



# FORMULATION AND EVALUATION OF HERBAL TOPICAL DRUG DELIVERY

**Shital Jadhav, Poonam Papule, Shrdha Phatak**

## ABSTRACT

*Herbal therapy and herbal drug predominates in traditional medicine practiced in developed world plant derived substances and herbal medicines have recently attracted the great interest towards their versatile application. The main objective the present study is to formulate and evaluate herbal ointment with antimicrobial activity by using Azadirachta Indica and Bombax Ceiba polymer. The ointment base was prepared and formulation of ointment was done by incorporation of the polymer in the base by levigation method. Ointments were prepared by using different concentration of the polymer such as using 1% Azadirachta Indica, 1% Bombax Ceiba and their combination (0.5% Azadirachta indica and 0.5% Bombax ceiba) w/w. After completion of formulation it was evaluated for its Physicochemical parameter like colour, odour, pH, Homogeneity, solubility, Consistency, washability, Spreadability, viscosity, extrudability, skin irritation test, diffusion study. The herbal formulations were evaluated for its Antimicrobial activity against Staphylococcus aureus and prepared formulations were also stable at 5°C and Room temperature. Overall result of this study reveals that this is an effective herbal ointment.*

**KEYWORDS:** Azadirachta Indica, Bombax Ceiba, Staphylococcus aureus

## INTRODUCTION <sup>1, 2,3,41</sup>

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 interaction. Traditional medicines if used judiciously can save a lot of time spent in the treatment and thus reducing global burden. Uses of plants and traditional practices will continue to play a significant role in the socio-cultural life of village communities. The herbal drugs and Excipients have more precise action and have no side effects and are economic. Herbal medicine refers to the use of any plants seeds, berries, roots, leaves, bark or flowers for medicinal purposes. Herbal medicine, also called botanical medicine or phyto-medicine, refers to the use of any plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes. Long practiced outside of conventional medicine, herbalism is becoming more main stream as up-to-date analysis and research show their value in the treatment and prevention of disease. Plants had been used for medicinal purposes long before recorded history.

Natural remedies are more acceptable in the belief that they are safer with fewer side effect that the synthetic one. Herbal formulations have growing demand in the world Market. Recently World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary healthcare. Whole herbs contain many ingredients, and it is likely that they work to produce the desired medical effect. In earlier study, medicinal plants have been reported to be very beneficial in wound care, promoting the rate of wound healing with pain, discomfort, and scarring to patient.

## MATERIALS AND METHODS

### Chemicals and reagent

Paraffin Wax (Molychem Mumbai), Cetostearyl Alcohol (SDFCL Mumbai), Lanolin (Oxford Laboratory), Paraffin soft yellow (Dipa chemicals Chhikalthana), Molish Reagent (Oxford Laboratory, Palghar), Ethanol ( Molychem Mumbai), Acetone( Molychem Mumbai), Azadirachta Indica (Cidco New Nanded), Bombax Ceiba (Usmannagar Nanded).



### Equipments

Digital pH-Meter, Digital Balance, Brookfield viscometer UV-Visible Spectrophotometer, Autoclave, Incubator, Hot air oven, Digital colony counter, Centrifugation machine, Sonicator, DSC, FTIR

### Collection of Plant:

The leaves of Neem plant were collected from cidco New Nanded (Maharashtra). And the Bark of Bombax Ceiba Were collected from the local areas of Usmannagar, Dist. Nanded (Maharashtra)

### Preparation of Neem Extract<sup>18</sup>

The leaves were dried at 40-45°C under shade for 5 days. After drying, the leaves were ground into powder by using grinder. Then take 50 g of dried leaf powder were electronically weighed into 250 ml of conical flask. To this 250 ml of methanol was added and kept for 24 hr with periodic shaking. Filtered and filtrate was collected. The procedure was repeated three times. The collected filtrate was collected.

