Research Article
CODEN (USA): IJPLCP

[Rajasree et al., 3(10): Oct., 2012]
ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Formulation and evaluation of antiseptic polyherbal ointment

P.H.Rajasree[2*], Vidya Vishwanad[1], Merin Cherian[1], Jincy Eldhose[1] and Ranjith Singh[2]

1, Department of Pharmaceutics, Amrita School of Pharmacy, Amrita Institute of Medical Science and Technology, Cochin (Kerala) - India

2, Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore, (TN) - India

Abstract

Herbal therapy and herbal drugs predominates in traditional medicine as well as in alternative medicine practiced in the developed world. Among the various indications where traditional herbal medicines are used, skin and skin related disorders is ranked top. Thus, the main objective of the present study is to formulate and evaluate a poly herbal ointment with antiseptic activity. Ointments were formulated using methanolic extracts (by continuous hot percolation-soxhletation) of *Azadirachta indica*, *Chromolaena odorata*,*Mimosa pudica*,*Samadera indica* and were evaluated for its physicochemical property, antibacterial and antioxidant activity. Ointments were prepared using different concentrations of the extracts such as 2%, 4%, 6% w/w by fusion method using emulsifying ointment as base. Formulations were then tested for its physicochemical properties like loss of drying, pH, spreadability, extrudability and diffusion study and gave satisfactory results. The prepared formulations were also stable at 4ºC, 25ºC and 37ºC. Further, polyherbal formulations were evaluated for its anti-bacterial activity against Staphylococcus aureus, Pseudomonas sp., Bacillus sp., by agar diffusion method by using Betadine (5%w/w) as the standard. All the formulations showed predominant activity against selected species. Formulations were also evaluated for anti-oxidant activity through reducing power assay, nitric oxide and hydrogen peroxide scavenging method. The results showed that the scavenging activity of the formulations increased with increase in concentration and this is due to the presence of flavanoids and tannins. The presence of both antibacterial and antioxidant activity reveals that the prepared ointment can also be used for wound healing. Hence an attempt was made to formulate a polyherbal ointment, and to evaluate for its physical parameter, in-vitro anti-oxidant activity and to compare its antibacterial activity with a marketed formulation (5% w/w Betadine).Overall result of this study reveals that this is an effective polyherbal antiseptic ointment.

Key-Words: *Azadirachta indica*, *Chromolaena odorata*, *Mimosa pudica*, *Samaera indica*, spreadability, extrudability

Introduction

Ayurvedic medicine is a time-tested system of medicine which has been in clinical use for centuries in India. Being a time-tested system, it has an edge over other existing systems of health management[1]. When two or more herbs are used in formulations, they are known as polyherbal formulations. Ayurveda and herbal medicine has roots in medicinal herbs and they have been practiced for centuries. Herbal medicine is making dramatic comeback and increasing number of patients are visiting alternative medicine clinics. Side effects of synthetic medicine are alarming and recent time has seen risk of herbal and herbal-synthetic drug interactions[2].

*** Corresponding Author**
E.mail:mailmerajasreeph@rediffmail.com,
ask4ph@rediffmail.com
Mob.: +91-8056754986

In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants, animal tissues, or microorganisms.The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants. To determine the potential and promote the use of herbal medicine, it is essential to intensify the study of medicinal plants that find place in folklore[3]. The herbal drugs are boon to our society.These herbal drugs are considered as a therapeutic weapon to fight against various diseases in birds humans and animals,without having any sideeffects Under the prevailing circumstances further

investigations into the concept of polyherbal formulations should be undertaken .so in the present work,we formulated a polyherbal ointment with better antimicrobial as well as anti-oxidant activity , can be used for skin infections .In recent years, there has been a great demand for plant derived products in developed countries. The literatures have reported that the usage of the traditional medicines brought a great benefit in skin related diseases. Hence the plant entities derived from the natural source need to be identified and formulated in to suitable dosage form for the management and treatement of various antimicrobial diseases. *Azadirachta indica* (Family-Meliaceae) known as Neem is well known for its medicinal properties. Its leaves possess broad spectrum of activity against Gram +ve and Gram –ve bacteria including M.tuberculosis, Vibrio cholera[4] .*mimosa pudica*(Family-Fabaceae) is known as touch me not plant possess anti-inflammatory,used in treating leprosy,leucoderma ,etc[5].Samadera indica(Family simurubaceae) is nipea bark tree having antifungal as well as anti microbial activities[6].Chromoleana odorata(Family-Asteracea) is Siam weed, and the methanolic extract of the plant have properties like anti bacterial, antifungal activity, anti oxidant activity and anti-inflammatory.The astringent properties of the leaf extracts of Chromolaena odorata on the blood vessel has made it a popular plant in the prevention of blood loss from wounds, also its anti-microbial properties has made it a popular choice in disinfecting and treating open wounds[7].

