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STUDY OF ANTIMICROBIAL POLYHERBAL OINTMENT OF LEAF JUICE EXTRACTS OF SELECTED PLANTS

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Abstract

Different parts *of Clerodendrum infortunatum* Linn. (Lamiaceae) has been used against convulsion, diabetes, microbial infections, worm infestation, liver diseases, pain, inflammation etc. *Paederia foetida* Linn. (Rubiaceae) has been used against rheumatism, diarrhoea, diabetes, cough, liver diseases, pain, worm infestation, ulcer, microbial infections, fever etc. *Euphorbia neriifolia* Linn. (Euphorbiaceae) has been used against CNS stimulation, abdominal sepsis, liver diseases, diabetes, cancer, inflammation, arthritis and skin diseases etc. The fresh leaf juice of plants are used against many diseases including healing of wound and treating microbial infections. In this study, the extracted juice was subjected to drying and concentrated to get a dry mass. The dry juice mass was fractionated using methanol (70%) after defatting with petroleum ether (60 ^{o}C – 80 ^{o}C). Fresh leaf juice mass and Methanol (70%) extract of each plant were investigated. Phytochemical screening revealed that the extracts contain alkaloids flavonoids, tannins, sugar, saponins, steroids, terpenoids and phenolic compounds. All extracts were investigated for antimicrobial activities. Hydophillic ointment USP was prepared and evaluated. Formulated herbal ointments were studied by agar cup method for antiinfective potential comparing with Neosporin ointment (antibacterial) & Micogel cream (antifungal). Ointments of Methanol (70%) extract of each plant showed highest activity in terms of ZOI against tested pathogens than the fresh leaf juice dry mass. Polyherbal ointment with the Methanol (70%) extract of plant-I, II & III in the proportion 40:30:30 showed better antimicrobial activity which may be due to high content of phytochemicals than the fresh leaf juice dry mass.

Keyterms: *Clerodendrum İnfortunatum, Paederia Foetida, Euphorbia Neriifolia,* Leaf Juice, Methanol(70%), Hydophillic Ointment USP, Antimicrobial Activity

INTRODUCTION

After pharmaceutical screening of some plants basing on traditional use by the tribal people and local vaidyas, it was found that the fresh juice of leaves are directly applied to infections and wounds on the skin for their cure. Plants are available locally which may have different biodiversity with many activities. Considering the knowledge of use since long, *Clerodendrum infortunatum* Linn.(Lamiaceae)[Plant-I], *Paederia foetida* Linn. (Rubiaceae) [Plant-II] and *Euphorbia neriifolia* Linn.(Euphorbiaceae) [Plant-III] were selected for study as herbal ointment against skin infections using the dry mass of fresh juice of leaves & the Methanol(70%) extract of the dry mass. *Clerodendrum infortunatum* Linn. is a large shrub or small tree in many parts of India and other countries.[1] Leaves and their juice are used in inflammation, deworming, vomiting, scabies, fever, helmintic infestation, hyperglycemia and to cure malaria, wound, bone fracture, dysentery, scorpion sting.[2,3] Leaves contain Alkaloids, flavonoids, steroid, saponins, terpenoid, phenolics, glycosides, phytosterols.[4,5] *Paederia foetida* Linn. a climbing plant is native to most of the parts of India, Nepal and other countries.[6] Leaves and their juice of *Paederia foetida* are used for the treatment of rheumatism, diarrhea,

inflammation, piles, toothache, asthma, bowel problems, diabetes, bacterial infections and seminal weakness.[7,8,9] Leaves yield glycosides, sterol, ursolic acid etc.[10,11] *Euphorbia neriifolia*, is a branched shrub with seen in many parts of India and Odisha.[12] Leaves and their juice of *Euphorbia neriifolia* are used as analgesic, anti-inflammatory, anti-diabetic, antiseptic, antispasmodic and purgative.[13,14] Leaves contain proteins, glycosides, alkaloids, phenolics, flavonoids, saponins, terpenoids, and lignin etc.[15]

The freshly extracted leaf juice of all three plants was studied pharmacologically. The plan was to standardize the ointment of individual extracts. An attempt was taken to prepare suitable polyherbal ointment.

MATERIAL AND METHOD

The chemicals used were of analytical grade.

Collection of plant material:

5 kg of fresh leaves of all plants were gathered separately from different sites of Dukura, Dist- Mayurbhanj, Odisha in March 2020(Fig-1). The plant was authenticated by Dr. R K Sharma, Professor in Botany, Seemanta Mahavidyalaya, Jharpokharia, Dist- Mayurbhanj, and Odisha. The specimen was deposited in The Omm Sai Institute of Paramedical Sciences, Dukura, Mayurbhanj, and Odisha.

Extraction and Phytochemical analysis: [16]

Freshly procured leaves were cut in to small pieces and grinded thoroughly and passed through a press/ juicer to collect the juice in bulk. The juice was strained through a fine cloth. The extracted juice was subjected to drying and concentrated in rotary evaporator till complete dryness at 40 ^{o}C. This extract (dry mass) was subjected for study directly and also fractionated.