### Preparation of Bombax Ceiba Extract<sup>20</sup>

The bark was collected and peeled off from branches and stem and wash it carefully to remove the foreign particles then cut into small piece with the help of sharp knife. Then wash with water and the small piece of Bark were soaked in cold water, then kept it overnight and crushed with the help of mortar and pestle. Then the sample is centrifugated for 10 min, after centrifugation the particle not settled down because it was clear solution. Then the sample was precipitated with acetone. The precipitated were separated by using muslin cloth then it was dried in a hot air oven at 50°C. After drying the sample crushed with the help of mortar and pestle then the powder obtained and it was passed through the sieve no.22

### Phytochemical Analysis

The methanolic extract obtained after extraction procedure was subjected to various Phytochemical screening as per the standard procedure to reveals the presence of various active phyto-constituents.

### Formulation of Ointment

**Table No.1 Formulation of Ointment Base**

Sr.No	Name of Ingredient	Quantity to be taken
1	Wool fat	1.5
2	Cetostearyl Alcohol	1.5
3	Hard paraffin	1.5
4	Yellow soft paraffin	24.5

**Table No.2 Formulation of Herbal ointment**

Sr.No	Name of Ingredient	Quantity to be taken		
		F1	F2	F3
1	Azadirachta Indica Powder	1	0	0.5
2	Bombax ceiba powder	0	1	0.5

### Procedure for preparation of ointment

a) Initially the Ointment base was prepared by weighing accurately grated hard paraffin which was placed in evaporating dish on water bath. After melting of hard paraffin remaining ingredients were added and stirred gently to aid melting and mixing homogenously followed by cooling of ointment base.

b) Herbal Ointment was prepared by mixing accurately weighed Neem and Bombax ceiba powder to the Ointment base by levigation method to prepare a smooth paste with 2 or 3 times its weight of base, gradually incorporating more bases until to form homogenous ointment, finally transferred in a suitable container.

**RESULT AND DISCUSSION****Phytochemical characterization of Azadirachta Indica polymer and Bombax Ceiba polymer****Table No. 3 Phytochemical Characterization of polymer**

Sr.No	Test	Azadirachta Indica	Bombax Ceiba
<b>1</b>	<b>Test for carbohydrates</b>		
a)	Molish's test	-ve	+ve
b)	Fehling Test	-ve	-ve
c)	Benedict Test	-ve	-ve
d)	Barford Test	-ve	-ve
<b>2</b>	<b>Test for protein Amino acid</b>		
a)	Biuret Test	-ve	-ve
<b>3</b>	<b>Test for Phenolic compound</b>		
a)	Ferric chloride Test	-ve	+ve
b)	Lead Acetate Test	-ve	+ve
c)	Iodine test	-ve	-ve
<b>4</b>	<b>Test for steroids</b>		
a)	Salkowski Test	+ve	+ve
<b>5</b>	<b>Test for terpenoids</b>		
a)	Liebermann-burchard test	+ve	+ve

**Physicochemical Characterization****1. Organoleptic Characteristics:****Table No 4: Organoleptic Characteristic of powder**

Sr. No	Parameter	Observation	
		Azadirachta Indica leaves polymer	Bombax ceiba bark polymer
1	Physical Appearance	Smooth	Smooth
2	Colour	Greenish	Brownish
3	Odour	Bitter	Characteristic
4	Taste	Bitter	Characteristics
5	Nature	Powder	Powder
6	Melting point	154-158	121°C
7	pH	5.6	5

**Micromeritics Properties of Azadirachta Indica & Bombax Ceiba****Table No.5: Micromeritics Properties**

Sr.No	Property	Observation(n=3)	
		Azadirachta Indica	Bombax ceiba
1	Bulk Density	0.376±0.04	0.659±0.03
2	Tapped density	0.576±0.009	0.844±0.04
3	Angle of Repose	22.45±37.71	25.63±44.32
4	Hausner's Ratio	1.436±0.028	1.118±0.29
5	Carr's Index	30.47±1.30	22.13±2.85



