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PREPARATION & FORMULATION OF POLYHERBAL OINTMENT FOR WOUND HEALING ACTIVITY

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ABSTRACT

Aim:- The present study aimed to moderate the wound healing activity of polyherbal plants like *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark in topical ointment formulation. **Method:-** The emulsifying ointment formulations containing extracts of the exceeding mentioned herb was formulated, evaluated and their wound healing activity was studied on experimentally induced excision open wounds in albino rats. **Result:-** The effects of the formulation on wound healing were assessed by the rate of wound closure, period of epithelialization. The methanol extract(by hot extraction process) of *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark 2%, 4%, & 5% is incorporated into 100 g. of a simple ointment base by melting and triturating method for preparation of polyherbal ointment formulation. The measurement of the wound areas were taken up-to 18th days and the percentages of wound closures were calculated. Blank ointment base and Framycetin ointment (1% w/w) served as the control and standard treatments. Topical application of 5% *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark of the prepared ointment on the excision wound in rats caused a significantly ($p<0.05$) higher rate of wound healing and reduced the epithelialization period in a dose-related approach. The polyherbal

ointment treated group showed complete epithelialization is compared to the (1%) Framycetin ointment. The formulated ointments were evaluated and effective in wound care and should be explored in harnessing the potentials of the plant in the treatment of Topical diseases.

Key words: Wound healing, Polyherbal formulation, Excision wound.

1. INTRODUCTION

Wounds may be defined as loss or breaking of cellular and anatomic or functional strength of living tissue. It may be formed by microbial, thermal, physical, or chemical immunological scratch to the tissue. When skin is damaged, cut, or punctured it is termed as an open wound and when force pain causes a mark, it is called closed wound, where as the burn wounds are caused by sunlight, heat, fire, radiation, electricity or chemicals [1-3]

World Health Organization is promoting traditional less expensive, complete medical care, especially in emergent countries. Out of the world's population Eighty percent relies on medicinal plants for their primary health care. WHO also accepted the significance of traditional herbal medicinal and has treated strategies, guidelines, rules and standard for botanical medicine. [4]

Worldwide the herbal industry is selection up at a fast rate. Herbs and botanical now show in more products and have more medicinal function than still previous to. India is permission with divers' agro-climatic situation and is a most important source for a wide range of medicinal plant. But the production probable is mostly underexploited. When we look turn around over 250 years before, people of some continents for the most part of restored to herbal and traditional medicine. As well in India too, Ayurveda work affords to botanical medicine and take care of the health of our country. Approximately the 25 to 40% of the dynamically active components of the synthetic medicine of allopathic medicine had start for higher peak plants of the world and the clues to find out them came from tradition medicine of particular cultures. While the allopathic medicines are very helpful in treatment short term and urgent health condition, it has been basically ineffective in treating some of the multifactorial chronic disease. The relative strength of recent system of medicine better than the established system is in its huge pharmacopeia technology development in surgical tools or process and in treatment of acute diseases. Although the actuality that a lot of new medicine are being additional in allopathic, only about the 30% of known 2000 disease cured,

50% are being symptomatically around, 20% of the allopathic medicine have significant side effect. [5-7]

Medicines based on herbal origin have been the organization of handling and treat for different diseases. Moreover, Indian folk medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds, skin infections, inflammation, diarrhea, leprosy, scabies, ulcers, venereal disease, snake bite, etc. More than 80% of the world's population still depends upon traditional medicines for different skin disease. Herbal drugs in wound organization involve debridement, disinfection and providing a moist atmosphere to give assurance the establishment of the proper environment for ordinary healing procedure. A huge number of plants are used by folklore traditions in India for carry out on treatment of cuts, wounds and burns. [8]

Erythrina indica is commonly known as Indian coral tree or Tropical coral tree in English,Pangara in Marathi, Paribhadra in Sanskrit & Mandara in Hindi. *Erythrina indica* Linn. (Febaceae) is a medium-sized, spiny, deciduous tree normally growing to 27 mtall, found in Bengal and many parts of India especially in southern India. The leaves are pinnate with a 20 cm petiole and three leaflets, each leaflet up to 20 cm long and broad. It has dense clusters of scarlet or crimson flowers and black seeds. Flowers bright red on leafless branches, in dense stout racemes. Sepals 5, Petals 5, papilionaceous corolla. Stamens10, alternately longer and shorter. Ovary one celled, many ovuled. Special parts of plant are used in traditional medicines as a nervine sedative, collyrium, in opthalmia, anti-asthmatics, antiepileptic, antiseptic and as an astringent. Bark is used in fever, liver ailment and rheumatism' wound heal. Leaf paste useful for muscular pain in cattle. Juice from the leaves is mixed with honey and ingested to kill tapeworm, round-worm and threadworm. [9-11]