Hence, an effort has been made to establish the scientific validity to investigate the possible antimicrobial and antioxidant activity of the formulated ointments made from the methanolic extracts of the above four herbs.From this investigaton and the results ,this polyherbal ointment posses significant antimicrobial as well as anti-oxidant activity can be used for the treatement of burns,wounds,rashes etc.

Material and Methods[2-5]

Collection of plants

The plants *Azadiracta indica, Chromalaena odorata, Mimosa pudica, Samadera indica* were collected from Perumbavoor, Ernakulam,Kerala, India.

Chemicals and reagents

Emulsifying wax (I. P, Lobe Chem.), Liquid paraffin (I.P, Nice Chemicals), White soft paraffin (I.P, Lobe Chem.), Methanol, n-hexane, Sulphuric Acid, Drangendroff's reagent, Molisch's reagent, Acetone.

Equipments

Soxhlet apparatus, Incubator, Digital balance, Bunsen burner, pH meter, Glass wares, UV Spectrophotometer.

Media

Nutrient Agar and Muller Hinton Agar Media

Organism

Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus sp

Extraction

The collected plants *(Azadirachta indica, Mimosa pudica, Samadera indica, Chromolaena odorata)* were extracted by6 continuous hot percolation (soxhletation). 50g of powdered leaves of the above four plants were defatted using petroleum ether. The marc obtained from each of the powdered plant parts were successfully extracted separately with 250 ml of methanol by using soxhlet apparatus. The extraction was carried out for 24 hours. After extraction, the solvents were distilled out; the concentrated residues were analyzed by chemical tests.[8]

Phytochemical analysis

The methanolic extract obtained after soxhletation was subjected to various phytochemical screening as per the standard procedure to reveals the presence of various active phytoconstituents.[9]

Formulation of ointment

Working formula (emulsifying ointment base)

Emulsifying wax	- 300g
White soft paraffin	- 500g
Liquid paraffin	- 200g

Procedure

Required quantities of emulsifying wax, liquid paraffin and white soft paraffin were weighed and melted. To this, adequate quantities of methanolic extract of the mentioned four plants were added and stirred well until a homogeneous mass were obtained[10]. The compositions of different polyherbal ointment are listed in Table II.

Evaluation

Physicochemical parameters[11]

Preliminary evaluation of formulations at different concentrations was carried out as follows:

Colour and odour

Colour and odour was examined by visual examination.

Loss on drying

Loss on drying was determined by placing ointment in petridish on water bath and dried for 105^{O}C.

pH

The pH of various formulations was determined by using Digital pH meter. One gram of ointment was dissolved in 100 ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values were depicted in Table-III.

Spreadability

Spreadability is a term expressed to denote the extent of area to which the ointments readily spreads on application to skin or affected part. A special apparatus has been designed by9 Multimer to study the spreadability of formulations. The spread ability was expressed in terms of times in seconds taken by two slides to slip off from ointment and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, result the better spread ability. Spread ability was calculated by using the formula.

$$S = (M.L/T)$$

Where, S = Spreadability, M = Weight tied to upper slide, L = Length of glass slides and T = Time taken to separate the slides

Extrudability

A simple method was adopted for this study. The formulations were filled in the collapsible tubes after the ointments were set in the container. The extrudability of the different ointment formulations was determined in terms of weight in grams required to extrude a 0.5 cm of ribbon of ointment in 10 second.

Diffusion study

The diffusion study was carried out by preparing agar nutrient medium of any Concentration. It was poured into petridish. A hole bored at the centre and ointment was placed in it. The time taken for the ointment to get diffused was noted.

Stability studies

The stability studies were carried out for the prepared formulations at different temperature conditions (4° C, 25° C and 37° C) for 3 months.

Evaluation of antimicrobial activity[12]

Procedure

Microorganisms

Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus sp.

Standard used

5% w/w Betadine ointment.

Sample preparation

About 10mg of ointments (2%, 4%, 6% w/w) were weighed and dissolved in DMF (dimethyl formamide) and used for activity studies.

