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FORMULATION AND EVALUATION OF POLYHERBAL ANTIBACTERIAL OINTMENT

Debajyoti Bhattacharya[1], Anup Kumar Roy, Ruchi Shakya, Telagu rajesh, Subhasish Mondal[2]

[1]*Department of Industrial Pharmacy, Acharya & B.M. Reddy college of Pharmacy, Bangalore.*

[2] *Division of Medicinal Biochemistry, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India.*

Corresponding author, Mailing address: Department of Industrial Pharmacy, Acharya & B.M. Reddy college of Pharmacy, Bangalore, E-mail: royanupkr1960@gmail.com

ABSTRACT

The aim of the present study is to formulate and evaluate poly herbal antibacterial ointment using hexane, ethyl acetate and methanolic (80%) extract prepared from both plant and marketed herbal oils (Turmeric, anise oil, camphor oil, coconut oil, neem oil)used in conventional folk medicine in India. Nine formulations of poly herbal antibacterial ointment were formulated by emulsification method and physicochemical properties of them were evaluated. After that the formulations were compared with both synthetic and herbal marketed antibiotics in their antibacterial activities by calculating their zone of inhibition against both gram positive and gram negative bacterial strains by well diffusion method. F5 and F7 had shown significant antibacterial activity than other formulations with compared to the marketed formulations. Stability studies had been done of the formulations according to the ICH guide lines and the viscosity, spreadabiliy and pH were checked and found that there were no or little change in those properties.

Keywords: - Successive extraction, Emulsification, Zone of inhibition, ICH.

1. Introduction

Herbal medicine is a triumph of popular therapeutic diversity. Plants have been used as medicine from time immemorial because they have fitted the immediate personal need are easily accessible and in expensive. As per world health organization report, 21000 plants are used for medicinal proposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is a largest producer of medicinal herbs and its called as a botanical garden of world. Indian material medica includes 2000 natural products of therapeutic importance of which 400 are mineral and animal origin and rest are vegetable origin. They are

approximately 1250 medicinal plants which are used in formulating therapeutic preparation[3]. The use of herbal medicine rises due to the failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has lead to the screening of several medicinal plants for their potential microbial activity Worldwide infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. The negative health trends calls for a renewed interest in the infectious disease in the medical and public health communities and renewed strategies in treatment and prevention[7]. While we live in harmony with most bacteria, and indeed rely upon many bacteria for their beneficial properties, certain pathogenic bacteria do give rise to serious, often deadly, diseases. Since the advent of the antibiotic era in the early 1940s with the clinical use of penicillin, an ever-growing arsenal of antibiotics has provided an effective therapy against major bacterial pathogens. The Ayurvedic approach to the prevention and treatment of microbial infection recognizes the emergency use of modern drugs, but recommends traditional herbal combinations and extracts known to balance the individual and improve health, as well as herbs that help to combat or prevent microbial infections[8]. Bacterial infection is the invasion of the body by pathogenic micro-organisms and disease occurs. Transmission of the infection can be done by contact, inhalation, ingestion, inoculation and congenital process. The lodgments and multiplication of a parasite in or on the tissues of a host constitute infection. It does not invariably result in disease. Infact, disease is nothing but a rare consequence of infection which is a common natural event. Bacterial disease can be classified as communicable diseases, contagious diseases and non communicable disease .Bacterial diseases of the skin are usually transmitted by the contact with an infected individual, insects, soil and water[8].Although the skin normally provides a barrier to infections develop. Antibacterial activity.

2. Method

Collection of Plants and oils

The plant material was collected from the Department of Horticulture, University of agricultural science, Bangaluru. The oils of the different plants were collected from respective companies.

FORMULATION OF OINTMENT

Poly herbal antibacterial ointment was prepared by the emulsification method. In this method the emulsifying wax and paraffin oil were melted together on a water bath at a temperature of 70˚C and the extracts along with other excepients were heated almost at the same temperature as that of the melted base. The solution was slowly added to the melted base with continuous stirring until product was cooled down and a semisolid mass was prepared known as ointment.