Calotropis procera (Giant milkweed) is also known as sodom apple, calotrope, French cotton, small crown flower (English),rui(Marathi) algodón de seda,bomba (Spanish), cotton-france, arbre de soie, and bois canon (French) *Calotropis procera* (Ait.) R. Br., a wild growing plant of family *Asclepiadaceae*, is well known for its medicinal properties. Arka (*Calotropis procera*) an important drug of Ayurveda is known in this country from the earliest time. It is mentioned by the earliest Hindu writers and the ancient name of the plant which occurs in the vedic literature was Arka alluding to the form of leaves, which was used in the sacrifical rites. Different parts of this plant have been reported to exhibit anti-inflammatory, analgesic, and antioxidant properties. It is found in waste lands and grows as a

weed in agricultural lands. In ancient Ayurvedic medicines the plant *Calotropis procera* was known as "Rakta arka". [12]

F. religiosa belongs to a class of drugs called rasayana. Rasayana are rejuvenators, antioxidants and relieve stress in the body. In India it is known by several vernacular names, the most commonly used ones being Asvatthah (Sanskrit), Pimple(Marathi) Sacred fig(Bengali), Peepal (Hindi), Arayal (Malayalam), Ravi (Telgu) and Arasu (Tamil) *F. religiosa* is a large deciduous tree with few or no aerial roots. It is often epiphytic with the drooping branches bearing long petioled, ovate, cordate shiny leaves. Leaves are bright green, the apex produced into a linear-lanceolate tail about half as long as the main portion of the blade. The bark is flat or slightly curved, varying from 5 to 8 mm in thickness, outer surface is grey or ash with thin or membranous flakes and is often covered with crustose lichen brown or ash coloured, surface has shallow irregular vertical fissures and uneven due to exfoliation of cork, inner surface smooth, yellowish to orange brown and fibrous. [13]

2. OBJECTIVE

To evaluate the wound healing activity of polyherbal plants like *Erythrina indica* leaf, *Calatropsis procera* root *& Ficus religiosa* bark.

3. MATERIALS AND METHODS

3.1 Plant Material [14]

The Fresh *Erythrina indica* leaf, *Calatropsis procera* root *& Ficus religiosa* bark were collected in September, 2013 from area, Maharashtra state Gadhinglaj, samangadh Forest. The plant material was authenticated by Prof. a staff of department of Botany, Shivaji university of Kolhapur. The plant materials were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container.

3.2 Preparation for Plant Extraction: [15, 16]

The authenticated fresh *Erythrina indica* leaf, *Calatropsis procera* root *& Ficus religiosa* bark were dried under shade and used for the preparation of extract. The *Erythrina indica* leaf, *Calatropsis procera* root *& Ficus religiosa* bark was coarsely powdered with the help of mechanical grinder and passed through the sieve. The powder was stored in an air tight container for further use. The powder obtained was added to following soxhlet extraction by using soxhlet apparatus and cold maceration method. The following are extract used as solvent for extraction process.

3.2.1 Chloroform extract: The marc left after soxhelation extraction was dried and then extracted with chloroform (55-56^{0}c), until the extraction was completed. After achievement of extraction, the solvent was evaporated by heating on a water bath. Dark greenish colour residue was obtained. The residue was then stored in desiccators.

3.2.2 Methanol extract

The marc left after chloroform extraction was dried and then extracted with methanol 95%, until the extraction was completed. After achievement of extraction, the solvent was evaporated by heating on a water bath. Dark brown colour residue was obtained. The residue was then stored in desiccators.

3.2.3 Aqueous extract

The marc left after methanol extraction was dried and then extracted with chloroform water by cold maceration process for 7 days with moderating starring. At the end of 7th days, it was filtered through muslin cloth and filtrate was concentrated. The remaining solution was evaporated by heating on a water bath. The brown colour was obtained. The residue was then stored in desiccators.

Yields of each extraction are given in Table No.1.It was found that % of yield is greater in Methanol extract by Soxhelation extraction process.