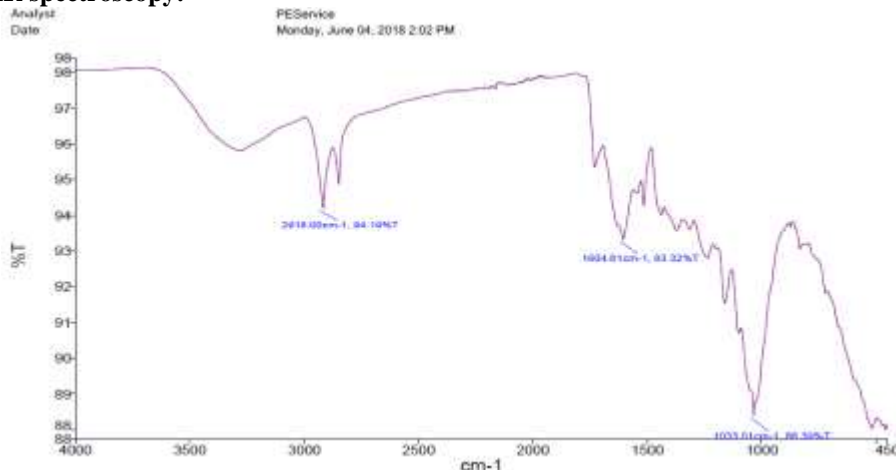
**Determination of Microbial Load**

**Table No.6: Total Viable Aerobic count**

Micro-organism	Media used	Sample	Microbial count (CFU)	Total count/gm (CFU/GM)
Bacteria	Casein soya bean digest agar	Bombax Ceiba	44	440
		Azadirachta Indica	6	60

**Instrumental Analysis**

**1) Identification by FTIR spectroscopy:**



**Fig no. 1 FTIR spectrum of Azadirachta Indica polymer**

**Table No.7 Characteristics peak of Azadirachta Indica Polymer**

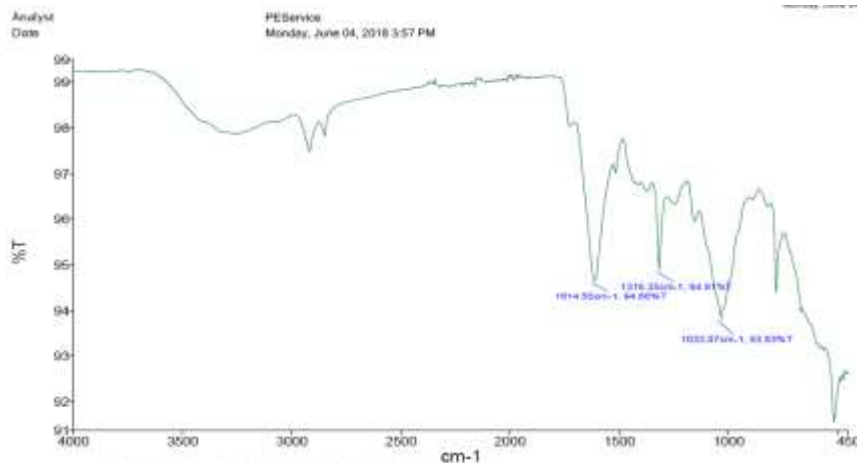
Functional Group	Characteristic peak(cm <sup>-1</sup> )	Obtained peak(cm <sup>-1</sup> )
CH-streaching	2600-3000	2918.0
C=C	1600-1700	1604.81
CH-O	1000-1200	1033.01



**Fig no.2 FTIR spectrum of Bombax Ceiba polymer**

**Table No.8 Characteristic peak of Bombax Ceiba Polymer**

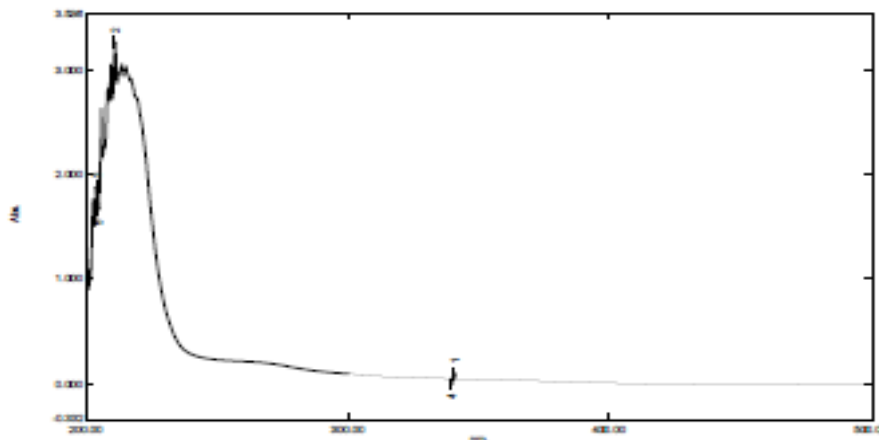
Functional Group	Characteristic peak( $\text{cm}^{-1}$ )	Obtained peak( $\text{cm}^{-1}$ )
CH-stretching	2600-3000	2801
C=C Aromatic	1600-1700	1613.49
CO-C stretching	1050-1150	1029.98
CO-stretching	900-1300	1316.09
CH Bending	680-860	780.05

