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STUDY ON HYDROALCOHOLIC EXTRACT OF EUGENIA JUMBOLANUM LEAVES, PIPER NIGRUM FRUIT, ZINGIBER OFFICINALIS RHIZOME AS POLY HERBAL OINTMET AGAINST PSORIASIS

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ABSTRACT

The main object of the present study was to formulate and evaluate the poly herbal ointment of ***eugenia jumbolanum leaves, piper nigrum fruit and zingiber officinalis rhizome*** *against psoriasis. In the present study the physiochemical, phytochemical, formulation of poly herbal ointment and pharmacological activity were investigated successfully. The crude parts of a various plant were extracted with different solvents, based on polarity in that the ethanol extract was taken for further studies because of the presence of many secondary metabolites. The physiochemical and phytochemical screening study showed satisfactory result. All the formulations were showed satisfactory result in consistency, ph, melting point and viscosity .Among six formulation the formulation f6 shows good inhibition activity for psoriasis , may be due to increase in the concentration of base which increases the surface area and rate of penetration. The acute toxicity study of ethanol extract does not show any marked sign of toxicity and mortality up to 2000 mg/kg body weight. The* ***immunomodulatory activity*** *of ethanol extract of poly herbal has been reported for their immunosuppressive activity. Because the secondary metabolites balance the immune system and will help in the treatment of psoriasis. Anti psoriasis activity of poly herbal ethanolic extract showed increasing percentage of orthokeratotic regions.*

KEY WORDS: *Ethanol extract, poly herbal ointment, Anti psoriasis activity*

INTRODUCTION

Eugenia jambolana Lam. (Syn. Syzygium cumini Skeels or Syzynium jambolana Dc) belonging to the family Myrtaceae is a large evergreen tree up to 30 m high. Bark pale brown, slightly rough on old stems. Leaves are opposite, simple, entire, elliptic to broadly oblong. Flowers white 7.5-13 mm across in branched clusters at stem tips. Fruit are variable in size up to 2.5 cm long, ellipsoid or oblong, crowned with truncate calyx-limb, black with pink juicy pulp. According to Ayurveda, its bark is acrid, sweet, digestive, astringent to the bowels, anthelmintic and in good for sore throat, bronchitis, asthma, thirst, biliousness, dysentery, blood impurities and to cure ulcers [1,2] Piper nigrum (family Piperaceae)"The King of spices" among various spices. Black pepper is grown in many tropical regions like Brazil, Indonesia and India. Piper nigrum is commonly known as Kali Mirch in Urdu and Hindi, Pippali in Sanskrit, Milagu in Tamil and Peppercorn, White pepper, Green pepper, Black pepper, Madagascar pepper in English . Pepper is used worldwide in different types of sauces and dishes like meat dishes[3,4]. It contains major pungent alkaloid Piperine Piperine exhibits diverse pharmacological activities like antihypertensive and antiplatelets , antioxidant, antitumor, antiasthmatics , antipyretic, analgesic, anti-inflammatory, anti-diarrheal, antispasmodic, anxiolytic, antidepressants , Piperine also found to stimulate the pancreatic and intestinal enzymes which aid to digestion [5,6]. Many therapeutic activities of this spice are attributed to the presence of piperine apart from other chemical constituents. The fruits of Piper nigrum are used to produce white and green peppers. Piper nigrum is also used as a flavoring agent .

Zingiber officinalis Roscoe, commonly known as ginger belongs to family Zingiberaceae is cultivated commercially in India, China, South East Asia and West Indies .Ginger is extensively used around the world in foods as a spice. For centuries, it has been an important ingredient in Chinese, Ayurvedic and Tibb-Unani herbal medicines. Fresh ginger has been used for cold-induced disease, nausea, asthma, cough, colic, heart palpitation, swellings, dyspepsia, loss of appetite, and rheumatism [7]To dispel nausea, fresh ginger was mixed with a little honey, topped off with a nip of burnt peacock feathers

COLLECTION AND AUTHENTICATION

The plants specimen (*Wirghtia tinctoria, Hemidesmus indicus*) for the proposed study was collected in Chennai, Tamilnadu. It was identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Tambaram, Chennai. A vocher specimen No. PARC/2010/605, PARC/2011/882 has been deposited for further references.