The extract (dry mass) was defatted with petroleum ether (60 ^{o}C – 80 ^{o} C) and then filtered. The residue was air dried and dispersed in 1000ml of methanol (70%) [Methanol: water = 70:30] and the flask was shaken by a shaker to disperse the extract and dissolve the soluble components. The flask was kept for 2 hrs on shaking mode and then filtrate was collected, concentrated to get powder. Both the dry mass of fresh juice and the Methanol (70%) extracts as coded (Table-1) were subjected to phytochemical and pharmacological investigation. Percentage yield of extracts is given in Table-3.

Table 1: Name of plants selected and coding of extracts for study

Plant code	Name of plant	Extract for use	Extract Code
I	*Clerodendrum infortunatum* L.	Fresh juice extract(dried mass)	Ci-F
		Methanol 70% fraction of Fresh juice extract	Ci-M
II	*Paederia foetida* L.	Fresh juice extract(dried mass)	Pf-F
		Methanol 70% fraction of Fresh juice extract	Pf-M
III	*Euphorbia neriifolia* L.	Fresh juice extract(dried mass)	En-F
		Methanol 70% fraction of Fresh juice extract	En-M

Phytochemical investigation of extracts:[17]

Standard procedures were followed for qualitative analysis of phytochemicals present in the extracts. Methods included Molisch test (carbohydrate), Benedict's Test (Reducing sugar), Ninhydrin test(protein), Dragondorff's test(alkaloids), Ferric chloride test(tannins and phenols), General test (glycosides), Alkaline reagent test (flavonoids), Liebermann Burchard test(steroids and terpenoids), Foam test(saponins). Observations are noted in Table-4.

Formulation and evaluation of ointment base:

Formulation of ointment base: Hydrophilic Ointment USP was used for preparation of medicated ointments. The ointment was prepared as per the official method.

Evaluation of Prepared Hydrophilic Ointment USP:

Prepared Hydrophilic Ointment USP was evaluated for following parameters.

Psychosensory evaluation: Hydrophilic Ointment USP packed in collapsible tubes were evaluated manually by 10 student volunteers were given a tube of ointment to assess the physical parameters and the findings as noted in Table-5.

Rheological evaluation: Hydrophilic Ointment USP was evaluated for spreadability (using a simple apparatus suggested by Mutimer *et al*.)[18], viscosity using Brookfield Viscometer and extrudability as force for removal of base from tube

Physical evaluation: Hydrophilic Ointment USP was evaluated for pH, Softening range by melting point apparatus, Bleeding[19] inside the tube and Globule size analysis by microscopic method. The observations are noted in Table-6.

Thermodynamic evaluation: Hydrophilic Ointment USP was evaluated for Phase separation, for stability through Freeze-thaw cycling through 4 ˚C and 45 ˚C and storage at different higher temperatures. Stability of Hydrophilic Ointment USP was studied at 25 ˚C, 30 ˚C and 40 ˚C storage. The globule size distribution is noted in Table-7 and shown in Fig-2.

Preparation and evaluation of herbal ointment*:*

Preparation of herbal ointment*:* Calculated amount of each extract was properly weighed and triturated with 2.5% DMSO solution for proper dispersion and solubulization. The dispersion was added to the Hydrophilic Ointment and triturated in a mortar to get the herbal ointments(15%w/w).

Evaluation of herbal ointment*:* Hydrophilic Ointment USP and herbal ointments were evaluated by toxicological studies and antimicrobial screening.

Toxicological study: The research on animals (rabbit and Wistar albino rats) was conducted according to the animal study guidelines as approved by IAEC of Jeeva Life Sciences, Telengana having IAEC Approval No. CPCSEA/IAEC/JLS/18/07/22/028.

Draize rabbit patch test: The base was mixed with 2.5% DMSO was applied to wounded skin of rabbit, covered for 24 hours and observed for irritancy, erythema or oedema. Ointments were applied repeatedly and evaluated after 48 hrs and 72 hrs also.[20]

Human patch test: Students (on own willingness) participated for study by applying on skin of forearm.

Antimicrobial screening:[21]

All medicated ointments were evaluated for antibacterial and antifungal activities by standard agar cup method. Medicated ointments were undergone antibacterial screening using two bacterial strains [*Staphylococcus aureus*(SA) and *Pseudomonas aeruginosa* (PA)] and antifungal screening using two fungal strains [*Candida albicans* (CA) and *Asperigillus niger* (AN)]. All the microorganisms were obtained from the aseptic laboratory maintained in the Microbiology P.G. Department, North Odisha University, Baripada, Odisha. Neosporin ointment(GSK) and Micogel cream(Cipla) were used as standard antibacterial and antifungal creams respectively.