**Fig no.3 FTIR spectrum of Azadirachta Indica + Bombax Ceiba Polymer****Table No.9 Characteristic peak of Azadirachta Indica and Bombax Ceiba polymer**

Functional Group	Characteristic peak( $\text{cm}^{-1}$ )	Obtained peak( $\text{cm}^{-1}$ )
C=C Aromatic stretching	1600-1700	1614.50
CO-stretching	900-1300	1316.25
CO-C stretching	1050-1150	1033.07

## 2) UV Spectrophotometric analysis

a) **Determination of  $\lambda$  max:**(Azadirachta Indica):10 mg of powder dissolved in 100 ml phosphate buffer pH 6.8 to get the 100 $\mu\text{g}/\text{ml}$  stock solution. The ultraviolet spectrum was determined by scanning stock solution of 100 $\mu\text{g}/\text{ml}$  from 200-800 nm. The  $\lambda$  max of solution was found at 215 nm. as shown in fig

**Figure 4: UV Spectrum of Azadirachta Indica**

### 3) XRD Analysis

The XRD technique was used to determine the crystal structure of polymer. Azadirachta Indica polymer shows well defined characteristics peak which is in amorphous form and the Bombax Ceiba polymer was show crystalline form.

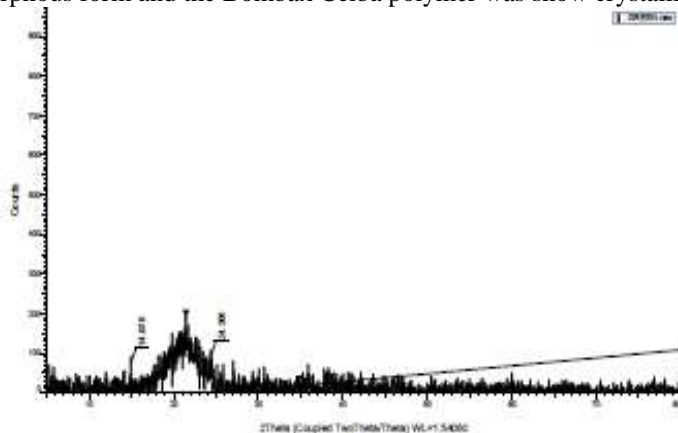


Fig no 5: XRD result for Azadirachta Indica polymer

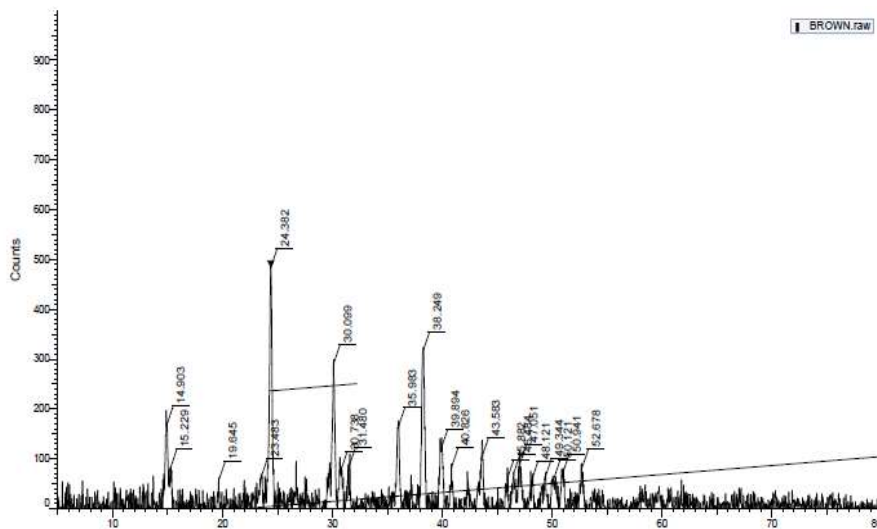


Fig no 6: XRD result for Bombax Ceiba polymer



4) Differential Scanning Colorimetry (DSC)