Extraction of *Eugenia jumbolanum* leaves, *Piper nigrum* fruit, *Zingiber officinalis* rhizome:-

Plant materials was dried and powdered, the powdered material will be pulvarised and equal amount of the each samle (Eugeniajumbolanum leaves , Piper nigrum fruit, , Zingiber officinalis rhizome) was extracted with 70% ethanol(v/v)[8].The solvent was filtered and distilled off. Final traces of solvent were removed under vacuum. The process was repeated until the complete extraction of the solvent.

Physicochemical analysis: The physiochemical analysis such as ash value, extractive value, swelling index and loss on drying were carried out as per the procedure given in the standard books. The result was shown in Table 1

Phytochemical studies: The phytochemical screening such as alkaloids, flavanoids, tannins, tri terpenoids, steroids, glycosides, saponin etc were analysed as per the procedure given in the standards. The result was shown in Table 3.

FORMULATION OF OINTMENT

The required amount of hard paraffin and cetostearyl alcohol was melted on water bath. To this wool fat and white soft paraffin was incorporated, stirred until all the ingredients were melted. The mixture was stirred thoroughly until cold. The required quantity of ointment base was weighed and incorporated the appropriate medicaments in the different ratios; as per the requirements of the different formulations. Triturate the semi-solid medicaments with a small amount of the base; on an ointment slab, with the help of a stainless steel ointment spatula until a homogeneous product is formed [9]. The remaining quantities of the base were added until the appropriate medicaments and uniformly mixed with it.

EVALUATON OF OINTMENT[10]

Colour and Form of physical state: The ointment was observed visually for colour and physical appearance of the ointment. The results were shown in **Table 4**

pH: pH of ointment was measured using digital pH meter at room temperature. 10 %w/v diluted solution of the ointment was prepared to study its alkalinity or acidic nature of the formulated cream. The results were shown in **Table 4**

Consistency: Oinment should be smooth, no solid particles. The result was shown in Table 5

Melting point : A small amount of the ointment was heated gently and melting point was determined with the help of a thermometer. The result was shown in Table 5

Solubility: Solubility was tested in water and boiling water. Should be soluble in 9 parts of water and 17 parts of boiling water. The results were shown in Table 5.

Viscosity measurements of ointment formulation: Rheological behavior of the formulation was studied by keeping the ointment at room temperature (25°C), and at elevated temperature (45°C) in an oven for one month. The viscosities of the ointment are determined by using spindle no.7 at different r.p.m. (1, 5, 10, 50 and 100) with the aid of Brookfield viscometer. The results were shown in Table 6

Sensitivity: It was tested by patch test. The product was applied on 1 cm^2 patch of the skin of the mice, it was observed for its sensitivity.

Irritation test: It was carried out by applying product on the skin of mice for 10 minutes and the applied area was tested for its irritating and non irritating property.

Grittiness: A pinch of ointment was rubbed on skin of mice and it was observed with magnifying glass to observe any rashes or eruption present on the skin.

Spreadability: A pinch of product was applied on the skin of mice and it was checked for its easy spreadable nature on the skin after application. The result was shown in Table 7.

Table1. Composition of the ointment

S. No	F1	F2	F3	F4	F5	F6	F7	F8
Ehanol (70%v/v)extract	1.25g	1.25g	1.25g	1.25g	1.25g	1.25g	1.25 g	1.25 g
Wool fat	1.243g	1.237g	1.243g	1.237g	1.243g	1.247g	1.205g	1.218g
Cetosteryl alcohol	1.243g	1.237g	1.243g	1.237g	1.243g	1.237g	1.205g	1.218g
Hard paraffin	1.243g	1.237g	1.243g	1.237g	1.243g	1.202g	1.235g	1.218g
White soft paraffin	20.521g	21.039g	20.521g	21.039g	20.521g	22.039g	20.498g	20.709g

PHARMACOLOGICAL SCREENING

Experimental Animals: The acute toxicity study was carried out by using Swiss albino mice of either sex, weighing about 25–30g. This study was performed as per OECD-423 guidelines (Organization for economic co-operation and development). Animals were kept in a temp controlled environment (23 ± 2°C) at 12 hours light/dark cycle. All the protocols were performed in accordance with Institutional Animal Ethics Committee of C.L.Baid Metha College Of Pharmacy.

Experimental protocol was approved by the Institutional Animal ethics Committee IAEC. Ref.No: XII/CLBMCP/04/2000/CPCSEA/IAEC/22.2.11.

Acute toxicity study: In acute toxicity study, toxicity effect of drug can be evaluated and the LD_{50}, ED_{50} and the therapeutic index was determined for the drug under investigation.