Culture media: For bacterial strains: Nutrient agar(NA) and Nutrient broth(NB) and for fungi: Sabouraud dextrose agar(SBA) and broth(SDB) for fungi.[22]

Screening method: The Agar cup method was followed aseptically. Three agar plates were developed for extracts of each plant for antibacterial study and three agar plates were developed for extracts of each plant for antifungal study.

For antibacterial study**:** NA media was made, sterilized and put into sterile petri dishes to get 4 mm uniform thickness. Overnight Nutrient Broth (NB) cultures of organisms were seeded to make a lawn.

For antifungal study**:** SDA plates were seeded with CA and AN by lawn culture.

Four holes (6mm dia.) were made on plates. Holes were filled with aliquot amount of prepared herbal ointments, standard ointments/creams and controls (base with 2.5% DMSO) considering antibacterial and antifungal screening into account. The plates were incubated at 37 °C for 24 hrs. For bacterial and 28 ± 2 °C for 72 hrs. For fungi. The plates were observed for clear zone of inhibition around each hole and zone diameters were measured. An average zones of inhibition were recorded (n=3) in Table-8.

Selection of final formulation:

Methanol 70% extract of each plant having 15% of extract showed comparatively higher zone of inhibition in case of both antibacterial and antifungal studies. This might be due to presence of flavonoids and phenolics in high proportion in the Methanol 70% extract than the leaf juice extracts (dry mass). The proportion of zone of inhibition shown by the Methanol(70%) extracts was Ci-M> En-M> Pf-M. It was proposed to prepare four polyherbal ointments using the above three extracts.

Formulation and preparation of polyherbal ointment:

Polyherbal ointments (four formulations) containing 15% of total extract in the following proportions were prepared by trituration method.

Table 2: Composition of polyherbal cream

Polyherbal ointment	Proportion of Methanol(70%) extracts in percentage		
	Ci-M	Pf-M	En-M
PHO-1	20	40	40
PHO-2	30	35	35
PHO-3	35	30	35
PHO-4	40	30	30

Method: The extracts were weighed and dispersed in 2.5% DMSO solution one by one. The dispersion was added to the base taken in a mortar. The base was triturated to get a smooth preparation in which the extract dispersion was thoroughly mixed. The prepared ointments were transferred to sterile container and stored for further use.

Evaluation of polyherbal ointments:

i) Antimicrobial activity study: Prepared polymerbal formulations were subjected to antimicrobial activity study by the method described as earlier and the results are recorded in Table-9 and shown in Fig-3. From the zone of inhibition study it was observed that the formulation PHO-3 & PHO-4 showed better antimicrobial activity in comparison to other two. PHO-3& PHO-4 were selected for wound healing capacity study.

ii) Evaluation of infected wound healing response:[23]

A. Study of activities of polyherbal ointment on bacterial infected wound:

In this study wound was infected by a suitable bacteria (*Staphylococcus aureus*) and the effect of PHO-3 & PHO-4 on wound healing was studied.

Animals: Eight healthy Wistar albino rats of same age group were acclimatized for a period of eight days and were maintained on uniform diet and management throughout the experiment period.

Procedure : The area of dorsal plane of thoracolumber region was prepared and skin depth rectangular wounds of 300 mm^2 (approx.) were surgically induced. A loopful of inoculums of overnight bacterial culture of *S. aureus* was applied for infecting(after 48 hrs) the wounds of separate animals. Then treatment was started. Animals were grouped into four with two in each group.

Gr-a- Animals were treated with ointment base.

Gr-b- Animals were treated with standard Neosporin ointment.

Gr-c- Animals were treated with polyherbal ointment [PHO-3]

Gr-d- Animals were treated with polyherbal ointment [PHO-4]

Preparations were applied on the infected surface once a day after cleaning with surgical cotton wool. One animal from each set was used for sixth day healing observation and the other animal for twelfth day healing observation. After sixth day and twelfth day of treatment, wound tissues were collected for histopathological study.

B. Study of activities of polyherbal ointment on fungal infected wound:

In this study wound was infected by a suitable fungi (*Candida albicans*) and Micogel cream was used as standard. Similar method was adopted as described under antibacterial study. After sixth day and twelfth day of treatment, wound tissues were collected for histopathological study.

Histopathological study:[24] Collected wound tissues from infected wound area during antibacterial and antifungal treatment were subjected to histopathological study. The collected tissue was fixed in 10% formalin, stained by Hematoxylin & Eosin and finally photographed by Olympus microscope. The respective observations are shown as Fig-4a to 4i for bacterial infected wounds and Fig-5a to 5i for fungal infected wounds.

RESULT & DISCUSSION

Results of phytochemical investigation:

Yield of Ci-F was 10.39% W/V; Pf-F was 9.87%W/V and En-F was 9.12%W/V. These extracts(dry mss) were obtained from the fresh juice of leaves. However the methanol 70% extract of the dry mass was obtained in high amount due to presence of both methanol and water such as Ci-M[82.23%W/W], Pf-M[85.54%W/W] and En-M[81.72%W/W]. Most of the components were dissolved in the mixed solvent.