4. Preliminary Phytochemical Analysis [17,18]

A preliminary phytochemical screening was passed out for separate extract employing the standard procedure to reveal the presence of alkaloids, glycosides ,carbohydrates ,steroids, flavonoids, saponins, tannins & amino acids. .It was found that phytoconstituent are more found in Methanol extract by Soxhelation extraction process.

Phytochemical Analysis of polyherbal extracts are given in Table No. 2

5. Composition of different polyherbal formulation

The composition of different polyherbal extracts are given in Table No. 3

6. Antimicrobial Activity:

The antimicrobial activity of different polyherbal formulations was assessed by measuring the zone of inhibition by using nutrient agar medium. *Bacillus cereus, Staphylocous aureus, Escherichia coli, Pseudomonas aeruginosa*.as test organisms.

The antimicrobial activity is performed by following steps as given below.

6.1 Sample preparation: The extracts were weighed & prepared polyherbal formulation (F1 to F4) of different concentrated by using same solvent & 0.05 ml was used for activity studies.

6.2 Control preparation: The same solvents which are used for extraction process are used as control for the antimicrobial studies.0.05 ml solvents are used for activity study.

6.3 Standard preparation [19]

Amoxicillin serve as a standard control for antimicrobial activity.(Amoxicillin10µg/ml)

6.4 Preparation of medium and nutrient broth [20]

Weighed about 0.4gm of nutrient soup and dissolved in 30ml of distilled water. Then the soup was hanged in each of test tube. The Muller Hinton agar medium was prepared which contain 9.7gm of MHA was hanged in 250ml of water. Then broth the medium and soup were for sterilization. After sterilization, the nutrient soup was permitted to cool and then the organisms were inoculated for 4 hours. The MHA medium were poured in the petridish before cooling and allowed to solidify for about 3-4 hours.

6.5 Methodology

The antibacterial activity was determined according to the method described by Okeke (2001)with some modifications [96.] *Bacillus cereus* culture was swabbed on the surface of sterile nutrient agar plate in triplicate. In agar plate five wells were prepared with the help of sterilized cork borer of 6mm diameter namely a,b,c,d,& e. The (a) center well is served as standard, (b) served as control, (c) served as 2% polyherbal extract, (d) served as 4% polyherbal extract & (e) served as 5% polyherbal extract . Each well was filled aseptically with 0.05ml std, control & polyherbal formulations by using micropipette. Similarly the antimicrobial activity of, *Staphylocous aureus, Escherichia coli & Pseudomonas aeruginosa* was carried out. These plates were incubated at 37^{o}C overnight for observation. The presence of inhibition was noted and compared with the standard. The susceptibility of the test organism to the tested plant extract was resolute by observing the zone of inhibition around each well.

6.6 Microbiological screening [21,22]

Antimicrobial activities of different extracts of polyherbal plant were evaluated by the agar well diffusion method.

6.6.1 Agar well diffusion method [23]

Agar well-diffusion method was followed to find out the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria. Five wells (6mm diameter and about 2 cm a part) were made in each of these plates using sterile cork tool. Polyherbal extract of different concentration 2, 4 & 5 mg/ml was used. About 0.05 ml of polyherbal formulation is subjected into well by using micropipette and permitted to diffuse at room temperature for 2hrs. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured and calculated. The readings were taken in three different fixed directions and the average values were recorded.

6.6.2 Determination of relative percentage inhibition[24]

The relative percentage inhibition of the polyherbal extract with respect to standard control was calculated by using the following formula

Relative percentage inhibition of the test extract =

$$\frac{100 \times (x-y)}{(z-y)}$$

Where,

x: total area of inhibition of the test extract

y: total area of inhibition of the solvent

z: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using area = πr^2

Where, r = radius of zone of inhibition

The antimicrobial activity of different polyherbal ointment is listed in Table No. 4 & graphical representation is given in fig. No.1.

7. Acute Oral Toxicity Study

The toxicity study aimed to investigate the safety profile of methanol extract of *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark. The result of acute oral toxicity

study of methanol extract indicate that, the extract up to 2000mg/kg body weight did not produce any sign of toxicity or the mortality in any group during or after treatment. Thus, the present study establishes the reliable safety profile of the methanol extract of *Erythrina indica* leaf, *Calatropsis procera* root *& Ficus religiosa* bark, administered orally in albino rats.