Procedure Acute toxicity study procedure was carried out as per the guidelines by Organization for Economic Co-operation and Development (OECD) 423. In this experiment three groups of albino Swiss mice n=6 were used. In the study, the drug effect was evaluated in a single dose level. The animals were divided into 6 groups (n=6).

Group I (Control): Received 2% CMC (vehicle).

Group II: Received Combination of hydroalcoholic extract of *Eugenia jumbolanum, Piper nigrum, Zingiber officinalis* suspended in 2% CMC at a dose of 2000 mg/kg body weight orally.

The animals were observed continuously for one hour and observed during 24 hours after administering the test drug for any changes in general behavior like alertness, aggressiveness, grooming, gripping, touch response, tremors, respiration or other physiological activities like convulsion, lacrimation, writhing etc. were observed. At the end of the study the toxicological effect was assessed on the basis of mortality noted after 24 hours. The observed general behaviors were shown in Table 8.

Evaluation of immunomodulatory activity

Carrageenin induced pleurisy in mice for leucocyte migration assay:

Pleurisy is induced in rats by paw edema of 0.5 ml of 1% (w/v) suspension of carrageenin in sterile normal saline. Ethanol extract of *Eugenia jumbolanum, Piper nigrum, Zingiber officinalis* were administered at a dose 250 mg/kg and 500 mg/kg orally 1 hr before and 6 hr after the carrageenan injection. Blood sample

was collected 24 hr after carrageenan injection from each rat. Volume is measured and total and differential leucocyte counts were determined. Saline treated group serve as positive control. The results were shown in Table 9.

Anti-psoriatic activity

Mouse tail model for psoriasis : Male albino NMRI mice weighing 25-27g are used. The tails are treated locally with ointment of different formulations to the proximal part of the tail. For the contact time of 2 h a plastic cylinder is slipped over the tail and fixed with adhesive tape. At the end of contact time the cylinders were removed and the tail washed. Animals were treated once daily, 5 times in a week, for 2 weeks. Five to 8 animals are used per dosage group. Two hours after the last treatment the animal are sacrificed and the tails prepared histologically (fixation in 4% formalin, paraplastic embedding). Longitudinal sections of about 5µm thickness are prepared and stained hematoxylin-eosin. Level of orthokeratotic region was measured. The results were shown in Fig. 1.

Treatment regimen: Standard group: Retino- A 0.05% (Tretinoin cream U.S.P.)

Control group: Saline solution, Group 2: Ethanolic extracts (70%v/v) of *Eugenia jumbolanum* leaves, *Piper nigrum* fruit, *Zingiber officinalis* rhizome (1%), Group 3: Ethanolic extracts (70%v/v) of *Eugenia jumbolanum* leaves, *Piper nigrum* fruit, *Zingiber officinalis* rhizome (2%).

RESULTS AND DISCUSSION.

PHYTOCHEMICAL STUDY

Ash value, Extractive value and Loss on drying: As a part of phytochemical study, ash value, extractive value and loss on drying of the coarsely air dried powder of the *Eugenia jumbolanum* leaves, *Piper nigrum* fruit, *Zingiber officinalis* rhizome was carried out and the results were reported in Table 2. This data are helpful for identifying and ascertaining the quality of the collected crude drug.

Table 2: Physico-chemical standards for poly herbal

S. No	Parameters	*Poly herbal* (%w/w)
1.	**Ash Values**	
	Total ash	8±0.010
	Acid insoluble ash	2.2±0.034
	Water soluble ash	6.11±0.157
2.	**Extractive Values**	
	Alcohol soluble extractive value	15.24±0.407
	Water soluble extractive value	12.2±1.135
	Chloroform soluble extractive	11.11±0.224
3	**Loss on drying**	8.10±.030

The results were expressed in mean ± SD of three independent values.

Preliminary phytochemical screening: Phytochemical screening of ethanol extract of *Eugenia jumbolanum* leaves, *Piper nigrum* fruit, *Zingiber officinalis* rhizome showed the presence of flavonoids, steroids, tannins, alkaloids, saponins, glycosides, phenolic compounds, protein, amino acids and terpenoids. The result was shown in Table 3.