FORMULATION CHART

Table 1: Extracts present in the formulations

Extracts	F1	F2	F3	F4	F5	F6	F7	F8	F9
Turmeric	T/H	T/EA	T/M	T/H,T/EA	T/EA,T/M	T/H,T/M	T/H	T/EA	T/M
Camphor	Ca/H	Ca/EA	Ca/M	Ca/M	Ca/EA, Ca/M	Ca/H, Ca/M	Ca/H	Ca/EA	Ca/M
Anise	A/H	A/Ea	A/M	A/H, A/Ea	A/Ea, A/M	A/H,A/M	A/H	A/Ea	A/M
Neem	N/H	N/Ea		N/H,N/Ea	N/Ea,N/M	N/H,N/M	N/M	N/EA	N/EA
Coconut	-	-	Co/M	-	Co/M	Co/M	-	Co/M	-

Table 2 :Formulation chart

Extracts	F1 %	F2 %	F3 %	F4 %	F5 %	F6 %	F7 %	F8 %	F9 %
Turmeric	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Camphor	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Anise	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Neem	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Coconut	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Emulsifying	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Liquid paraffin	Qs	Qs	Qs	Qs	Qs	qs	Qs	qs	Qs
Glycerine	3	3	3	3	3	3	3	3	3
Rose oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methyl paraben	3	3	3	3	3	3	3	3	3
Propyl paraben	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75

* All weights are in %w/w

EVALUATION OF OINTMENT

ACID VALUE

5 g of the cream was accurately weighed and dissolved in 50 ml of a mixture of equal volumes of ethanol (95%) and ether, previously neutralized with 0.1 M potassium hydroxide using phenolphthalein solution as indicator. 1 ml of phenolphthalein solution was then added and titrated with 0.1 M potassium hydroxide until the solution remains faintly pink after shaking for 30 s. The acid value was calculated from the expression.

Acid value = 5.61 n/w

Where, n = the volume (ml) of 0.1 M potassium hydroxide required;

w = the weight in gram of the substance.

SAPONIFICATION VALUE

2 g of ointment was accurately weighed and introduced into a 200 ml borosilicate glass flask fitted with a reflux condenser. 25 ml of 0.5 M ethanolic potassium hydroxide was added with a little pumice powder and boiled under reflux on a water-bath for 30 min. 1 ml of phenolphthalein solution was then added and titrated immediately with 0.5 M hydrochloric acid (a ml). The operation was repeated by omitting the substance being examined (b ml). Saponification value was determined by using the formula:

Saponification value =28.05 (b-a)/w, Where, w = weight of the substance in gram.

VISCOSITY

Viscosity of the different formulations was determined using Brookefield viscometer with spindle TF-96 at 60 rpm at temperature 37±0.5 °C.

SPREADABILITY

Spreadability was done by using slide over slide method. In this method required quantity of cream formulation was applied to one slide and to the other slide particular weight was tied. The time taken to slip off the slide from one slide was noted. The spreadability coefficient was calculated by using the formula, S=ML/t

where, M = weight tied to the slide

L = length of the slide t = time

pH

1 g of ointment mixed with 9 ml of water and pH of the resulting mixture taken with a pH meter.

CONSISTENCY

The consistency was determined by dropping a cone attached to a holding rod from a fixed distance of 10 cm in such away that it should fall on the centre of the glass cup filled with the ointment.

HOMOGENICITY

All the developed formulations were checked for homogencity for its appearance and presence of gritty particles or aggregate by visual inspection. It was done by agar well diffusion method.

Table 3: COMPARISON IN ANTI BACTERIAL ACTIVITY BETWEEN THE FORMULATIONS AND THE STANDARD ANTIBIOTIC, MARKETED HERBAL OINTMENT

SL NO	Gram positive	Gram negative	Standard (Synthetic)	Standard (Herbal)
1	*Bacillus subtilis*	*Escherichia coli*	Streptomycin	Btex ointment
2	*Staphylococcus aureus*	*Klebsiella pneumoniae*	Gentamycin	Btex Ointment

Table no : Bacterial pathogens used for Comparison of antibacterial activity of the formulations and the marketed antibiotic(Synthetic and Herbal)

3.Method

Nutrient agar was swabbed (Sterile cotton swabs) with 8 hour old broth culture with respective bacteria.Wells (10mm diameter and 2 cm a part) were made with cork borers. Formulations and standard antibiotics were placed in the wells and allowed to diffuse at room temperature for 2 hours.

The diameter of the inhibition zone was measured and activity was compared with standard antibiotics with respect to their zone of inhibition

STABILITY STUDIES

Stability testing of drug products begins as a part of drug discovery and ends with the commercial product. To assess the drug and formulation stability, stability studies were done. The stability studies were carried out for the most satisfactory formulation. The most satisfactory formulation was sealed in a polyethylene container and kept at 30 ± 2 ℃ and at RH 65% ± 5% in thermo lab humidity chamber(GINKYA IM3500series)for 60 days. At the end of 2 months, the sample was analysed for the pH, Viscosity and spreadability.