Preparation of medium and nutrient broth

Weighed about 0.4gm of nutrient broth and dissolved in 30ml of water. Then the broth was suspended in each of test tube.The MullerHintonagar medium was prepared which contain 9.7gm of MHA was suspended in 250ml of water. Then both the medium and broth were for sterilization.After sterilization, the nutrient broth was allowed to cool and then the organism were inoculated and incubated for 4hours.The MHA medium were poured in the petridish before cooling and allowed to solidify for about 3-4 hours.

Methodology

The bacterial culture was spread on the culture medium and a well was bored in the middle of the agar. Then different samples and standard solutions of 0.05ml was poured inside these wells and plates were incubated at 37oC overnight for observation. The presence of inhibition was noted and compared with the control.The susceptibility of the test organism to the tested plant extract was determined by observing the zone of inhibition around each well.

***In-vitro* antioxidant study**[13]

Reducing power assay method

Reagents

0.1%Ferric chloride, 1%Ferricyanide, Phosphate buffer6.6, Trichloro acetic acid.

Procedure

Preparation of standard ascorbic acid solution

Weighed accurately 50mg of ascorbic acid and made up to 50ml with methanol.

Preparation of sample solution

50mg of ointments (2%, 4%, 6% w/w) was dissolved in 50ml of methanol. From the above, different concentrations (0.5, 1, 2, 3, 4, 5ml) were pipetted out and made up to 10ml with methanol. Added 2.5ml of phosphate buffer 6.6 and 2.5ml of potassium ferricyanide to each of the test tubes and was incubated at 40oC for 20min. After incubation, 2.5ml of Tri chloro acetic acid was added and centrifuged the reaction mixture for 5min.To 2.5ml of this reaction mixture 0.5ml ferric chloride and 2.5ml water were added. The absorbance was measured using Double beam spectrophotometer at 700nm.

Preparation of control

10ml of methanol was taken. To it 2.5ml of potassium ferricyanide and 2.5ml of buffer 6.6 were added. The above reaction mixture was incubated at 40oC for 20min and centrifuged for 5min.To this add 0.5ml ferric chloride and 2.5ml of water.

Nitric oxide scavenging method

Reagents

Sodium nitroprusside, phosphate buffer solution, Griess reagent were procured. Fine chemicals and all the solvents used were of A R grade.

Procedure

Preparation of test sample

50mg of ointments was dissolved in 50ml of distilled methanol to obtain a solution of 1mg/ml. From this stock solution, different working dilution were prepared to get concentration of 100, 200, 300, 400, 500 µg/ml.

Methodology

Nitric Oxide was generated from sodium nitroprusside was measured by the Griess reagent. Sodium nitroprusside5mM in phosphate buffer of pH 7.4 saline was mixed with different concentrations of the ointments (100, 200,300,400,500 µg/ml) dissolved in water and incubated at 25 ºC for 150 minutes. At different time interval, sample (1.5 ml) of the incubated solution were removed and diluted with 1.5 ml Griess reagent (1%sulphanilamide, 2% H3PO4, and 0.1%naphthylethylenediamene dihydrochloride).

Evaluation

The absorbance was read at 564nm. Ascorbic acid was used as the standard. The difference in the absorbance between test and control of Nitric Oxide were calculated and expressed as percent scavenging of Nitric Oxide radical. Capability to scavenge the nitric oxide radical was calculated by using equation

Hydrogen peroxide scavenging activity

Solution of hydrogen peroxide (40mM) was prepared in phosphate buffer of the pH 7.4.The concentration of hydrogen peroxide was determined by absorption at 230nm using spectrophotometer.The ointments 1mg/ml in methanol were added to hydrogen peroxide solution (0.6ml, 40mM).The absorbance at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The % of the hydrogen peroxide scavenging by the samples and the standard compounds were calculated.

Results and Discussion

Literatures revealed that the selected four herbs *Azadirachta indica, Mimosa pudica, Chromolaena odorata, Samadera indica* have antioxidant and antibacterial activity. Hence an attempt was made to formulate a polyherbal ointment, and to evaluate for its physical parameter, in vitro antioxidant activity and to compare its antibacterial activity with a marketed formulation (5% w/w Betadine).

Extraction and the phytochemical screening was done using methanol as the solvent. Phytochemical screening confirmed the presence of various phytoconstituent like carbohydrate, glycosides, flavanoids and tannins.