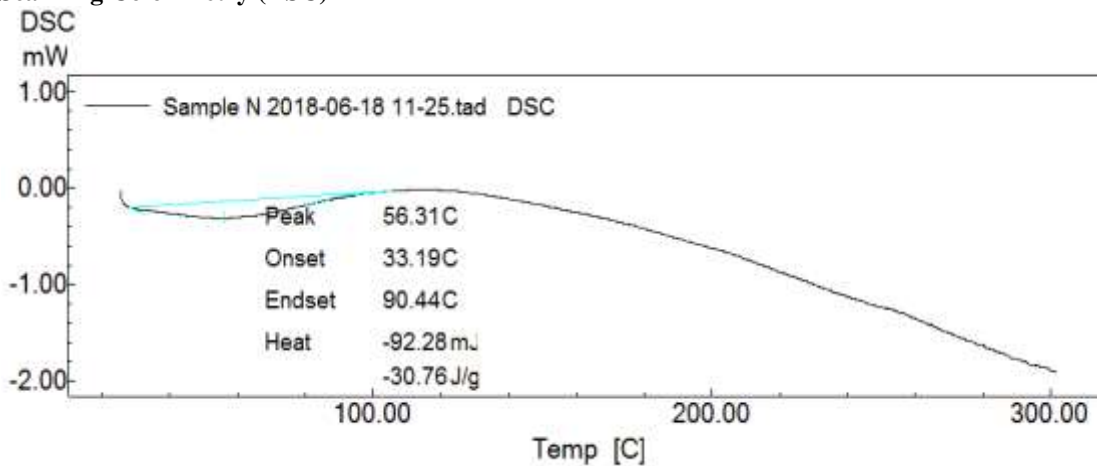


Fig no.7: DSC thermogram of Azadirachta Indica Polymer

Table No.10 Thermal parameter of Azadirachta Indica Polymer

Sr.no	Parameter	Result
	Onset temperature	56.31°C
	Peak temperature	33.19°C
	End set temperature	90.44°C

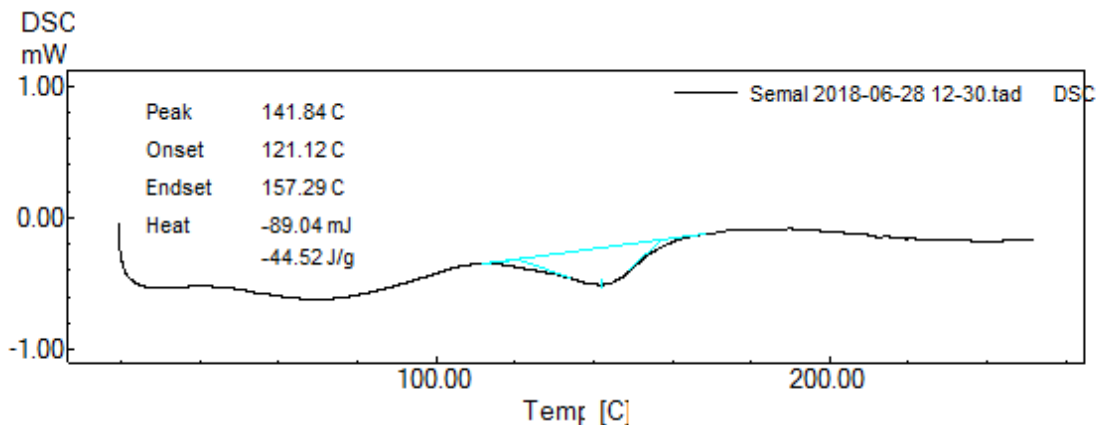
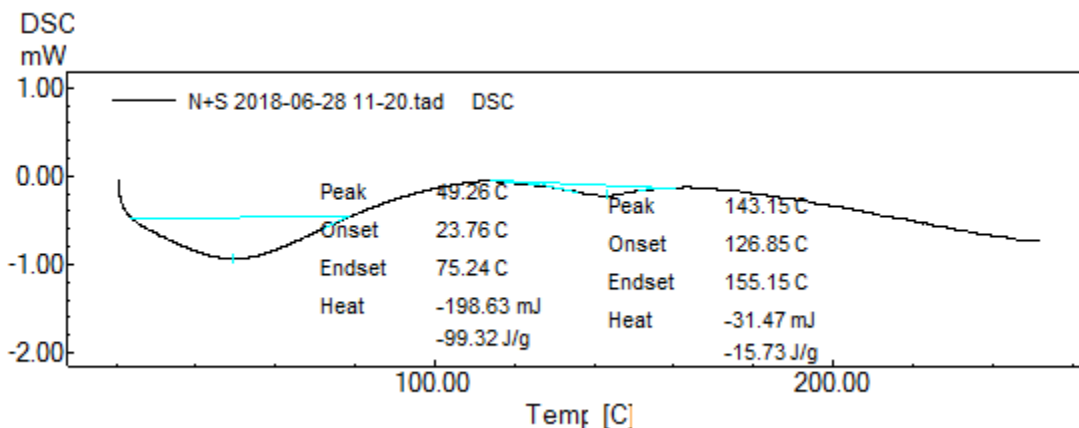


