glass beaker for homogenization
- The mixture was homogenized at 1000 rpm for 2 hours
- Finally, after homogenization, the polyherbal ointment was formulated
- The formulation was stored in a cool and dry place.

Fig 1:- Shows the 1% polyherbal ointment formulation

Preparation of 3% polyherbal ointment

- To prepare the ointment base, 85gm of yellow paraffin taken in a beaker. After that 5gm of hard paraffin was added into it. Additionally 5gm of lanolin and 5gm of cetyl alcohol was also added into the same beaker (weighed by using the digital weighing balance)
- The mixture was continuous stirred.
- The content in the beaker was kept on water bath approximately for 10 min at 70 °C temp to melt the componentswith continuous stirring
- 10ml of solution (hot mixture) was measured in the measuring cylinder and poured intothe mortar. The mixture was kept cooling at room temperature
- After cooling, 1.5gm of Bhasm was weighed and transfer to the mortar
- 1 mL extract of chaft flower, forest burr and kali musli was measured andtransferred to the motar
- The formulation was transferred into the glass beaker for homogenization
- The mixture was homogenized at 1000 rpm for 2 hours
- Finally, after homogenization, the polyherbal ointment was formulated
- The formulation was stored in a cool and dry place

Fig 2:- Shows the 3% polyherbal ointment formulation

Preparation of 5% polyherbal ointment

- To prepare the ointment base, 85gm of yellow paraffin taken in a beaker. After that 5gm of hard paraffin was added into it. Additionally 5gm of lanolin and 5gm of cetyl alcohol was also added into the same beaker (weighed by using the digital weighing balance)
- The mixture was continuous stirred.
- The content in the beaker was kept on water bath approximately for 10 min at 70 °C temp to melt the componentswith continuous stirring
- 10ml of solution (hot mixture) was measured in the measuring cylinder and poured intothe mortar. The mixture was kept cooling at room temperature
- After cooling, 2.5gm of Bhasm was weighed and transfer to the mortar
- 1 mL extract of chaft flower, forest burr and kali musli was measured andtransferred to the motar
- The formulation was transferred into the glass beaker for homogenization
- The mixture was homogenized at 1000 rpm for 2 hours
- Finally, after homogenization, the polyherbal ointment was formulated
- The formulation was stored in a cool and dry place

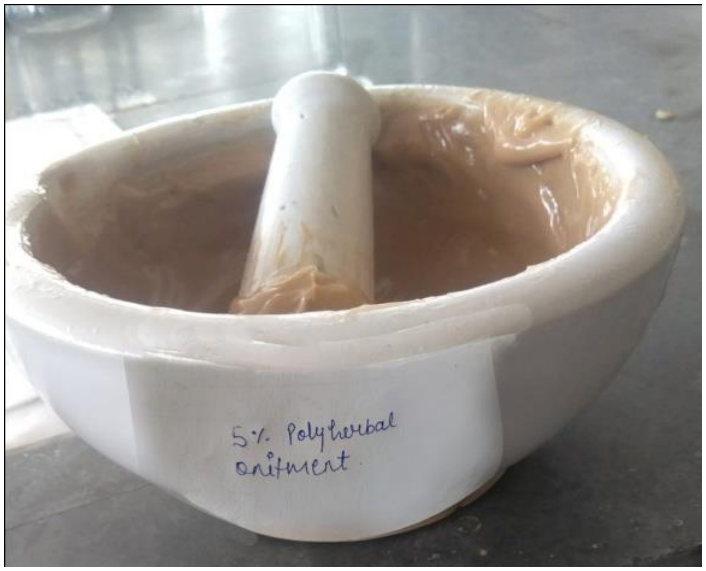

Fig 3:-Shows the 5% polyherbal ointment formulation

Prepration of 7% polyherbal ointment

- To prepare the ointment base, 85gm of yellow paraffin taken in a beaker. After that 5gm of hard paraffin was added into it. Additionally 5gm of lanolin and 5gm of cetyl alcohol was also added into the same beaker (weighed by using the digital weighing balance)
- The mixture was continuous stirred.
- The content in the beaker was kept on water bath approximately for 10 min at 70 °C temp to melt the components with continuous stirring
- 10ml of solution (hot mixture) was measured in the measuring cylinder and poured intothe mortar. The mixture was kept cooling at room temperature
- After cooling, 2.8gm of Bhasm was weighed and transfer to the mortar
- 1 mL extract of chaff flower, forest burr and kali musli was measured andtransferred to the motar
- The formulation was transferred into the glass beaker for homogenization
- The mixture was homogenized at 1000 rpm for 2 hours
- Finally, after homogenization, the polyherbal ointment was formulated
- The formulation was stored in a cool and dry place

Fig 4:-Shows the 7% polyherbal ointment formulation

Evaluation of the prepared cream:

The resulting cream was tested for its organoleptic properties (odour, colour, and shape), pH, smear nature, and ease of removal [17].