In the present study, polyherbal ointments were prepared by fusion method using emulsifying ointment as the base. The formulations were then evaluated for their physical parameters, in -vitro antioxidant and compared with marketed 5%w/w Betadine ointment for its antibacterial activity. These physical parameters were within the acceptable range. The stability studies were carried out and inferred that the formulations showed no signs of instability. The antibacterial activity of prepared ointments were compared with 5%w/w Betadine ointment using selected species of microorganism such as Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus sp and it showed that formulations like F2 and F3 showed greater activity Staphylococcus aureus and Bacillus sp compared to 5% Betadine. So, antimicrobial study shows that the prepared ointments has better activity against Staphylococcus aureus and Bacillus sp compared to standard 5% Betadine ointment. Anti-oxidant activity inferred that the formulated ointments showed similar activity as that of standard ascorbic acid and hence revealed that this activity is due to the presence of flavanoids and tannins.

Hence the study concludes that an efficient antiseptic ointment with antimicrobial and antioxidant activities can be formulated from the methanolic plant extracts of *Azadirachta indica,Mimosa pudica, Chromolaena odorata, Samadera indica* which can also be used for wound healing and various skin infections.

Acknowledgement

The authors are thankful to Principal Dr. K. G. Revikumar, Amrita School of Pharmacy for providing the necessary facilities in the Colleges, Sincerely thanks to Mrs. Vidya Vishwanad, Assistant professor Department of Pharmacuetics, Amrita school of Pharmacy for her valuable support.We would also like to thank our colleagues, lab assistants and Ms. Litha Thomas (M.Pharm 2nd year Pharmaceutics) for hersupport.

References

1. BHUx: A Patent Polyherbal Formulation to Prevent Atherosclerosis.
2. www.ayurlifestyle.com.
3. Nihal Singh Verma, Sumeet Dwivedi, Debadash Panigrahi and S.K. Gupta (2011). Anti-bacterial activity of root bark of *Nyctanthes arbor-tristis* Linn., *International Journal of Drug Discovery and Herbal Researc*h ,1(2)April-June:61-62.
4. Abhijeet Pandey, Jui V. Jagtap, Aditi A. Patil, Richa N. Joshi and B. S. Kuchekar (2010). Formulation and evaluation of anti-bacterial and anti-fungal activity of a herbal ointment containing *Aloe-vera, Azadirachta indica and Curcuma longa*, *Journal of Chemical and Pharmaceutical Research,* 2(3):182-186.
5. Sadia Afreen Chowdhury et al. Cytotoxicity, Antimicrobial and antioxidant studies of the different plant parts of *Mimosa Pudica*, J. *Pharm. Sci.* 1(1&2): 80-84.
6. www.wikipedia.com.
7. www.wikipedia.com.

8. Kokate C.K. (1997). Practical Pharmacognosy, Vallabh Prakashan, Delhi, 4th Edition, 107 - 111.
9. Ramesh B. Parmar, Ashok H. Baria1, Shital D. Faldu1, H. M. Tank, Darshan H. Parekh (2009). Design and Evaluation of Poly-herbal Formulation in Semisolid Dosage Form for its Antibacterial Activity. *Journal of Pharmacy Research*, 2(6):1095-1097.
10. Prabhudutta Panda (2010). Formulation and evaluation of topical dosage form of *Pandanus fascicularis* Lamk and their wound healing activity, *Drug Invention Today*, 2(9):417-420.
11. Himal Paudel Chhetri, Nisha Shrestha Yogol, Jyoti Sherchan, Anupa K.C, S. Mansoor, Panna Thapa (2010). Formulation and evaluation of antimicrobial herbal ointment, Kathmandu University Journal of Science, Engineering and Technology, vol 6:102-107.
12. Arun K. Mishra, Amrita Mishra, Pronobesh Chattopadhyay (2010). Formulation and in-vitro evaluation of antioxidant activity of o/w sunscreen cream containing herbal oil as dispersed phase, *International Journal of Biomedical Research*, 1(5).

Table 1: composition of polyherbal ointments

Ingredients	F1 (2%)	F2 (4%)	F3 (6%)
Azadirachta indica methanolic extract	2gm	4gm	6gm
Chromolaena odorata methanolic extract	2gm	4gm	6gm
Mimosa pudica methanolic extract	2gm	4gm	6gm
Samadera indica methanolic extract	2gm	4gm	6gm
Emulsifying ointment	q.s to 100gm	q.s to 100gm	q.s to 100gm

Table II: Phytochemical screening of the methanolic extract of *Azadirachta indica, Chromolena odorata, Mimosa pudica, Samadera indica*