Acute toxicity study is carried out according to OECD guideline 423, none of rat shows sign of toxicity upon single administration of *Erythrina indica* leaf, *Calatropsis procera* root *& Ficus religiosa* bark extract (2000mg/kg,). The result is shown in Table No. 5.

8. Preparation of Topical Formulation

Depends on % yield concentration, phytoconstituent & antimicrobial activity Methanol extract prepared by Soxhelation extraction process is used for experimental process. Topical poly herbal formulation was prepared by using suitable emulsifying bases (simple ointment). These ointments are 2%, 4% & 5% methanol extracts prepared by soxhalation process of plants like *Erythrina indica* leaf, *Calatropsis procera* root *& Ficus religiosa* bark & ointment is prepared by fusion method.

9. Evaluation of Wound Healing Activity[25]

To evaluate the wound healing ability of the prepared formulation, we measured parameter like:

- Rate of wound contraction (Excision wound model)
- Epithelization time (Excision wound model)
- Histological evaluation of healed tissue
- Determination wound breaking strength (Incision wound model)

9.1 Animals: Healthy Wistar albino rats of either sex and of about the same age, weighing among 150-250 gm were used for the study. They were independently housed, maintained clean polypropylene cages containing paddy pod bedclothes and fed with standard diet and water *ad labium* in animal house facility and maintained under usual experimental conditions. The experimental protocol has been approved by institutional animal ethical committee, TKCP College of Pharmacy, Warnanagar, Dist-Kolhapur

9.2 Drugs and Chemicals

Framycetin 1% w/w(soframycin, Aventis Pharma, (Goa) was procured from local retail

pharmacy, which is used as standard drug. Simple ointment used as the ointment base. By using this, ointment is prepared in three concentrations (2.%w/w, 4%w/w and 5%w/w).

9.3 Experimental procedure

Excision was inflicted on the rats according to methods described by Morton and Malone (1972) under light ether anesthesia. The dorsal fur of the animals was shaved with an electric cutter. Full skin thickness was excised from the clear area to get a wound measuring about 500 mm 2 by using toothed forceps and pointed scissors. [26]

The animals were divided into five groups of six animals each as mentioned below

Group I- Control group with wound and treated with ointment base.

Group II- Standard group with wound and treated with ointment framycetin 1% w/w.

Group III- Test group with wound and treated with methanol extract of 2.%w/w *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark

Group IV- Test group with wound and treated with methanol extract of 4%w/w *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark

Group V- Test group with wound and treated with methanol extract of 5%w/w *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark

All the formulations were applied once a day till the complete epithelialization preliminary from the day of cutting. Wound healing property was evaluated by wound contraction percentage and wound closure time. The wound surface area was measured at once by placing a transparent paper over the wound and tracing it out, area of this impression was calculated using the graph sheet. The same procedure is employed every third day until healing was complete [27,28]

9.4 Evaluation of parameters

9.4.1 Rate of wound contraction: Wound contraction indicates the rate of reduction of unhealed area during the healing process. Thus, the faster rate of wound closer indicates the better efficacy of medication. The progressive reduction in wound area of different groups of animals over 18 day, by methanol extract. The ointment formulations with methanolic extracts of *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark showed significant promotion of wound-healing activity with statistically significant (*p<0.05) in all the five groups of animal which were depicted in the Table 6 .

During wound healing period and at the preset time intervals, the wound area was traced manually. The percentage of wound contraction was calculated by the formula (1-wound area at the studied day/wound area at initial day) x100. Reduction in the wound area was expressed as percentage of the initial wound diameter. The average wound areas together with standard deviation of each formulation listed in Table No.6. & also graphical represent is shown in fig. No.2.

9.4.2 Epithelialization Time[29]

It was monitored by noting the number of days required for the mark to fall off from the wound surface without leaving a raw wound behind.The epithelization time is given in Table No. 7

9.4.3 Histological Evaluation[25]

A comparison of Histopathological studies of regenerated tissue section of control, standard and wound treated with different ointment based formulation are fig No.3. Microscopic examination of the section prepared from the wounds of control, standard and tested group exhibited the following characteristics.

a. Control I (Simple ointment)

The tissue showed poor inflamed connective tissue with chronic inflammatory cells between the collagen fibers, incomplete wound healing. Many thin wall blood vessels are present.

b. Standards (1% Framycitin sulphate ointment)

It shows higher collagen deposition. Tissue showed good fibrous connective tissue with scattered inflammatory cells and fibroblast. These result shows that the faster and completed wound healing.

c. Formulation 1 (2% P.H.M.S.F.)