Both the extracts of each plants contained similar phytoconstituents. However as the weight of Methanol 70% extract obtained was less, it showed these extracts might be having the phytoconstituents in higher proportion on weight basis than the dry mass. The extracts of all three plants contain Alkaloids, Glycosides, Saponins, Flavonoids, Terpenoids, Carbohydrates, Tannins, Proteins, Phenolics.

Evaluation of Prepared Hydrophilic Ointment USP:

Hydrophilic Ointment USP was evaluated for different parameters as noted in Table-3 (psychosensory observations); in Table-4(physical parameters); in Table-5 and Fig -2(globule size analysis after being stored at varied conditions).

From the overall observation, it was seen that the prepared base was stable at higher temperatures, showed satisfactory results as per the observations by students and have good flow. The base is official in USP and showed all satisfactory results on evaluation.

Toxicological studies showed no sign of irritation (erythema and oedema) in Draize rabbit patch test and no itching, oedema or inflammation in the occluded area of skin of human under study.

Findings of biological screening:

Ci-F ointment showed ZOI 16.05-17.21mm. and Ci-M ointment showed ZOI 23.71-24.65mm against bacterial strains. Similar results were observed in case of extracts of other two plants. Pf-F ointment(14.30-15.72mm) and En-F ointment(15.23-15.56mm) showed lesser ZOI than corresponding Methanol(70%) extract Pf-M ointment(20.35-20.76mm) and En-M ointment(21.34-22.67mm) respectively. Similarly while considering the antifungal activity of ointments Ci-F ointment(12.15-14.43mm), Pf-F ointment(10.52-11.34mm) and En-F ointment(12.38-13.01mm) showed lesser ZOI than corresponding Methanol(70%) extract ointments i.e. Ci-M ointment(22.17-22.35mm), Pf-M ointment(15.78-16.16mm) and En-M ointment(18.09-20.8mm) respectively. In comparison, the standard antibacterial ointment showed 31.42-33.14 mm and antifungal cream showed 28.63-29.74mm of ZOI. This showed that Methanol (70%) extracts of all plants were more effective than corresponding fresh juice dry mass. However Ci-M ointment showed maximum effect. The effects might be due to flavonoids and phenolics which are responsible for better antimicrobial potential of the extracts.

Hence all three Methanol(70%) extracts were selected for preparation of polyherbal ointments.

Evaluation of Polyherbal ointments:

Four polyherbal ointments were formulated taking varied proportion of the three Methanol(70 %) extracts. All the ointments were evaluated for antimicrobial activities and infected wound healing response.

Antimicrobial activity results of polyherbal ointments:

PHO-1 and PHO-2 showed lesser ZOI against bacterial and fungal strains than PHO-3 & PHO-4 in terms of ZOI as shown in Table-7. Higher activity of PHO-3 & PHO-4 might be due to higher proportion of Ci-M extract in these ointments. PHO-4 showed higher ZOI than PHO-3 against both bacterial and fungal strains.

Results of infected wound healing response:

Wounds were infected with bacterial and fungal strain cultures to develop infection after 48 hours. The respective wounds were treated with the prepared polyherbal ointments PHO-3 & PHO-4 along with the standard. Wound tissues were collected after 6 days and 12 days and subjected to histopathological study. Tissue biopsy as developed slides were studied microscopically and following observations were made.

Histopathological study of tissues of bacterial infected wound:

- The cross section microscopic study of the normal skin showed normal epidermis and dermis. The intact skin revealed normal histology and normal skin architecture. No evidence of pathological appearance observed under microscope (Fig-4a).
- After treatment with standard antibacterial, tissue biopsy after six days showed absence of infiltrating inflammatory cells or granuloma with healing by deposition of collagen fibers and maturation of fibrous connective tissue (Fig-4b). On 12^{th} day the focal ulceration was covered by granulation tissue. The epidermis and dermis showed normal appearance with healing area. (Fig-4c).
- Multiple section of skin biopsy showed hemorrhages with little amount of fibrino-necrotic material and granulation tissue after 6days of treatment with control(Fig-4d). Abundant collagenous deposion with granulation tissue and fibrino-necrotic material was observed after 12 days(Fig-4e).
- Tissues at 6^{th} day treatment with PHO-3 showed ulceration of skin with mild edema. The ulcer started healing with granulation tissue(Fig-4f). At 12^{th} day ulcers were covered by granulation tissue. No granuloma was found. Histology showed progressive healing with neo-epithelization(Fig-4g).
- Tissues at 6^{th} day treatment with PHO-4 showed presence of granulation tissue. No inflammatory change or granuloma was found. Growth of healing tissue observed and was better than that of PHO-3 (Fig-4h). At 12^{th} day healing of ulcer was done with fibrosis. There was extensive deposition of collagen fibers. No inflammatory reaction or granuloma was found. Keratinization was noticed on nearly complete grown epidermis. A prompt healing was observed than that of PHO-3 (Fig-4i).