Fig no. 8: DSC thermogram of Bombax Ceiba Polymer

Table no.11 Thermal parameter of Bombax Ceiba Polymer

Sr.no	Parameter	Result
1	Onset temperature	121.12°C
2	Peak temperature	141.84°C
3	End set temperature	157.29°C



**Fig no. 9: DSC thermogram of Azadirachta Indica + Bombax Ceiba (mixture)**

**Table no.12 Thermal parameter of Azadirachta Indica + Bombax Ceiba (mixture)**

Sr.no	Parameter	Result	
1	Azadirachta Indica	Onset temperature	23.76°C
		Peak temperature	49.26°C
		End set temperature	75.24°C
1	Bombax Ceiba	Onset temperature	126.85°C
		Peak temperature	155.15°C
		End set temperature	143.15°C

**Evaluation of Ointment**

**1) Appearance**

**Table no.13 Appearance of Ointment**

Sr. No	Physical Parameters	Result		
		F1	F2	F3
1	Colour	Dark Green	Slightly brownish	Faint Green
2	Odour	Characteristics	Characteristics	Characteristics
3	Nature	Semisolid	Semisolid	Semisolid
4	Consistency	Smooth	Smooth	Smooth
5	Grittiness	No grittiness	No grittiness	No grittiness

**2) pH determination:**

**Table No 14: pH of formulation**

pH (N=3)		
F1	F2	F3
6.6±0.278	6.38±0.075	6.44±0.089

**3) Homogeneity**

All formulations Produce uniform distribution of polymer in Ointment. It was confirmed by visual appearance and touch.

**4) Consistency**

Consistency of all formulation was checked by applying Ointment on skin and it was found that all formulation of ointment has good consistency.

**5) Washability**

All formulation was applied on skin and washing with water observe following observation



**Table No.15 Washability of ointment**

F1	F2	F3
++	+	+

**Easily Washable (++)**, washable (+)

F1 was easily washable when applied on skin as compared to F2 and F3 formulation.

#### 6) Spreadability test<sup>2</sup>

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. One glass slide was fixed on wooden block. By this method Spreadability was measured on the basis of slip and drag characteristics of cream and ointment. The excess amount of ointment was placed on fixed ground slide, another glass slide having the same dimensions. The Ointment was sandwiched between two slides, and 50 kg weight was placed on the top of this glass slide to for 5 min to expel air and compress the glass slides of uniform thickness and excess cream and ointment was scraped off from boundaries or edges. Then the top of the slide pull 20 gm of weight with the help of thread or string attached to the one end of hook. The time (in seconds) required by the top slide to cover up a distance of 7.5 cm be noted. A shorter interval indicates better Spreadability.

Spreadability was calculated by using the formula,

$$\text{Spreadability} = m \cdot l / t$$

**m = Weight tide to upper slide**

**l = length moved on the glass slide**

**t = time taken**

**Table no 16: Spreadability of Ointment**

Sr. No	Formulation code	Spreadability (g.cm/sec)
1	F1	10
2	F2	14
3	F3	12

#### 7) Viscosity

Viscosity of the formulation was determined by Brookfield DV-E Viscometer using spindle no 64.