The tissue showed poor collagenization, fibroblasts, and many blood vessels are present, incomplete the wound healing.

d. Formulation 2 (4% P.H.M.S.F.)

It shows good collagen deposition and better healing as compared with control group. Epithelization tissue was observed. Parameter like fibroblasts, collagen, and neo-vascularization were higher in compared with controlled group.

e. Formulation 3 (5% P.H.M.S.F.)

It shows moderate collagen deposition but better healing as compared with control group. Parameter like fibroblasts, collagen, were higher in compared with controlled group.

9.4.4. Incision Method: (Determination of wound breaking strength): The wound healing intended by the breaking strength of the healing skin, treated with different formulation on the 16th day, discovered that the wound treated with control I and Formulation I had the minimum strength, while the braking strength of the tissue treated with formulation III of 5% extract of P.H.M.S.F higher than the control I and Formulation I. The breaking strength of polyherbal plant ointment is given in Table No. 8

10. Statistical analysis

All the values are expressed as mean ± S.E.M for groups of six animals each. Analyzed by one way ANOVA and compared **by Dunnett<'s t-test** test. The values are statistically significant at three levels, ***p<0.001. **p<0.01. *p<0.05. But ns if p >0.05.

11 RESULTS AND DISCUSSION

This present study shown that the selected three herbs that are *Erythrina indica* leaf,*Calatropsis procera* root& *Ficus religiosa* bark was made to create a different concentration of polyherbal formulation, and to evaluate for its antimicrobial activity & wound healing with a standard antibiotic like Amoxicillin & framycitin sulphate 1%.These three plants are extracted by maceration process using a aqueous, chloroform & methanol solvents & again continuous hot extraction(soxhelation) process by methanol solvent and the Phytochemical screening was done. Phytochemical screening established the presence of various phytoconstituent like alkaloids, carbohydrate, glycosides, flavanoids, amino acids, steroid, saponin, protein, phenolic compound and tannins.In the present work, the extract was studied for antimicrobial activity & was compared with standard antibiotic like Amoxicillin using selected species of gram positive bacteria such as *Bacillus cereus, Staphylocous aureus* & gram negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa* and it was showed that formulation like F4 showed greater antimicrobial activity. On these results the 5 % extract of polyherbal plants like *Erythrina indica* leaf,*Calatropsis procera* root& *Ficus religiosa* bark was topically prepared & wound healing activity is compared with 1% framycitin sulphate for 18 days. The highest wound closer was observed by P.H.M.S.F. F3. The mean percentage closure of wound area was calculated on the 3, 6, 9, 12, 15 and finally 18 days. The wound healing activity was found to be comparable with that of the reference

standards and control bases. The percentages closure of excision wound area in animals treated with P.H.M.S.F.F3 was compared with that of the commercial products of Framycitin sulphate ointment. Out of the three formulations the P.H.M.S.F F3 was shown (99.56%) maximum wound healing activity. The epithelization time is almost near to the standard ointment. On histopathological study of 5% polyherbal the ointment wound healing growth cellular structures and tissue layers in broken tissue are restored as systematically as probable to its normal state. The collagen deposition is better healing as compared with control group. The braking strength of wound treated with 1% Framycitin Sulphate ointment is was similar to that 5% P.H.M.S.F & was nearly similar result that on the breaking strength of healed tissue. These study confirms that methanol extract of 5% polyherbal plants like *Erythrina indica* leaf,*Calatropsis procera* root& *Ficus religiosa* bark are very good wound healing property.

Table No. 1: Percentage yield of various extract of *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark

Sr. No.	Solvent	Aqueous extract % Yield	Chloroform extract % Yield	Ethanol Methanol extract % Yield	Methanol extract (Soxhelation) % Yield
1.	*Erythrina indica* leaf	13.9	12.4	14.2	14.8
2.	*Calatropsis procera* root	12.8	09.2	13.2	13.4
3.	*Ficus religiosa* bark	13.2	11.3	13.4	13.6

Table No.2. Phytochemical screening of the Methanol (Soxhalation) extract of Erythrina indica leaf,Calatropsis procera root & Ficus religiosa bark

