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## Research Article

# Formulation And Evaluation Of Herbal Anti-Bacterial Crack Heel Ointment Using Thuja Occidentalis Leaves Extract And Euphorbia Caducifolia Juice

Priyanka Raut\*, Manisha Mishra\*, Gayatri Bawane<sup>1</sup>, Ayushi Ambade<sup>2</sup>, Alshapha Anjum Khan<sup>3</sup>, Abhilasha Thakur<sup>4</sup>, Isha Patle<sup>5</sup>

Department of Pharmaceutical Chemistry, Manoharbai Patel Institute Of B. Pharmacy, Kudwa, Gondia..

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## ABSTRACT

Herbal preparation has its origins in ancient cultures. It involves the medicinal use of plants to treat disease and enhance general health and wellbeing pharmaceutical medications are based on manmade versions of naturally occurring compounds found in plants. For example, crack heels can be cured by *Thuja occidentalis* extract and *Euphorbia Caducifolia* Juice. The present research has been undertaken with the aim of formulate and evaluate the herbal ointment containing *Thuja occidentalis* extract and *Euphorbia Caducifolia* juice. The ointment formulations were prepared by using Turmeric, camphor, wool fat, hard paraffin, Cetostearyl alcohol, white soft paraffin and required amount of Rose water. Wool fat, strearyl alcohol, hard paraffin and white soft paraffin forms the base of an ointment. The physical parameters of formulated ointment like color, homogeneity, pH, viscosity, spreadability and removability were evaluated. The comparison between herbal preparation and marketed preparation were studied. The herbal ointment containing *Thuja occidentalis* extract and *Euphorbia caducifolia* juice has better antibacterial activity.

## INTRODUCTION

The body's first line of protection against exposure to the outside world is the skin. The skin is composed of three layers: the dermis, hypodermis, and epidermis.<sup>[1]</sup>

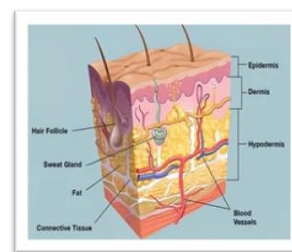



Fig no.1 :- Anatomy of skin<sup>[2]</sup>

\*Corresponding Author: Priyanka Raut

Address: Department of Pharmaceutical Chemistry, Manoharbai Patel Institute Of B. Pharmacy, Kudwa, Gondia.

Email : [rautpriyanka695@gmail.com](mailto:rautpriyanka695@gmail.com)

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The human foot is a vital organ that experiences high levels of friction and environmental exposure. It has been reported that *Staphylococcus* bacteria is the cause of foot odor, which results from bacterial breakdown.<sup>[3]</sup>

### Crack Heels:

Another name for cracked heels is heel fissure. They are caused by dry skin and are accompanied by thicker skin around the heel edge, occasionally with yellow or brown calluses.<sup>[4]</sup>

### Common causes that can cause crack heels:

1. Always wearing shoes with open backs because skin expands more easily.
2. Skin conditions like psoriasis and dermatitis (Dry skin makes skin break easily).<sup>[5]</sup>

### Complications of cracked heels:

1. Discolorations can occur due to the thick derma.
2. If left not treated, cracked heels can cause bleeding. Can lead to an infection.<sup>[6]</sup>

Herbal Crack heel ointment can contain plant extracts, including those of *Thuja occidentalis*, *Euphorbia caducifolia*, *Calendula officinalis*, *Berberis aristata*, *Bergenia ligulata*, *Leptadenia reticulata*, *Mallotus philippensis* and *Rubia*. The primary objective of the study was to determine whether or not phytochemical components were present in all of the medicinal plants.<sup>[7]</sup>



Fig.no.02: Depiction of foot crack<sup>[8]</sup>

## BACTERIAL INFECTION:

Bacterial contamination in foot breaks happens when microbes enter the skin through fresh injuries or crevices. *Staphylococcus aureus* and *Streptococcus* species are two common types of bacteria that can cause infections in the feet.<sup>[9]</sup>

Symptoms of a bacterial infection in foot cracks may include:

1. Increased pain or discomfort
2. Redness and swelling around the cracked area<sup>[10]</sup>

Bacterial contaminations are sicknesses that can influence your skin, lungs, brain, blood and different pieces of your body. If left untreated, foot cracks may result in bacterial infections.<sup>[11]</sup>

### Ointment:

An ointment is a semisolid readiness applied to the skin or mucous films for restorative or defensive purposes. It normally comprises of a base, which can be water-solvent or greasy. Ointments are thicker than the creams and lotions.<sup>[12]</sup>

There are several types of ointments:

1. Medicated Ointments
2. Emollient ointment
3. Protective ointment
4. Herbal ointment
5. Ointment For Wound healing
6. Ointment for Acne<sup>[13]</sup>

### Ointment Bases:

The following are some typical Ointment bases:

1. Hydrocarbon Bases
2. Retention Bases
3. Water-Dissolvable Bases
4. Oleaginous bases
5. Hydrophilic Treatment Bases
6. Anhydrous Bases<sup>[14]</sup>

### Advantage:

- (a) They are water soluble; hence, very easily can be removed from the skin.
- (b) Helps in good absorption by the skin.
- (c) Good solvent properties.<sup>[15]</sup>

### Disadvantages:

- (a) Limited uptake of water. Macrogols dissolve when the proportion of water reaches about 5%.  
 (b) Solvent action on polyethylene and Bakelite containers and closures.[15]

## MATERIAL AND METHODS

Sr.no.	MATERIAL	SOURCE
1	<i>Thuja Occidentalis</i>	Local area of Gondia
2	<i>Euphorbia Caducifolia</i>	Local area of Gondia
3	Wool fat	MIBP,Gondia
4	Hard paraffin	MIBP,Gondia
5	Cetostearyl alcohol	MIBP,Gondia
6	White soft paraffin	MIBP,Gondia
7	Camphor	MIBP,Gondia
8	Turmeric	MIBP,Gondia
9	Rose water	MIBP,Gondia

**Table no.01: List of Active Herbal Ingredients and Excipient with their sources**

### Instruments:

SR.NO.	INSTRUMENT	MANUFACTURER
1	Soxhlet apparatus	Labline
2	pH meter	Systronics Globe Instrument
3	Brookfield viscometer	Brookfield engineering laboratories
3	Digital balance	Kerro P3
4	Digital Autoclave	ASI-254
5	B.O.D Incubator	HMG Gondia
6	Mixer Grinder	Jyoti
7	Hot air oven	Metalab scientific Industries
8	UV spectrophotometer	SHIMADZU model no-1700
9	Heating mantle	Biotechnics India
10	Rectangular water bath	Labline

**Table no.02: List of Instruments used**

### Method:

**Preparation of plant extract:** The dried plant material (leaves) is used for extraction. The fresh plant of part dried by air-cured method which is carried in the shade outdoors, after complete dried of leaves. Mix finely divide powder by grinding method. Extraction of *Thuja Occidentalis* done by hot-continuous method/Soxhlet extraction method, in which 50 gm of finely divided powder is filled in extractor. Ethanol is used as a solvent in extraction which is filled in boiling flask and condenser are connected to it for condensation process. It take 24 hrs to complete extraction process and to collect extract in a boiling flask. Collected extract evaporate on water bath for 2hrs

and we get concentrated extract.<sup>[16]</sup> % Yield = weight of extract/ weight of ground powder material\*100.<sup>[17]</sup>



**Fig no.03: Soxhlet extraction**

**Thin layer chromatography:** Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel Gas stationary

phase. The mobile phases, results and chromatograms are depicted in following figure:<sup>[18]</sup>

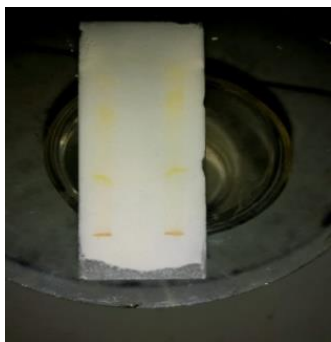


Fig no.04:TLC of *Thuja Occidentalis*



Fig no.05:TLC of *Euphorbia Caducifolia*

### Development of herbal ointment formulation:

Ointments are prepared by following methods:

- Fusion method

By the fusion method, all or some of the components of an ointment are combined by being melted together and cooled with constant stirring until congealed.

- Clean all the glassware and dry them properly as per SOP.
- Weigh all the ingredient properly.
- Take hard paraffin and cetostearyl alcohol. Melt them in porcelain dish and kept on water bath.
- To above melted mixture add wool fat, white soft paraffin, camphor and extract then stir well.
- Add turmeric and rose water to the oily phase. Maintain 70-75°C. Stir continuously until a uniform ointment is formed.
- After melting all ingredients, remove it from a porcelain dish.
- Pour it into an air tight container and place it in a cool and dry place<sup>[19]</sup>

### FORMULA:

Sr.no.	Ingredients	Quantity for 100g
1	Wool fat	5g
2	Hard Paraffin	5g
3	Cetostearyl alcohol	5g
4	White soft paraffin	85g

Table no 03: Control batch formulation of Herbal Ointment

### BATCHES PREPARED

Sr. No	Ingredients	Uses	F1 (20g)	F2 (20g)	F3 (20g)
1	<i>Thuja occidentalis</i> Extract	Anti-fungal property	0.5g	0.75g	1g
2	<i>Euphorbia caducifolia</i> Juice	Moisturizing and healing property	1g	0.75g	0.5g
3	Cetostearyl alcohol	Ointment base	1g	1g	1g
4	White soft paraffin	Ointment base	14.50g	14.50g	14.50g
5	Wool fat	Ointment base	1g	1g	1g
6	Hard paraffin	Ointment base	1g	1g	1g
7	Turmeric	Antioxidant property	0.25g	0.25g	0.25g
8	Camphor	Cooling effect	0.75g	0.75g	0.75g
9	Rose water	Moisturizing effect	q.s	q.s	q.s