Constituents	Name of the test	Methanolic extract of *Azadirachta indica*	Methanolic extract of *MimosaPudica*	Methanolic extract of *Chromolaena oderata*	Methanolic extract of *Samadera indica*
Carbohydrates & Reducing sugars	Molisch's test	-	+	+	+
	Fehling's test	-	+	+	-
	Benedict's test	-	+	-	+
	Barfoed's test	-	-	-	-
Proteins	Millions test	-	-	-	-
	Biuret test	-	-	-	-
	Xanthoprotien test	-	-	-	-
	Legal's test	+	-	+	-
	Keller Killiani test	+	-	+	-

Glycosides	Borntrager's test	+	-	+	+
	Modified Borntrager's test	+	+	+	+
Flavanoids	Shinoda test	+	+	+	+
	Lead acetate	+	+	+	+
	Sodium Hydroxide	-	+	+	+
Tannins	5% $FeCl_3$	-	-	+	-
	Lead acetate soln	-	-	+	+
	Bromine water	-	-	+	+
	Dil: Iodine soln	-	-	+	+

Table III: Physicochemical evaluation of formulated formulations

Physicochemical parameters	F1 (2%)	F2 (4%)	F3 (6%)
Colour	Dark green	Dark green	Dark green
Odour	Characteristic	Characteristic	Characteristic
Loss of drying	37%w/w	40%w/w	41%w/w
pH	7.02	6.88	6.60
Spread ability(Seconds)	12	12	13
Extrudability	180g	180g	182g
Diffusion study (after 60 min)	0.7cm	0.8 cm	0.7cm
Storage (4ºC,24ºC,37ºC)	Stable	Stable	Stable

Table IV: Antimicrobial activity of formulated ointment

Ointments	Zone diameter in cm		
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Bacillus sp.*
F1(2%)	1.1	0.9	_
F2(4%)	1.3	1.1	2.5
F3(6%)	1.3	1.2	2.4
Standard	1.2	1.5	1.0

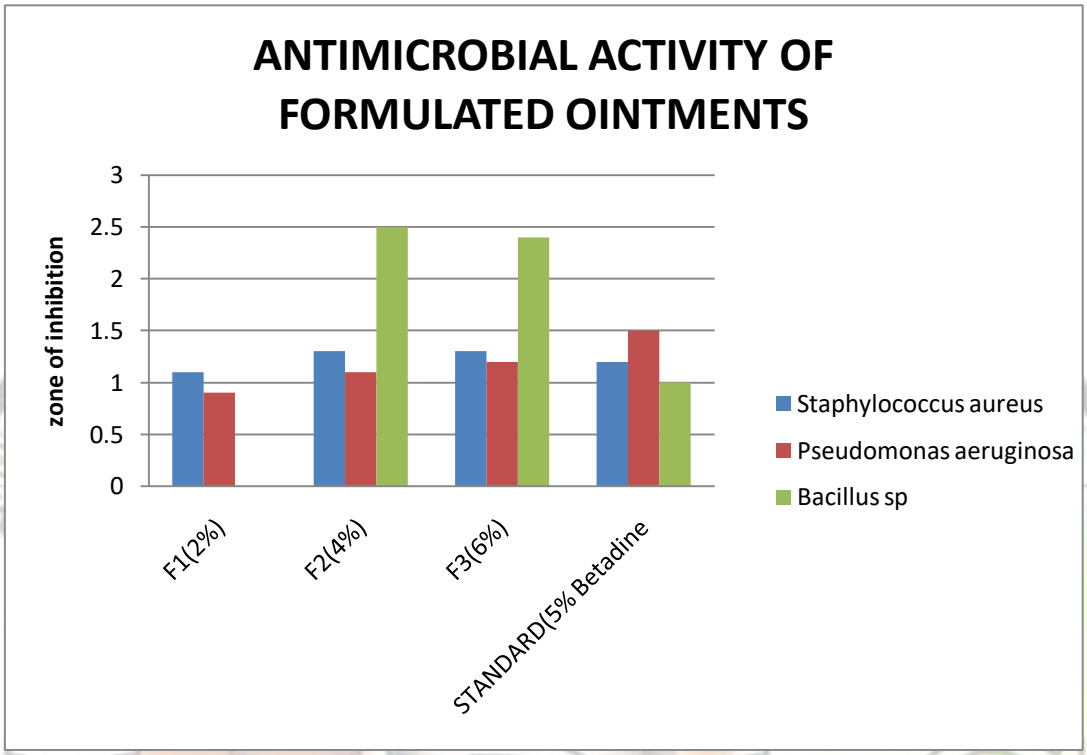

Fig. I: Antimicrobial activity of formulated ointments

Table V: Antioxidant activity of formulated ointments by reducing power assay

Concentration (µg/ml)	Absorbance			
	Standard	F1(2%)	F2(4%)	F3(6%)
50	0.346	0.213	0.156	0.222
100	0.369	0.260	0.210	0.253
200	0.412	0.276	0.313	0.392
300	0.578	0.346	0.510	0.472
400	0.687	0.410	0.682	0.593
500	0.786	0.511	0.703	0.639