In-Vitro Permeation Experiment Using the Franz Diffusion Cell:

Using a FranzDiffusion Cell (effective surface diameter 1.2cm, capacity 25mL, Singh Scientific Pvt. Ltd. India), an in-vitro permeation investigation of polyherbal cream was performed. The donor compartment of FDC received 250mg of the cream, whereas the receptor compartment received receptor fluid. The temperature of the receptor fluid was maintained at 37oC for the 12-hour trial. The sample intervals were maintained constant at 0.5, 1, 2, 3, 4, 5, 6, and 12 hours. The samples were analysed using a UV-Visible spectrophotometer [18].

Result and Discussion

Physicochemical properties of extracts

The physicochemical qualities of plant products such as kali musli, forest burr and chaff flower were assessed for various criteria. LOD, Foreign matter, Total ash, Acid insoluble ash, Water soluble extractive value, and Alcohol soluble extractive value are the criteria. The parameters were within the typical range of standard and conformed to the norms.

In-vitro anti-acne activity of plant extract

The disc diffusion technique was used to assess the anti-acne capabilities of a plant product's ethanolic extract. Using the in vitro disc diffusion technique, several concentrations of the extract were produced with DMSO and tested for anti-acne efficacy. The 40l solution is removed and tested for anti-acne action. Even at 0.4 mg/ul, chaff flower extract showed the most inhibition, whereas kali musli showed the least. As shown in table 1.

Table 1:- shows the anti-acne activity of the plant extracts.

Sr.no.	Drug concentration (mg/ul)	Zone of inhibition		
		kali musli	forest burr	chaff flower
1	0.4	2	-	-
2	0.8	3	-	-
3	1.2	7	3	-
4	1.6	8	5	2
5	2	8	6	4

FTIR of zinc and silver bhasma

- **zinc**

Table 2:- FTIR stretching of the Zinc bhasm

Sample	Major peak weavlenth	Functional group
Zinc	**877**	Strong aromatic
	1380	C-H alkyl methyal weak
	2009	R-N = C=S (C=N)
	2058	
	3830	Secondary amines