Sr.No.	Phytoconstituents	Herbal Plants Methanol(Soxhalation) extract		
		Erythrina indica leaf	*Calatropsis procera* root	*Ficus religiosa* bark
1.	Alkaloids	+++	++	++
2.	Saponins	+	+	-
3.	Glycosides	+++	+	++
4.	Carbohydrates	+	+	-
5.	Tannins and phenolic Compound	+	+++	++
6.	Fixed oils and Fats	+	-	+
7.	Flavonoids	+++	++	++
8.	Steroids	++	+++	++
9.	Proteins and Amino acids	+	-	+
10.	Terpenoids	+	-	+

Present = (+), absent = (-), (++) = appreciable, (+++) =very appreciable

Table No.3 Composition of different polyherbal formulation

Plants	**Extract by Maceration Process**			**Extract by Soxhelation Process**
Erythrina indica leaf *Calatropsis procera* root *Ficus religiosa* bark	Aqueous(F1)	Chloroform(F2)	Methanol(F3)	Methanol(F4)
	2%	2%	2%	2%
	4%	4%	4%	4%
	5%	5%	5%	5%

P.H.A.F.=F1-Poly Herbal Aqueous Formulation (2%,4% & 5% w/w)

P.H.C.F. =F2- Poly Herbal Chloroform Formulation (2%,4% & 5% w/w)

P.H.M.F.= F3- Poly Herbal Methanol Formulation (2%,4% & 5% w/w)

P.H.M.S.F.= F4- Poly Herbal Methanol Soxhalation Formulation (2%,4% & 5% w/w)

Table No.4: Zone of inhibition of diameter of different concentrated polyherbal formulation

Sr.No	**Polyherbal Formulation**	**Concentration**	**Inhibited Zone Diameter in mm. ±SEM**			
			Bacillus cereus	**Staphylocous aureus**	**Escherichia coli**	**Pseudomonas aeruginosa**
1	P.H.M.S.F. F1	2%	-	6	6	-
		4%	9	10	10	8
		5%	10	11	9	9
		Standard	18	19	18	20
		Control	-	-	-	-
2	P.H.M.S.F. F2	2%	-	-	-	-
		4%	6	7	-	6
		5%	8	9	7	8
		Standard	17	19	18	19
		Control	-	-	-	-
3	P.H.M.S.F. F3	2%	-	6	-	7
		4%	10	11	10	12
		5%	13	15	14	15
		Standard	18	19	19	20
		Control	-	-	-	-
4	P.H.M.S.F. F4	2%	7	-	8	7
		4%	15	12	16	16
		5%	19	16	20	19
		Standard	18	17	18	19
		Control	-	-	-	-

IZD- mean inhibition zone of diameter, n=3

Table No.5: Toxicity Study Result

Extracted Used	No. of Animal Used	Limit dose	Duration of effect	Mortality
Methanolic(Soxhalation)	12 Rats	2000mg/kg	48 hours	No

Table No.6: Topical application of ointments from extract of *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark on wound healing activity in rats. [% of wound healing = (1-wound area at the studied day/wound area at initial day) x 100] g

Group	Wound area in mm2 (Percentage of wound healing)						
	0 day	3rd day	6th day	9th day	12th day	15th day	18th day
Control I	511.2±1.12 (0.00)	482.79±1.56 (4.58)	412.32±0.79 (19.84)	302.83±0.13 (40.76)	251.64±0.15 (50.77)	135.92 ±1.78 (78.041)	83.24 ±0.57 (83.71)
Standard	513.10±0.00 (0.00)	438.56 ±1.13 (14.52)	305.08 ±1.07 (40.14)	202.93 ±0.62 (60.45)*	128.35 ±0.51 (74.98)*	40.16 ±0.82 (92.17)*	0±0.00 (100.00)*
P.H.M.S.F. F1	502.29±0 (0.00)	465.56±0.04(7.31)	432.56±1.62 (14.57)	308.68±0.28(39.03)	248.56±0.47 (50.91)	137.19±0.59 (72.90)	88.53±0.12 (82.51)
P.H.M.S.F. F2	506.85±0.15(0.00)	474.79±0.54 (6.23)	337.19±0.81(32.86)	250.84±0.22(50.06)*	145.13±1.31 (71.10)*	48.86±0.35(90.27)	4.13±0.06(98.17)*
P.H.M.S.F. F3	508.26±1.01(0.00)	451.59±0.83(11.14)	350.28±0.09(31.06)	225.35±0.59(55.66)*	130.09±0.72 (74.40)*	46.18±0.79(90.91)*	2.23±0.08(99.56)*