In the present investigation, the PHO-4 ointment showed prompt bacteria infected wound healing activity than PHO-3 with proliferation and increase in the rate of wound contraction as compared to the control animals.

Histopathological study of tissues of fungal infected wound:

- After treatment with standard antifungal, tissue biopsy after six days showed absence of infiltrating inflammatory cells or granuloma with healing by deposition of collagen fibers and maturation of fibrous connective tissue (Fig-5b). On 12th day the focal ulceration was covered by granulation tissue. The epidermis and dermis showed normal appearance with covering. Keratinization was noticed (Fig-5c).
- Multiple section of skin biopsy showed hemorrhages with little amount of fibrino-necrotic material and granulation tissue after 6days of treatment with control(Fig-5d). Abundant collagenous deposion with granulation tissue and fibrino-necrotic material was observed after 12 days. There was no evidence of malignancy, dysplasia and granuloma(Fig-5e).
- Tissues at 6th day treatment with PHO-3 showed focal ulceration covered by granulation tissue. tissue. Stroma revealed granulation tissue with mild edema and minimal inflammation. (Fig-5f). At 12th day of plate showed mild degree of inflammation composed of lymphoid cells. No inflammatory reaction or granuloma was found. The skin architecture showed focal ulceration covered by granulation tissue (Fig-5g).
- Tissues at 6th day treatment with PHO-4 showed presence of granulation tissue. No inflammatory change or granuloma was found. Growth of granulation tissue was observed. (Fig-5h). At 12th day very minimal ulceration of skin was observed. Stroma revealed granulation tissue composed of plump fibroblasts capillaries lined by plump endothelial cells. No inflammatory reaction or granuloma was found. The intact portion of the skin revealed normal histology. There was nearly complete healing of ulcer with granulation tissue. A prompt healing with PHO-4 was observed than that of PHO-3 (Fig-5i).

In the present investigation, the PHO-4 ointment showed prompt bacteria infected wound healing activity than PHO-3 with proliferation and increase in the rate of wound contraction as compared to the control animals.

		
***Clerodendrum infortunatum* L.**	***Paederia foetida* L.**	***Euphorbia neriifolia* L.**

Fig 1: Leaves of *selected plants*

Table 3: Physical parameters of extracts of Plant I, II & III

Sl. No.	Extract	Color	Consistency	% yield
1	Ci-F	Greenish black	Pillular	10.39 %W/V
2	Ci-M	Straw green	powder	82.23%W/W
3	Pf-F	Greenish black	Soft	9.87 % W/V
4	Pf-M	Greenish black	powder	85.54%W/W
5	En-F	Greenish black	soft	9.12 % W/V
6	En-M	Greenish black	powder	81.72%W/W

Table 4: Phytochemical screening of dry mass & Ethanol (70%) extract

Phyto chemicals	Plant-I		Plant-II		Plant-III	
	Ci-F	Ci-M	Pf-F	Pf-M	En-F	En-M
Alkaloids	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Amino acids & Proteins	+	+	+	+	+	+
Phenolic compounds	+	+	+	+	+	+

(+)sign stand for presence and (-ve) sign stand for absent of phytoconstituents

Table 5: Observations of psychosensory evaluation:

Parameters ▶	Extruda-bility•	Consi-stency▪	Spread-ability•	Appearance & Colour	Homoge-nicity
Base	Good	Soft	Good	Milky white	Homogenous

Table-6: Data showing findings of rheological, physical and thermodynamic evaluation:

Para-meters ▶	Spread-ability (g.cm./s)	Viscosity (cps)	Extrud-ability	Yield value	pH	Softening range in ˚C	Bleeding	Phase separation at ºC	Phase separation after freeze-thaw cycling
Base	156.8	28200	Good	415.3	5.4	46– 48	No	75	No

Table 7: Dispersed phase globule size distribution of Hydrophilic Ointment USP at different conditions

Conditions	Size range in µm				
	1-10	10-20	20-30	30-40	40-50
Initial	15	58	15	7	5
After freeze-thaw cycling	5	55	18	15	7
Storage at 25 °C	10	58	16	9	5
Storage at 30 °C	8	56	18	13	5
Storage at 40 °C	6	55	20	13	6