Table 3: Preliminary phytochemical screening of *Eugenia jumbolanum, Piper nigrum, Zingiber officinalis*

Extract/Fractions	Alkaloid	Carbohydrate	Glycoside	Protein	Amino acid	Saponin	flavonoid	Phenolic compound	tannin	Terpenoid	Steroid
Eugenia jumbolanum*, *Piper nigrum*, *Zingiber officinalis											
Ethanolic extract	+	+	+	+	+	+	+	+	+	+	+

Formulation and evaluation of ointment:
The ethanol extract of *Eugenia jumbolanum* leaves, *Piper nigrum* fruit, *Zingiber officinalis* rhizome were formulated in the form of ointment with different ratio for the evaluation of antisporiatic activity. The ointment was subjected to physicochemical and subjective evaluation. The results were shown in **Table 4-6.**

Table 4: Physicochemical evaluation

S. No	Formulation	Colour	Form of physical state	pH
1	F1	Brown	Viscous semisolid mass	6.8
2	F2	Brown	Viscous semisolid mass	7.2
3	F3	Dark brown	Viscous semisolid mass	6.9
4	F4	Dark brown	Viscous semisolid mass	7.0
5	F5	Orange red	Viscous semisolid mass	7.3
6	F6	Orange red	Viscous semisolid mass	6.7

Table 5: post evaluation study of ointment

S. No	Formulation	Consistency	Melting point (^{0}c)	Solubility
1	F1	Smooth, no solid particles	44	soluble in 9 parts of water and 17 parts of boiling water
2	F2	Smooth, no solid particles	38	soluble in 9 parts of water and 17 parts of boiling water,
3	F3	Smooth, no solid particles	40	soluble in 9 parts of water and 17 parts of boiling water
4	F4	Smooth, no solid particles	42	soluble in 9 parts of water and 17 parts of boiling water.
5	F5	Smooth, no solid particles	37	soluble in 9 parts of water and 17 parts of boiling water.
6	F6	Smooth, no solid particles	39	soluble in 9 parts of water and 17 parts of boiling water.

Table 6: Viscosity obtained for the formulated ointment (CPS)

Formulation	(Days)	0	2	7	22	14	30
F1	V1	28000	29000	31000	33000	30000	37866
	V2	27000	26000	25000	24000	24000	24000
F2	V1	29000	31000	27000	31000	29000	32000
	V2	27000	29000	24000	27000	25678	22456
F3	V1	28900	26000	28000	31000	28900	29000
	V2	24000	24678	24000	29000	27000	26000
F4	V1	33000	31000	27000	26000	33000	31678
	V2	29000	29000	25000	24678	24000	24000
F5	V1	32500	30000	29000	31000	32500	30000
	V2	30000	27900	27638	29000	27500	29000
F6	V1	29000	28000	33000	30000	30000	27900
	V2	27000	26000	32500	28000	29000	25000

V1-Temp at 25^{0}C
V2-Temp at 45^{0}C

Table 7: subjective evaluation of ointment

S. No	Formulation	Sensitivity	Irritation	Grittiness	Spreadability
1	F1	No	No	No	Easily spreadable
2	F2	No	No	No	Easily spreadable
3	F3	No	No	No	Easily spreadable
4	F4	No	No	No	Easily spreadable
5	F5	No	No	No	Easily spreadable
6	F6	No	No	No	Easily spreadable
	Report	**Pass**	**Pass**	**Pass**	**Pass**

The formulated ointment was evaluated by physical evaluation, viscosity, subjective evaluation and stability studies. The resulted ointment exhibited stable and good physical properties which can be used as the acceptable topical therapy for the treatment of psoriasis.

Pharmacological activity

Acute toxicity study: The ethanolic extracts separated from *Eugenia jumbolanum, Piper nigrum, Zingiber officinalis* respectively were screened for the acute toxicity and dose fixation. The extracts were given at the dose of 2000 mg/kg at different ratios.

Table 8 depicted that the tested extract does not show any marked sign of toxicity and mortality upto 2000 mg/kg body weight orally in mice for 24 hrs and was considered as safe for pharmacological activity.

Table 8: Acute toxicity study

Parameters	*Ethanolic extract of Eugenia jumbolanum Piper nigrum, Zingiber officinalis(* 2000mg/kg)
Aggressiveness	Absent
Alertness	Present
Convulsion	Absent
Pain response	Slow
Pinna reflex	Present
Pupils	Normal
Respiration	Normal
Restlessness	Present
Righting reflex	Absent
Salivation	Absent
Skin colour	Normal
Touch response	Present
Tremors	Absent
Urination	Normal
Writhing	Absent
Mortality	Absent
Tremors	Absent

Carrageenan induced pleurisy in mice for leucocyte migration assay

Effect of steroids, flavonoids, terpenoids on leucocyte migration by the Carrageenan induced pleurisy in mice. The results were shown in Table 9.