Values are expressed Mean±SEM of six readings; Significance evaluated by one-way analysis of variance (ANOVA) followed by Dunnett<'s t-test versus control group,*p<0.05, (n = 5). Values in parentheses indicate the percentage of wound healing

Table No.7: Effect of topical application of 5% methanol extract of *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark on Epithelialization Period Wound Healing

Sr. no.	Control I	Standard (1%Framycitin sulphate Ointment)	P.H.M.S.F1	P.H.M.S.F2	P.H.M.S.F3
1	21.20±0.58	15.80 ± 0.93	21.80±0.63	17.50±0.84	16.90±0.54

Values are the Mean±SD (n=5) ANOVA followed by Dunnet<'s t-test versus control group, p<0.05*.

Table No.8: Effect of topical application of Simple ointment base of 5% methanol extract of *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark on Breaking Strength of wound healing

Sr. no.	Control I	Standard(0.1% Framycitin Sulphate ointment)	2% P.H.O.	4% P.H.O.	5% P.H.O.
1	368.24±3.25	520.34 ± 3.32	340.13±1.20	508±1.84	511.20±2.54

Values are the Mean±SD (n=5) ANOVA followed by Dunnet<'s t-test versus control group, p<0.05*.

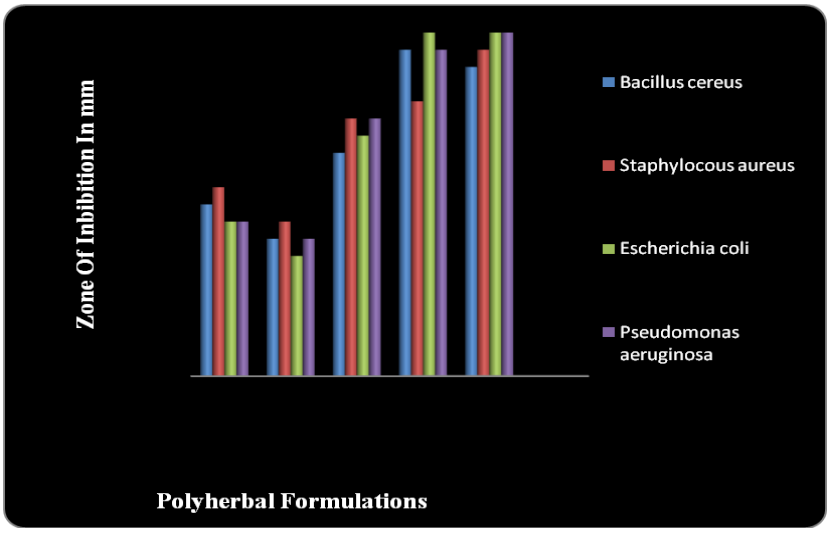


Fig. 1 Graphical Representation of Comparision of 5% Formulations F1 to F4 with standard.