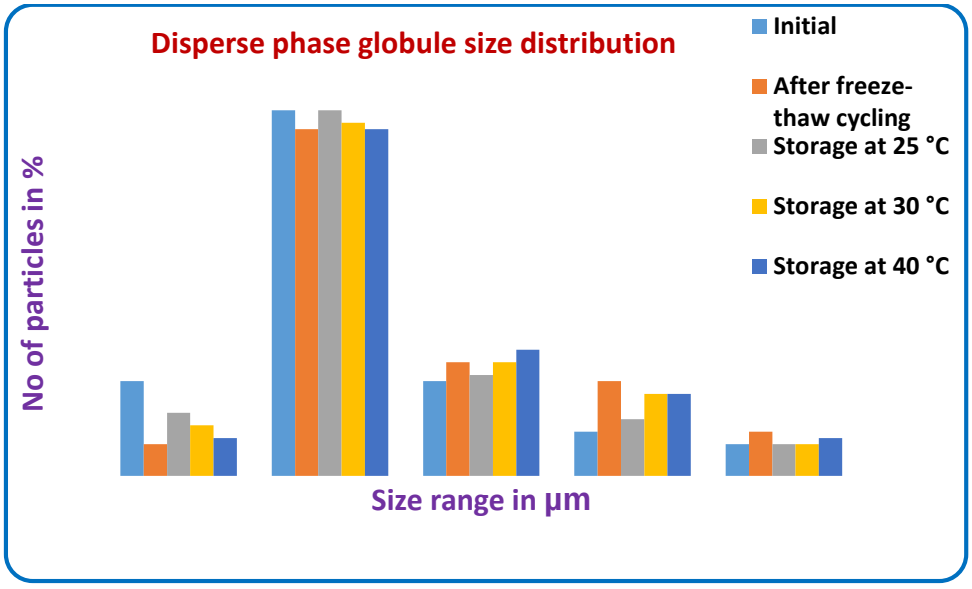


Fig 2: Dispersed phase globule size distribution of Hydrophilic Ointment USP at different conditions

Table-8: Zone of inhibition(in mm) shown by Herbal ointments against microbial strains:

S No	Preparations used	Zone of inhibition in mm[n=3]			
		against bacterial strains		against fungal strains	
		SA (Gr +ve)	PA (Gr -ve)	CA (Fungi)	AN (Mycelia)
1	Control	6.0± 0.00	6.0± 0.00	6.0± 0.00	6.0± 0.00
2	Standard#	33.14** ±0.42	31.42** ±0.35	29.74** ±0.16	28.63** ±0.05
3	Ci-F ointment	17.21 ±0.12	16.05 ±0.16	14.43 ±0.28	12.15 ±0.07
4	Ci-M ointment	24.65* ±0.13	23.71* ±0.21	22.35* ±0.24	22.17* ±0.18
5	Pf-F ointment	14.30 ±0.17	15.72 ±0.11	11.34 ±0.16	10.52 ±0.07
6	Pf-M ointment	20.76* ±0.25	20.35* ±0.21	16.16 ±0.13	15.78 ±0.17
7	En-F ointment	15.23 ±0.35	15.56 ±0.26	13.01 ±0.18	12.38 ±0.22
8	En-M ointment	22.67* ±0.20	21.34* ±0.15	20.08* ±0.36	18.09 ±0.15

Neosporin ointment (antibacterial) and Micogel cream (antifungal)

Values are expressed as mean ± standard deviation; Statistical significance vs Control ($p<0.05$, ** $p<0.01$) Vs Control; one way ANOVA,* followed by Student 't' test.

Table 9: Zone of inhibition(in mm) shown by polyherbal ointments

S No	Preparations used	Zone of inhibition in mm[n=3]			
		against bacterial strains		against fungal strains	
		SA (Gr +ve)	PA (Gr -ve)	SA (Gr +ve)	PA (Gr -ve)
1	Control	6.0± 0.00	6.0± 0.00	6.0± 0.00	6.0± 0.00
2	Standard[#]	33.42** ±0.35	31.31** ±0.78	29.54** ±0.63	28.14** ±0.47
3	PHO-1	24.32* ±0.38	23.17* ±0.28	18.59 ±0.41	17.21 ±0.38
4	PHO-2	23.05* ±0.51	22.75* ±0.43	20.32* ±0.78	19.71 ±0.04
5	PHO-3	25.68* ±0.35	24.15* ±0.05	22.08* ±0.46	21.75* ±0.25
6	PHO-4	27.34** ±0.35	26.27** ±0.42	24.43* ±0.15	23.87* ±0.63

[#] Neosporin ointment (antibacterial) and Micogel cream (antifungal)

Values are expressed as mean ± standard deviation; Statistical significance vs Control ($p<0.05$, ** $p<0.01$) Vs Control; one way ANOVA,* followed by Student 't' test.