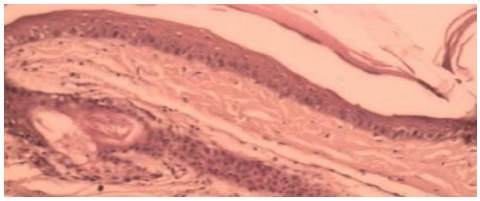
Table 9 : Carrageenan induced pleurisy in mice for leucocyte migration assay

S. No	Control	Ethanolic extract of *Eugenia jumbolanum Piper nigrum, Zingiber officinalis*	
		250 mg/kg	500 mg/kg
1	7600	8200	9200
2	7400	8100	8900
3	7100	8000	9200
4	7800	8100	9100
5	7800	8000	9200
6	7400	8100	9000
7	7516± 271.42	8083± 75.27**	9100± 126.49**

Flavonoids, terpenoids, steroids are the potent immunomodulators. Immune system have crucial role in the proliferative stages of psoriasis. So the immune modulator plays an immensive steps in the treatment of psoriasis. Terpenoids and steroids have been reported for their immunosuppressive activity. These compounds balance the immune system and will help in the treatment of psoriasis.

Anti psoriatic activity

Perry's scientific mouse tail model for psoriasis : *Ethanolic extract of Eugenia jumbolanum Ppiper nigrum, Zingiber officinalis* screened for their possible antipsoriatic activity using perry's scientific mouse tail model. Extract were applied topically in the form of ointment. Drug activity is defined by the increase in percentage of orthokeratotic regions (These are the regions in a cell having no nucleus and involved in protection from invaders like micro-organisms, UV rays, weak acids & bases). The results were shown in Fig. 1



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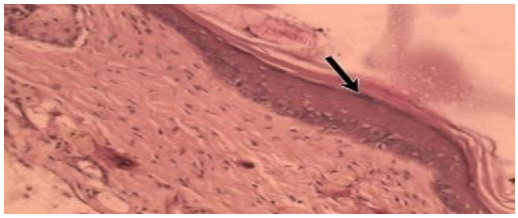
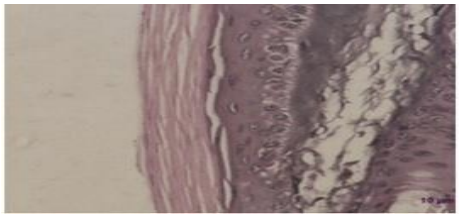
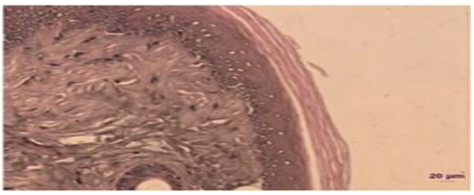


Standard





F6 formulation (Clearly visible orthokeratotic region)

Fig. 1: Longitudinal histological sections through the skin of mouse tails treated topically for 2 weeks, HE staining (original magnification 40×)

Granular layer of the epidermis is greatly reduced or absent in psoriatic lesions. Parakeratotic condition is seen in the adult mouse tail which is one of the hallmarks of psoriasis. Induction of orthokeratosis in the adult mouse tail is the basis behind the mouse tail test. Many drugs presently used in the treatment of psoriasis have been evaluated by the mouse tail test and were found to have shown good efficacies. Hence in the present study, we used the mouse tail test for evaluating the efficacy of herbal extracts and fractions. In the mouse tail test, formulation XII produced significant orthokeratosis when compared to control. Representative examples of the histological specimens underlying the histological section investigation were shown in **Fig. 1**

CONCLUSION

At present, psoriasis remains pathologies for which no complete cure is available. In the present study, formulation containing active constituents from different part of the herbs showed markable effect in anti-psoriatic study. Among six formulation the formulation F6 showed good result against psoriasis ,which may be due to increase in the concentration of base.which will increase the surface area and rate of penetration .On the basis of above observations, it may be concluded that the local application of this formulation as a valuable therapeutic approach to control psoriasis. However, further studies will be needed to elucidate the mechanism(s) involved in anti-psoriatic effect with references to phytoconstituents .

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