Control=0.992

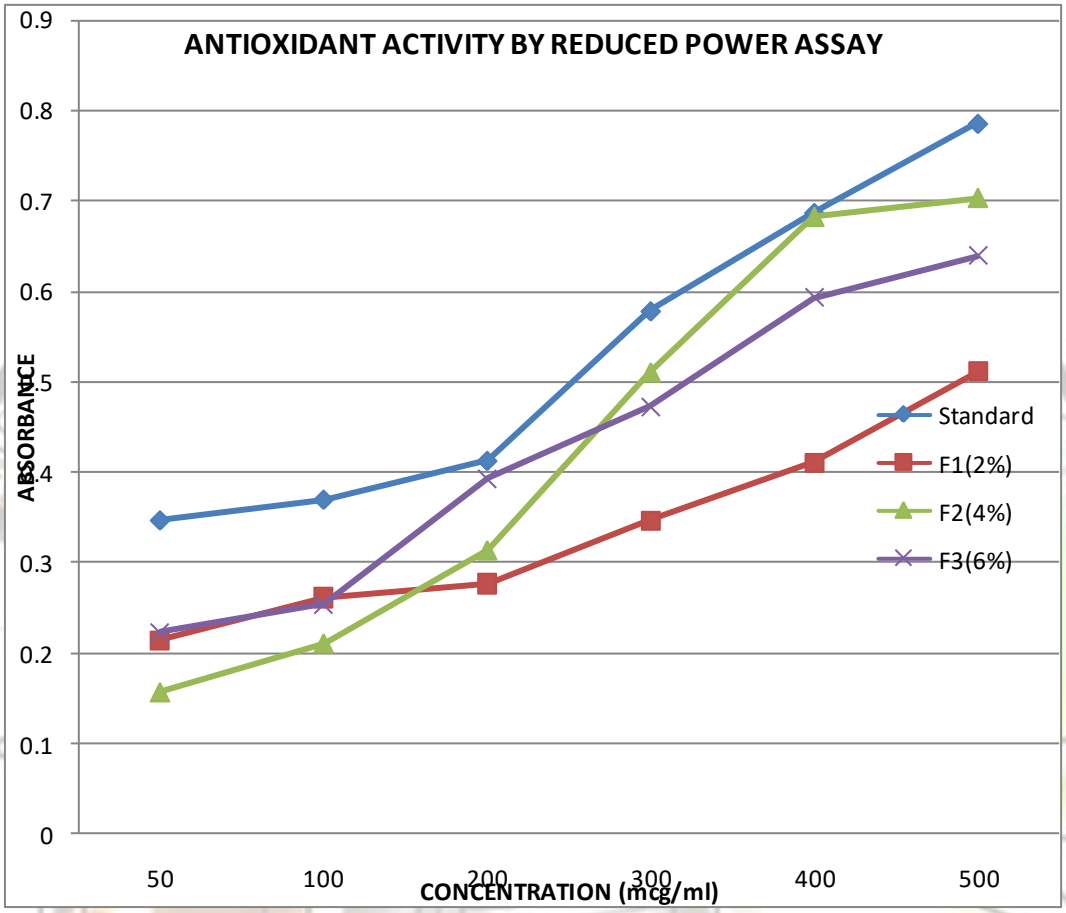

Fig. II: Graphical representation of antioxidant activity of formulated ointments by reducing power assay

Table VI: Antioxidant activity of formulated ointments by nitric oxide scavenging activity

Concentration (µg/ml)	Absorbance				% Inhibition			
	F1	F2	F3	STD	F1	F2	F3	STD
100	0.637	0.641	0.637	0.613	60.67	60.43	60.67	62.16
200	0.624	0.628	0.626	0.603	61.48	61.23	61.35	62.39
300	0.619	0.624	0.613	0.586	61.79	61.48	62.16	63.83
400	0.598	0.615	0.605	0.562	63.08	62.03	62.65	65.31
500	0.548	0.589	0.569	0.525	66.17	63.64	64.87	67.59