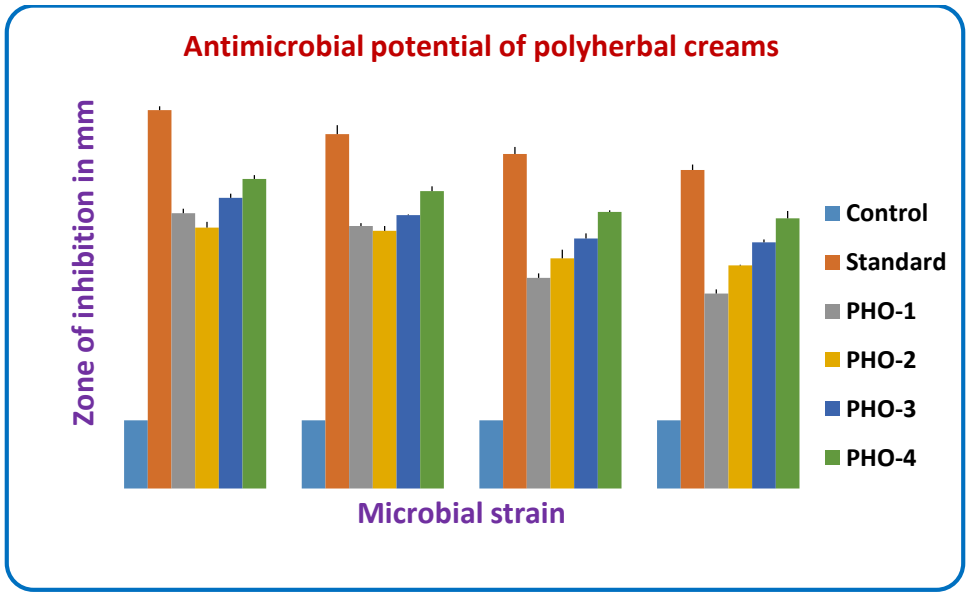


Fig 3: Zone of inhibition (in mm) shown by polyherbal ointment

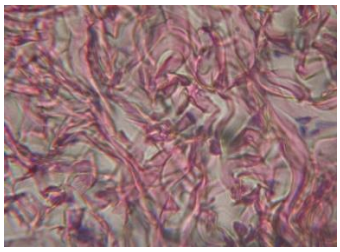
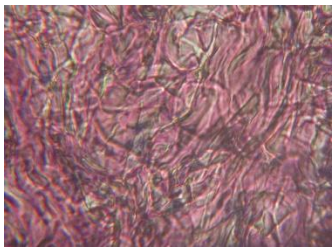
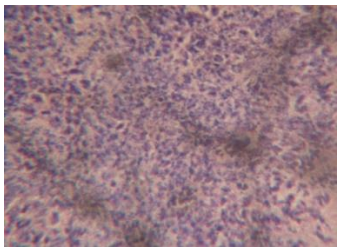
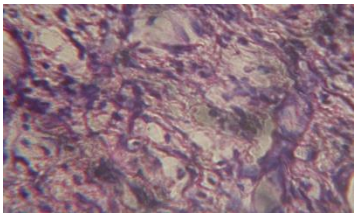
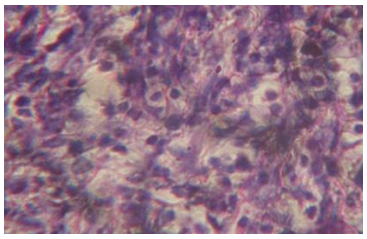
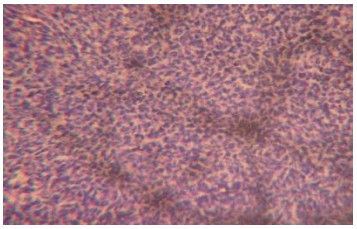
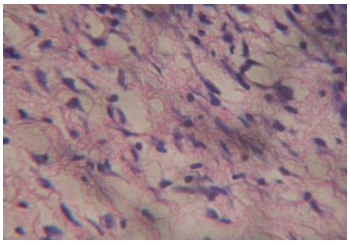
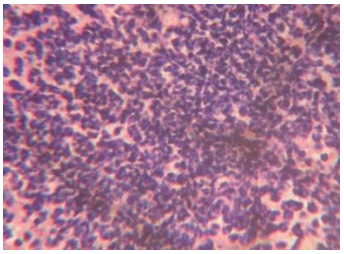
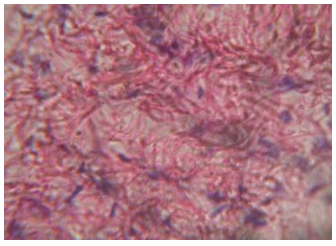
		
4a: Normal skin	**4b: Standard antibacterial treated (6th day)**	**4c: Standard antibacterial treated (12th day)**
		
4d: Control base treated (6th day)	**4e: Control base treated (12th day)**	**4f: Antibacterial PHO-3 treated(6 day)**
		
4g: Antibacterial PHO-3 treated (12 day)	**4h: Antibacterial PHO-4 treated (6 day)**	**4i: Antibacterial PHO-4 treated(12 day)**

Fig 4a to 4i: Histological section of granulation tissue of treated animals with antibacterial ointments(40X)