4. Result and Discussion

Table4: EVALUATION OF OINTMENT

Formulation Code	pH±SD	Viscosity	Spreadability (gcm/sec)	Consistency	Acid value	Saponification value	Homogenicity
F1	6.73±0.05	6466	12.5	10	5.61	42.07	Good
F2	6.9±0.05	5711	9.37	12	6.17	56.1	Good
F3	6.86±0.02	6842	12.5	12	7.01	49.08	Good
F4	6.94±0.01	5612	12.5	10	5.61	70.1	Good
F5	6.87±0.02	6472	7.5	12	6.73	84.15	Good
F6	6.9±0.05	5912	12.5	9	5.89	56.1	Good
F7	6.97±0.01	5730	9.37	10	6.73	42.07	Good
F8	7.01±0.05	6127	7.5	12	6.17	56.1	Good
F9	6.94±0.02	6520	12.5	10	5.09	63.11	Good

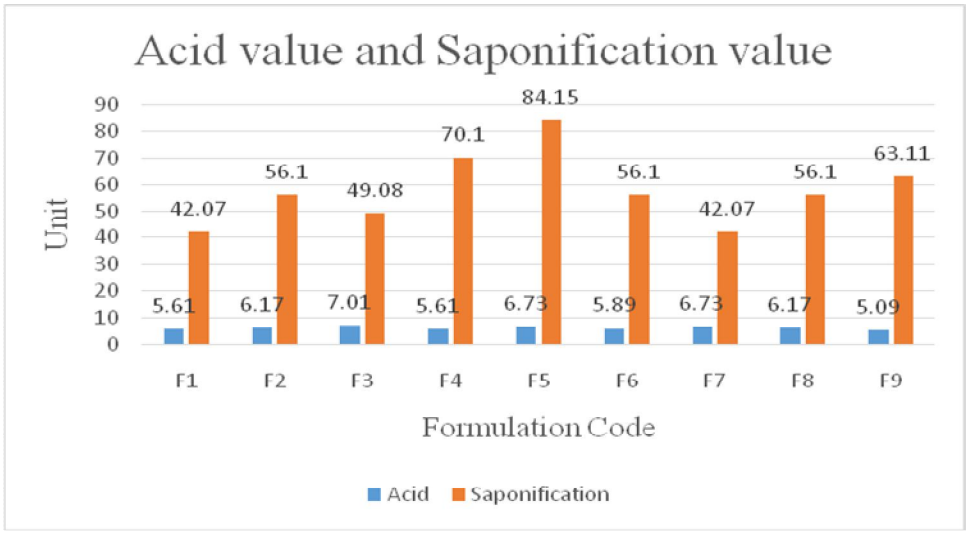

Fig1: Graph of acid value and saponification value of the formulations

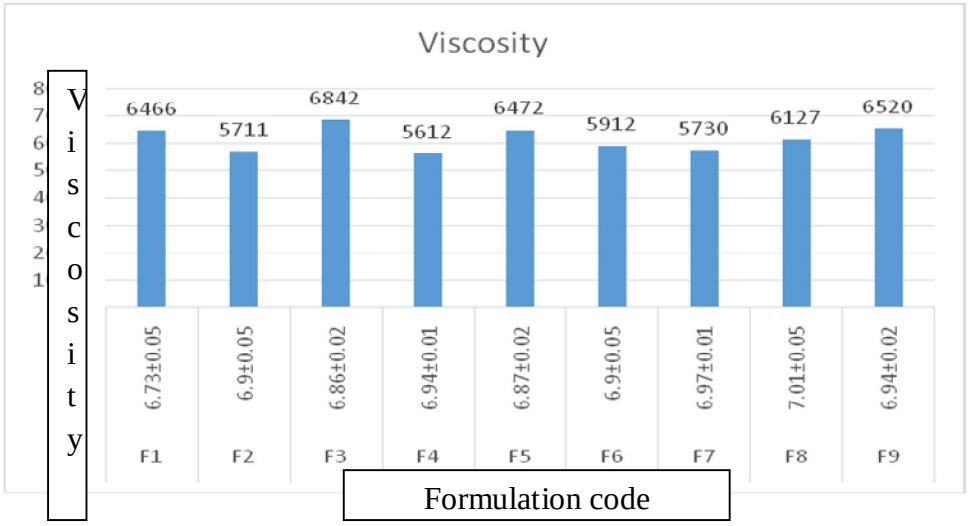

Fig2: Graph of viscosity of the Formulations