Control=1.62

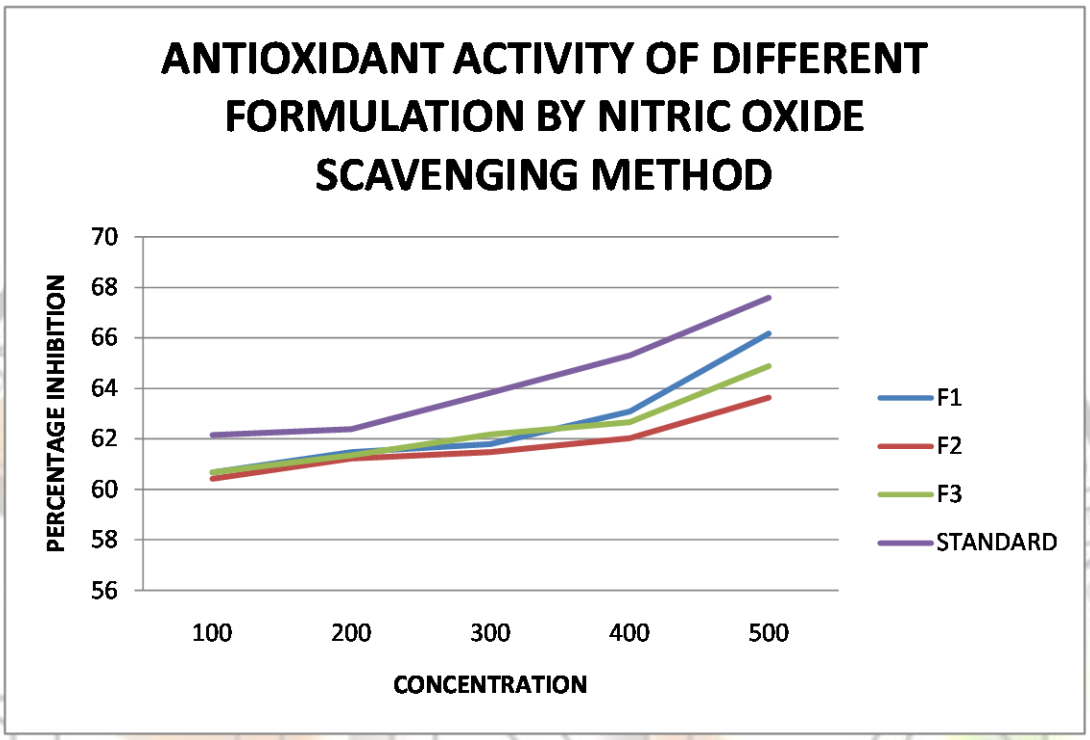

Fig. III: Graphical representation of antioxidant activity of formulated ointments by nitric oxide

Table VII: Antioxidant activity of formulated ointments by hydrogen peroxide scavenging activity

Concentration (µg/ml)	Absorbance				% Inhibition			
	F1	F2	F3	STD	F1	F2	F3	STD
100	1.179	0.610	0.515	0.495	45.00	71.5	76.00	76.95
200	0.770	0.456	0.451	0.394	64.13	78.76	78.99	81.64
300	0.551	0.427	0.441	0.295	74.33	80.11	79.45	86.25
400	0.455	0.369	0.399	0.195	78.80	82.81	81.41	90.91
500	0.372	0.366	0.376	0.134	82.67	82.95	82.48	93.75