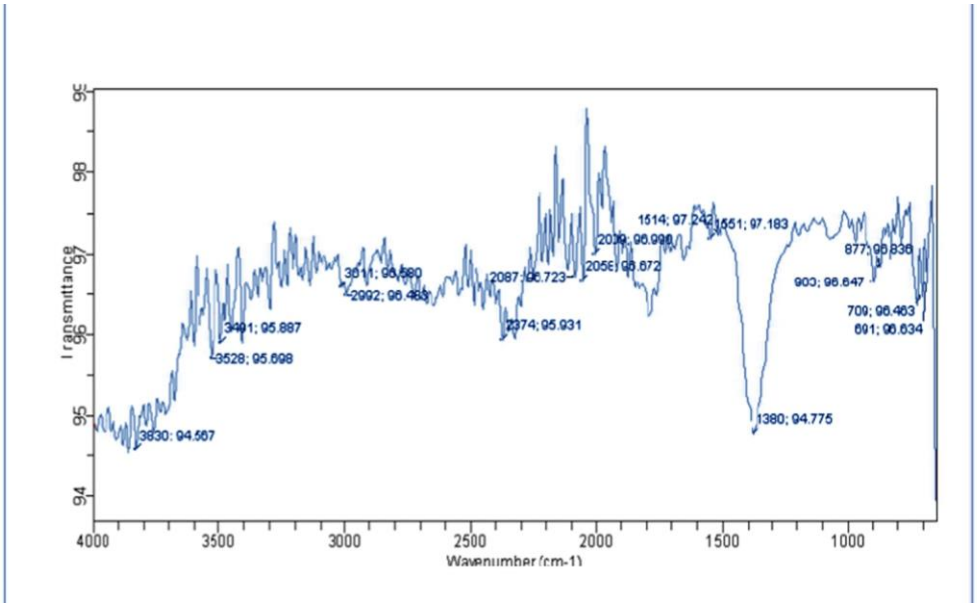

Fig 5:- FTIR stretching of the Zinc bhsma

- **silver**

Table 3:- FTIR streaching of the silver bhasma

Sr.no	Actual peak	Bond	Type of bond	Specific type of bond	Appearance
1	3898.77	-	-	-	Not found
2	2918.94	C-H	Alkyl	Methylene	Medium to strong
3	2850.88	C-H	Alkyl	Methyl	Mediumto strong
4	1022.64	C-N	Aliphatic amine	Any	Weak
5	480.05	-	-	-	Not found
6	444.15	-	-	-	Not found
7	434.14	-	-	-	Not found
8	42091	-	-	-	Not found

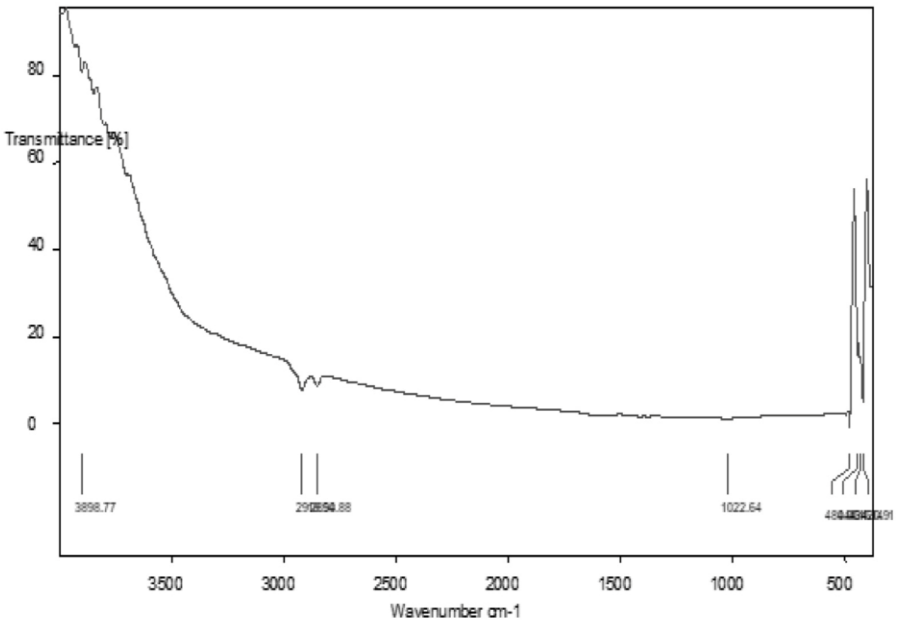

Fig6:- FTIR stretching of the Silverbhasma

SEM Analysis of zinc and silver bhasma

- **Zinc**

SEM image of zinc show rough road like structure ,which are unevenly scattered (Fig 6.3).

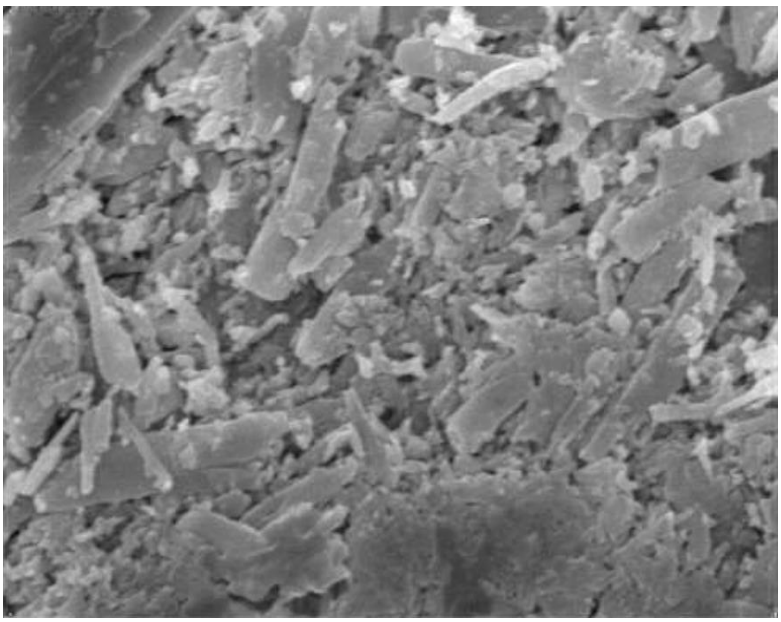

Fig 7:- SEM images of Zinc bhasma.