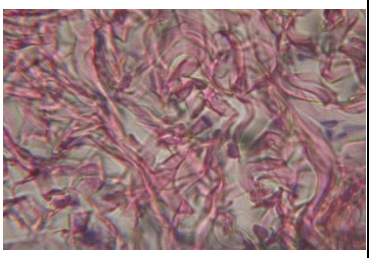
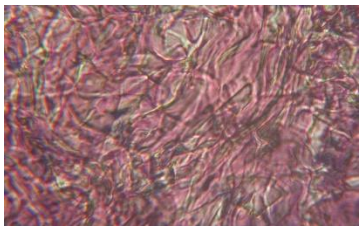
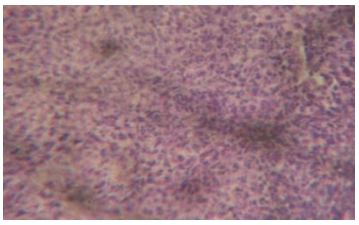
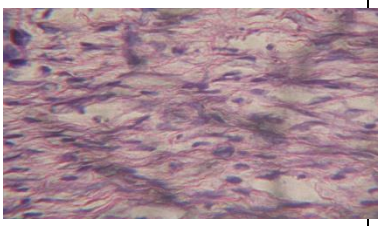
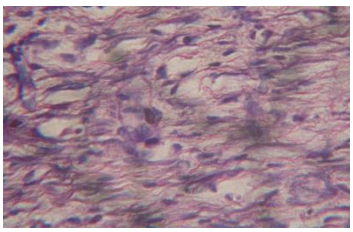
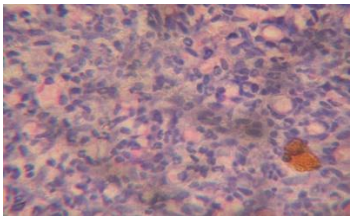
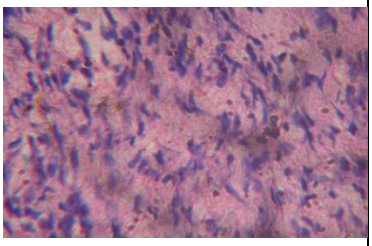
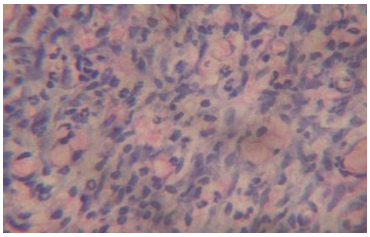
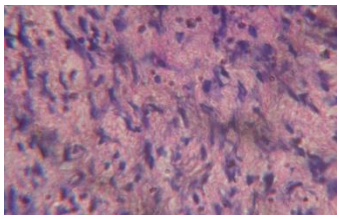
		
5a- Normal skin	**5b- Standard antifungal treated (6th day)**	**5c- Standard antifungal treated (12th day)**
		
5d. Control base treated (6th day)	**5e. Control base treated (12th day)**	**5f. Antifungal PHO-3 treated (6 day)**
		
5g. Antifungal PHO-3 treated (12 day)	**5h. Antifungal PHO-4 treated (6 day)**	**5i. Antifungal PHO-4 treated(12 day)**

Fig 5a to 5i: Histological section of granulation tissue of treated animals with antifungal ointments(40X)

CONCLUSION

Clerodendrum infortunatum Linn, *Paederia foetida* Linn. and *Euphorbia neriifolia* Linn. are well known plants. Different parts are medicinally used in different forms. Local vaidyas use the fresh juice of leaves of these selected plants. Less number of research has been found on use of fresh juice dry mass and extracts of these plants obtained from the place of work towards application against skin infections. The fresh juice was dried to get dry mass for study. Methanol(70%) extract was prepared from dry mass. The extracts were rich in many phytoconstituents including flavanoids and phenols. Extracts were incorporated in Hydophillic ointment USP which was prepared and properly evaluated. Ointments containing Methanol(70%) extracts(15%) have shown profound antimicrobial activity. The Methanol(70%) extracts due to more concentration of phytoconstituents particularly flavonoids and phenolics showed the higher antimicrobial potential. Methanol(70%) extract of *Clerodendrum infortunatum* showed higher antimicrobial activity. Polyherbal ointments were formulated. The ointment containing high proportion of Methanol(70%) extract of *Clerodendrum infortunatum* (PHO-4) showed higher ZOI in antimicrobial study in comparison to PHO-3. From wound healing potential study on infected wounds on rat skin surface better results on

wound healing was observed with PHO-4 ointment than PHO-3. The result concluded that the Methanol(70%) extract of leaves of all selected plants can be used effectively against skin infections as polyherbal ointment. If the ointments are properly designed and studied pharmaceutically and pharmacologically in a systemic manner including clinical studies, the ointment can be used therapeutically.

Statistical analysis:

All the data were presented as mean± SEM. The statistical differences were evaluated by ANOVA, followed by Student 't' test. Statistical significance on comparison with the control were considered by $p<0.01$ highly significant and $p<0.05$ significant.

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Abbreviations

AN - *Asperigillus niger*

CA - *Candida albicans*

Ci - *Clerodendrum infortunatum* L.

DMSO - Dimthyl sulfoxide

En - *Euphorbia nerifolia* L.

PA - *Pseudomonas aeruginosa*

Pf - *Paederia foetida* L.

PHO - Polyherbal ointment

SA - *Staphylococcus aureus*

USP - United States Pharmacopoeia

ZOI - Zone of inhibition

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