**Table no.17 Viscosity of Ointment**

Spindle No	RPM	Viscosity (cps) (N=3)		
		F1	F2	F3
64	10	54710±586.4	55096±422.53	54500±895.3
	20	28928±395.03	29860±215.48	29490±65.57
	50	11726±139.09	11796±32.15	11824±49.57
	60	9746±115.14	9882±21.16	9671±95.28
	100	5777±75.78	5982±17.89	5931±48.01

Viscosity of F3 formulation shown high viscosity as compared to F1 and F2 formulation.

#### 8) Extrudability study: It is the force required to extrude material out of tube; detemining % of Extrusion of Formulation.

**Fig.No.10: Extrudability of formulation****Table No.18 Extrudability Ointment**

Formulation	Net Weight of formulation in tube	Weight of formulation extruded	Extrudability amount%
F1	10	8.95 ± 0.14	89.5%
F2	10	8.25 ± 0.14	82.5%
F3	10	8.59 ± 0.19	85.9%

All formulations evaluated for Extrusion test in which F1 formulation has highest extrusion among so it indicates that F1 formulation has good extrudability.

#### 9) Antimicrobial test:<sup>49</sup>

Loopfull of provided culture of *Pseudomonas aeruginosa* and *staphylococcus aureus* were inoculated in sterile nutrient broth and 5 ml incubated and adjusted to 0.5 by MC Ferlands standards at 37°C for 24 hours. 0.1 ml of active culture of respective organisms was spreaded on sterile nutrient agar plates and well were cut with the help of borer. To these well labeled wells respective samples were added and also a blank of DMSO+ water was added to labeled well. These plates were kept in refrigerator for 30 min for diffusion, after 30 min plates were incubated at 37°C for 24 hours. After incubation result were recorded as zone of clearance and well in mm.

**Fig no 13 Zone of inhibition of F1, F2 and F3**

**Table No.19 Antimicrobial activity of formulated ointment**

Sr. no	Formulation	Concentration (mg)	Staphylococcus aureus	
			Zone of Inhibition	
1	F1	66.6 mg	11	
2	F2	33.3 mg	07	
3	F3	66.6 mg	08	

**10) Stability testing**

Accelerated stability testing of best batch formulation was conducted at room temperature, 5<sup>0</sup>C for 60 days and after 60 days formulation was evaluated for following parameters.

**Table no 20: Stability test Evaluation of F1 (after 60 days)**

Sr. No	Parameter	Temperature conditions	
		5 <sup>0</sup> C	R.T
1	Colour	Green	Green
2	pH	6.61±0.05	6.78 ±0.21
3	Viscosity	54710±586.4	55096 ±422.53
6	Washability	Easily washable	Easily washable
7	Consistency	Good	Good
8	Skin irritation	No irritation	No irritation

Hence the formulation is stable after 60 days.

**CONCLUSION**

Present study concluded that Azadirachta Indica and Bombax Ceiba powder was successfully obtained after extraction their percent yield was found as 10.15%, 1.38 % respectively. Dried powder extract of Azadirachta Indica and Bombax Ceiba is used for formulation of ointment. Obtained powder was preliminary evaluated for various test such as Phytochemical, Physicochemical properties, FTIR, DSC, XRD and UV spectroscopy etc. Successfully prepared ointments by Levigation method, using 1% Azadirachta Indica, 1% Bombax Ceiba and their combination (0.5% Azadirachta indica and 0.5% Bombax ceiba ).Formulations are evaluated for pH, Appearance, Spreadability, extrudability, antimicrobial test, skin irritation test for 12 hrs etc. percentage of extraction of F1 was found 89.5 % which was highest as compare to F2 and F3 formulations, it may be due to less viscosity of Azadirachta Indica powder as compare to Bombax Ceiba powder. pH of all formulations within the range of 6.3 to 6.6 so formulations are compatible with skin pH no skin irritations observed. The order of antimicrobial activity observed among the formulation as follows F1 > F2 > F3. F1 was selected as best batch formulation as it shows highest Zone of Inhibition among the F2 and F3 formulation. Stability test proves that formulations are stable after 60 days F1 formulations shows approximately similar results as that of previous, it proves that F1 formulation was stable.

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