- **Silver**

SEM image of silver shows cristtaline particle which are smaller in size.

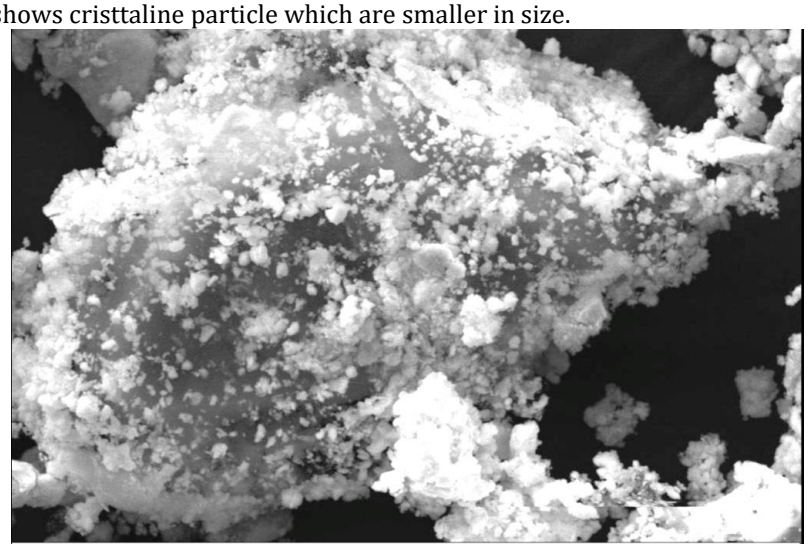

Fig 8:-SEM image of silver bhasma

Post formulation evaluation:-

The prepared ointment was subjected to different evaluation parameters and there results are shown in table 2.

Table 2:- shows the results of physicochemical properties of the formulation

S.NO.	Parameters	Formulation			
		F1 (Polyherbal ointment 1%)	**F2 (Polyherbal ointment 3%)**	**F3 (Polyherbal ointment 5%)**	**F4 (Polyherbal ointment 7%)**
1.	Colour	Light yellow	Yellowish	Yellowish brown	Brown
2.	Odour	Characteristic	Characteristic	Characteristic	Characteristic
3.	Consistency	Smooth	Smooth	Smooth	Smooth
4.	pH	6.66	6.86	7.07	7.25
6.	Viscosity	31.2±CPS	46.5±CPS	58.2±CPS	71.5±CPS
7.	Permeability	54.4%	49%	43.6%	39%
8.	Drug Content	0.00124 mg/ml	0.00328 mg/ml	0.00545mg/ml	0.00764mg/ml
9.	Stability Studies	Stable	Stable	Stable	Stable

In-vitro drug release

The formulation F3 was selected as best formulation on the basis of the results of physiochemical parameter results and the conc. at which the best zone of inhibition obtained. The F3 formulation was further subjected to In-vitro drug release using Franz diffusion apparatus and its result are shown in table 3.

Table 3:- shows the In-vitro drug release of the formulation

Sr.no.	sampling time point (h)	Abs (341nm)	Conc.	% DR
1	0.5	0.013	1.103	11.31
2	1	0.027	2.14	21.96
3	2	0.048	3.7	37.95
4	3	0.058	4.15	40.52
5	4	0.067	5.1	52.34
6	5	0.089	6.73	69.05
7	6	0.092	6.95	71.34
8	12	0.099	7.47	76.65

Conclusion

In this study, we used three herbs and two bhasma and created four different cream formulations, two of which are considered the best due to their form, and the other two formulations have the presence of hard paraffin, which makes the formulation hard, so two formulations are considered the final formulation. In vitro testing of kali musli, forest burr, and chaff flowers revealed that the highest zone of inhibition of these herbs is 4mm, 8mm, and 6mm, respectively, while the zone of inhibition of the created formulation is 9mm and 10mm, as indicated in the findings. The FTIR and SEM of bhasmas were also done. The quantity of medication released was calculated using an in-vitro skin penetration investigation. The proportion of medication release after 12 hours is determined to be 76.65%. Finally, we conclude that the produced composition and the herbs utilised exhibit anti-acne efficacy and excellent skin penetration.

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