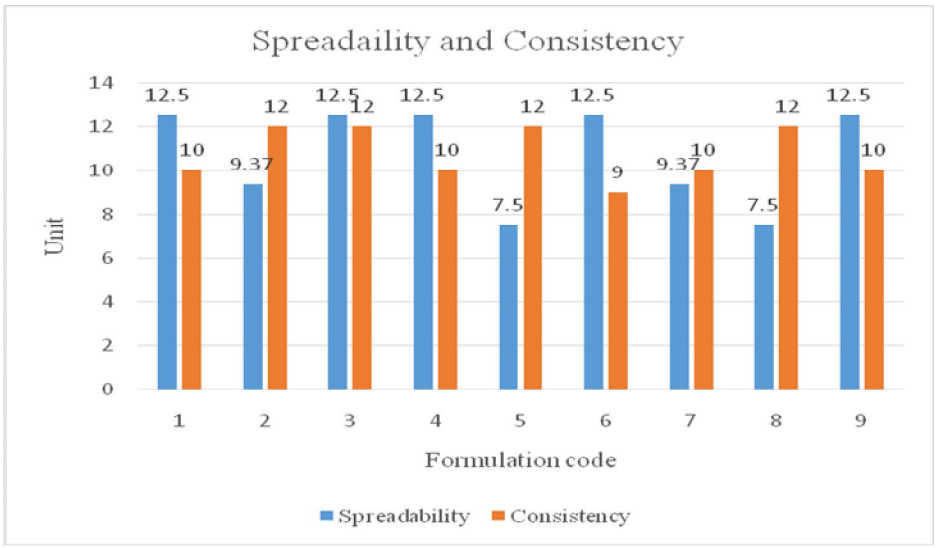

Fig 3: COMPARISON BETWEEN FORMULATED OINTMENT AND MARKETED SYNTHETIC ANTIBIOTICS AND HERBAL OINTMENT:

Table 5: Comparison of antibacterial activity between formulated ointment and the synthetic antibiotic

Formulation code	*E.coli*	*B.subtillis*	*Klabesella*	*S.aureus*
F1	3	2	3	3
F2	1	1	3.8	2.7
F3	0.5	1	3.2	2.5
F4	1	0.8	2	2.5
F5	2	0.5	3.7	3.5
F6	1	3	2	3
F7	6	3.2	3.7	3.7
F8	2.5	3	2.5	2.5
F9	4	2.5	3	2.7
Streptomycin	4.2	4.5	-	-
Gentamycin	-	-	6	7.5

Comparison of antibacterial activity of the formulation and marketed synthetic antibiotic

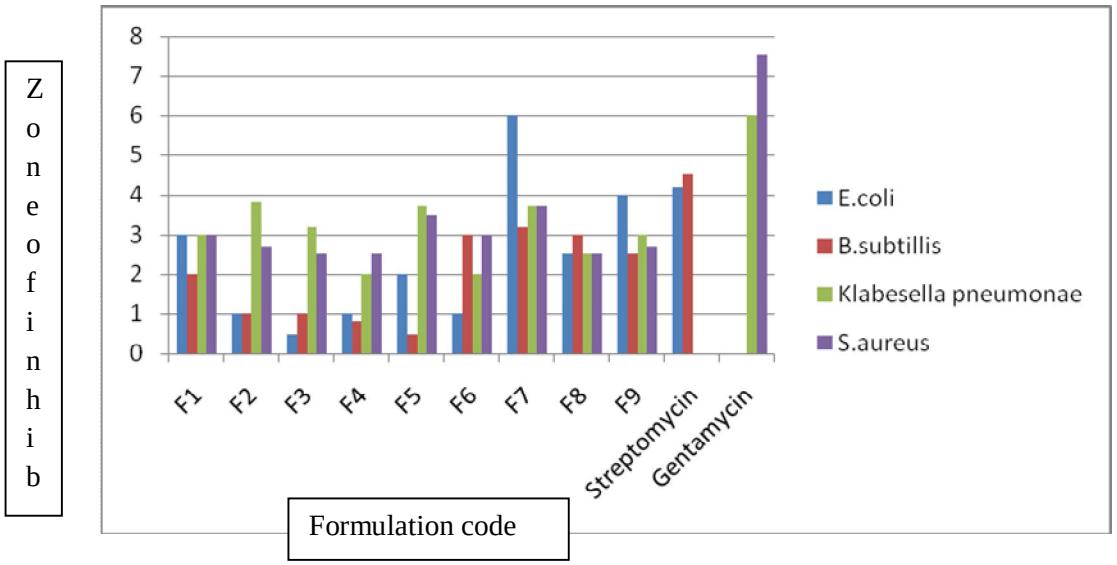

Fig 4: Graph of comparative antibacterial activity of the formulations and the standard marketed synthetic antibiotic