Control=2.147

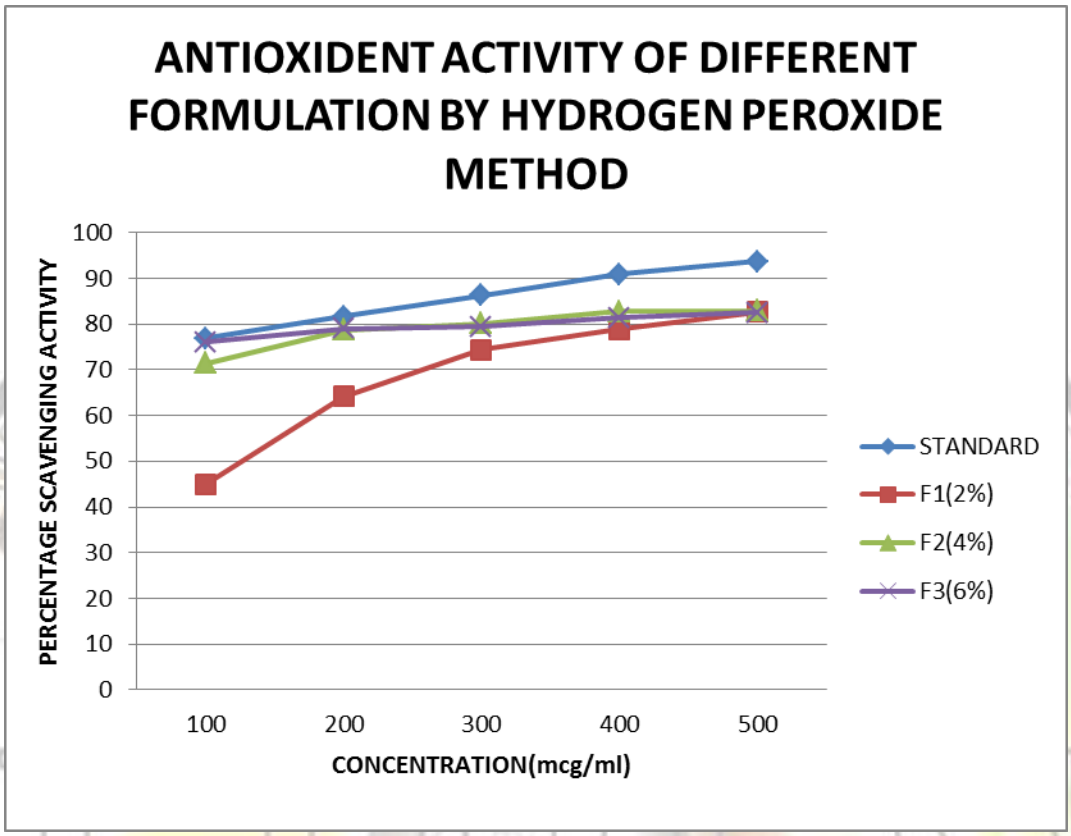

Fig. IV: Graphical representation of antioxidant activity of formulated ointments by hydrogen peroxide scavenging activity

Fig. V: Different concentrations of formulated polyherbal ointment

Azadiracta indica

Mimosa pudica

Chromolena odorata

Samadera indica bark

Fig. VI: Plants used in the formulation of the polyherbal ointment

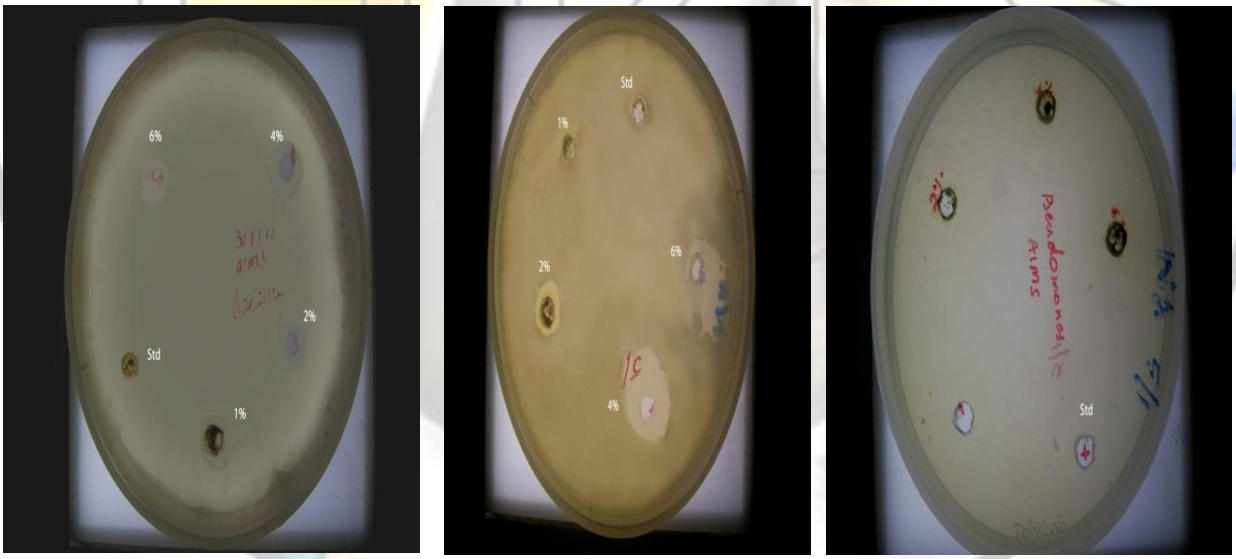

Fig. VII: Antimicrobial activity of formulated ointments