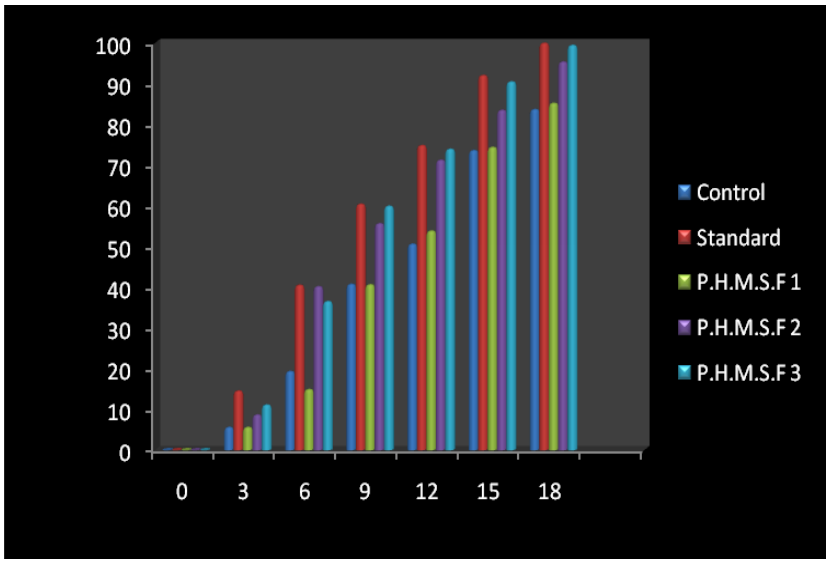
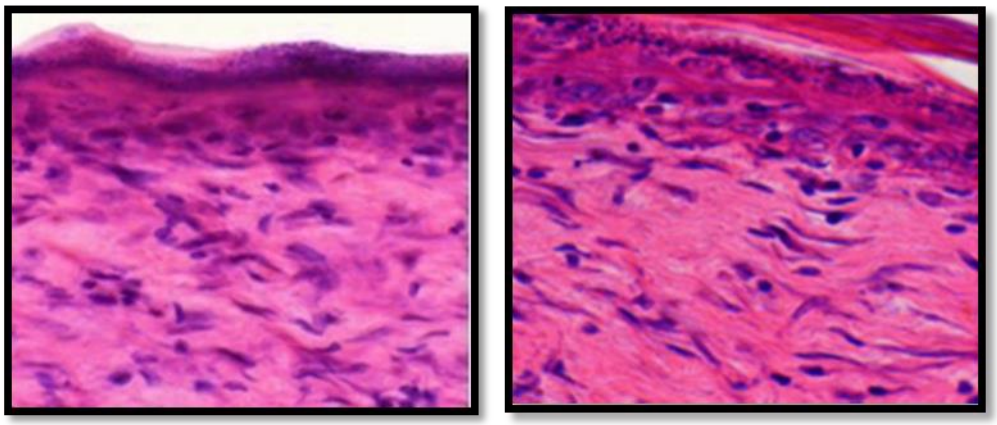
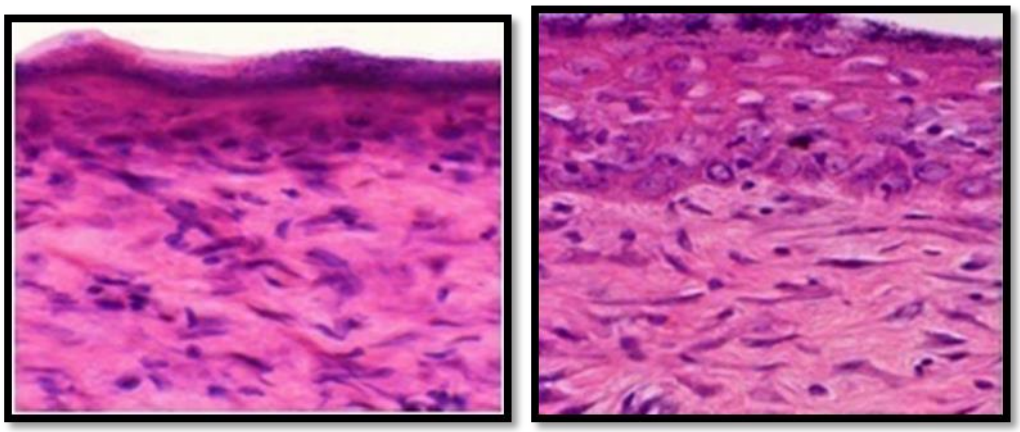


Fig.No. 2: The graphical represented of effect of topical application of 5% of methanol extract of P.H.M.S.F expressed as percent of wound contraction versus post wounding days.



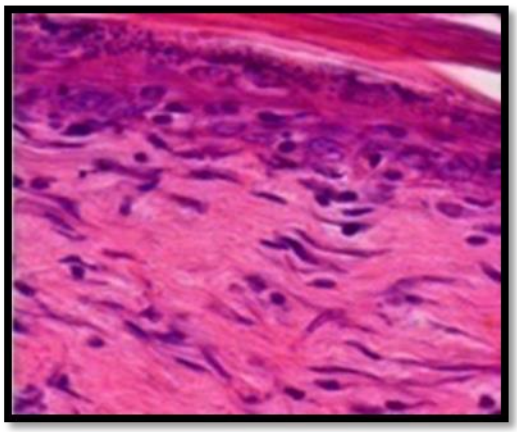
(A) **(B)**

Control (Simple Ointment) **Standard(0.1% Framycitin Sulphate Ointment)**



(C) **(D)**

Formulation I **Formulation II**



(E)
Formulation III

Fig No. 3: Histological view (100x) of Hematoxylin and Eosin (HE) stained skin section of rats treated with as on the 16th day (A)-Control (B) Standard (C)-Formulation I (2% P.H.M.S.F), (D) - Formulation II (4% P.H.M.S.F), (E) - - Formulation III (5% P.H.M.S.F)

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