Table6:Comparison of antibacterial activity between formulated ointment and the herbal antibiotic ointment

FORMULATION CODE	*E.Coli*	*B.subtillis*	*K.pneumonae*	*S.aureus*
F1	2	2.6	2	2
F2	2.7	3.5	2.5	2
F3	3	3.4	2.4	2.5
F4	2.7	2.1	1.4	2.3
F5	2	2.4	1.7	1.8
F6	2.5	1.8	3.5	2.6
F7	4.5	3.1	3.4	2.6
F8	2	2	3	2
F9	2.5	1.8	1.4	2.4
Btex ointment	3.9	2.9	3.1	3.9

Comparison of antibacterial activity of the formulation and the marketed herbal antibiotic ointment

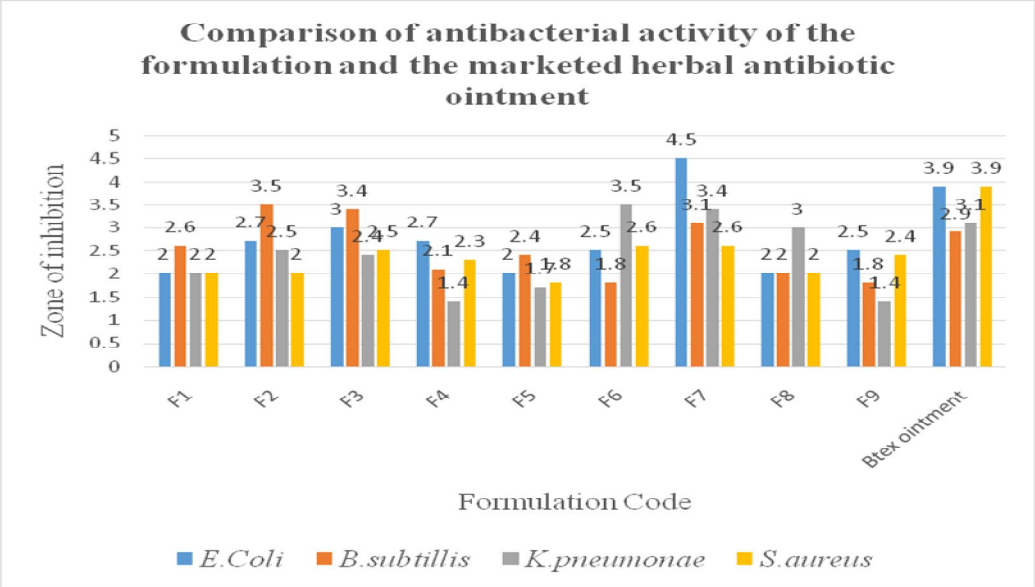

Fig 5:Graph of comparative antibacterial activity the formulations and the marketed herbal antibiotic ointment

Table 7: Stability studies

Parameters	At 0 day	At 30 days	At 60 days
pH±SD	6.97±0.01	6.8±0.02	6.97±0.01
Viscosity At 60 rpm (cps)	5730	5711	5713
Spreadability(gcm/sec)	9.375	9.12	9.06

Stability studies of the F7 formulation

5.Conclusion

In the present study an attempt was made to prepare a poly herbal antibacterial ointment by incorporating the extracts obtained from the rhizomes of Curcuma longa and the oils of *Pimpinella anesum*, *Cocos nucifera*, *Cinnamommum camphora* and *Azadirachta indica.* Collection and authentication of crude drug and crude oil was done. Extraction of each drug was done by using hexane, ethyl acetate and methanol(80%) successively. Formulation of poly herbal antibacterial ointment was

prepared by varying the extract while the concentration of the other constituents were constant by emulsification method. Evaluation of the physicochemical properties of each formulation was done. Comparison of antibacterial activity was done between formulations and marketed formulations (synthetic and herbal). The most satisfactory formulation-F7 had showed no significant change in physicochemical properties after stability studies at 4 ± 2°C and at R.T. for 2 months. Thus, the objective of the present work of formulating and evaluating poly herbal anti bacterial ointment had been achieved with success.

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