Table No.04: Formula of preparation of herbal ointment.<sup>[20]</sup>

## Evaluation of Herbal Ointment:

### 1. pH

The pH of all the formulated Herbal Ointment was measured by using digital pH meter. First, pH meter was calibrated with standard buffers (pH4 and 7). pH of products was measured 20min, 30min, 1 hour after preparation. The test was repeated three times.<sup>[21]</sup>

### 2. Appearance and Homogeneity

The developed individual and polyherbal Ointment were evaluated for physical appearance and homogeneity by visual observation.

### 3. Viscosity

Brookfield DV-III viscometer was used for the determination of viscosity. At first, viscometer was calibrated by Brookfield Viscal Kit. Ointment samples were placed at room temperature for 30min.

Then, they were poured in apparatus container. Number 74 spindle was attached then viscosity was determined at 25°C and 100–250 rpm. The results were reported in average after triplicate experiments.<sup>[22]</sup>

### 4. Spreadability

The spreadability of the Ointment formulations was determined by measuring the spreading diameter of 1 g of Ointment between two horizontal plates (20 cm x 20 cm) after one min. The standard weight applied on the upper plate was 125 gm.<sup>[23]</sup>

### 5. Skin Irritation Test

Skin irritation studies were carried out in order to detect irritation and sensitization under conditions of maximal stress which may occur over a prolong contact with the skin surface. Skin irritation test is done by using patch test on the back skin of volunteer. Ointment (F2) (2×2 cm<sup>2</sup>) was applied to the clean skin of the volunteer back. Volunteer was then kept under observation for a period of 4-6 hours to detect any sign of erythema, redness, sensitization or any other allergic reaction.<sup>[24]</sup>

### 6. Stability Study

Formulation of Antibacterial Herbal Ointment became stored in the refrigerator and oven. Also stored beneath room temperature for three weeks modifications acquired if any are noted.<sup>[25]</sup>

### 7. Antibacterial Study

The antibacterial screening of Herbal preparation was done by cup-plate method. The Ointments were tested against bacterial agents namely *S. aureus* and *E. coli*. A loopful of the pure bacterial culture was suspended in nutrient broth and incubated for 24 hours. Nutrient agar media was sterilized and poured into petri plates. After solidification, 0.1ml of the inoculum was spread over the agar evenly using a rod. 6mm diameter cavity was prepared and formulated Ointment is placed in the cavity. A standard antibiotic was used as the control. The inoculated plates are incubated for 24 hours. Later, the zone of inhibition around the disc was measured and recorded.<sup>[26]</sup>

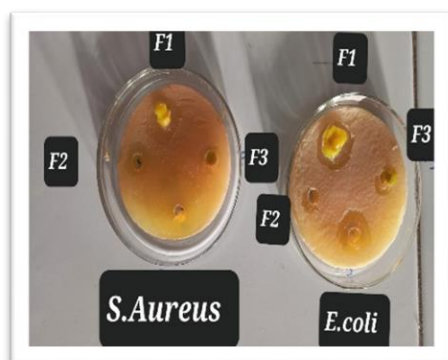


Fig.no.06: Antibacterial test



Fig.no.07: BOD Incubator

## RESULT AND DISCUSSION:

### Characterization and identification of plant extract

- **Physical properties:** Colour and odour was studied.

Colour	Dark Green
Odour	Aromatic

**Table no. 05 Physical properties of *Thuja Occidentalis* Extract**

Colour	Light Green
Odour	Pungent

**Table no. 06 Physical Properties of *Euphorbia Caducifolia* Juice**

- **Solubility studies:** Solubility in water, alcohol and Glycerin and Acetone was studied

Water	Alcohol	Glycerin
Soluble	Soluble	Soluble

**Table no. 07 Solubility study of *Thuja Occidentalis* Extract**

Ether	Alcohol	Acetone
Soluble	Soluble	Soluble

**Table no. 08 Solubility study of *Euphorbia Caducifolia* Juice**

## PRELIMINARY PHYTOCHEMICAL SCREENING OF PLANT EXTRACT

Preliminary phytochemical test for identification of phytoconstituents in *Thuja Occidentalis* and *Euphorbia Caducifolia*.

Sr.no.	Plant constituents	Test/Reagents	<i>Thuja Occidentalis</i>	<i>Euphorbia Caducifolia</i>
1	Alkaloids	Mayer's test	+	+
2	Flavonoids	Shinoda test	+	+
3	Glycosides	Aqueous NaOH test	+	+
4	Steroids	Salkowski's test	-	-
5	Cardiac glycosides	Keller kelani's test	-	-
6	Saponins	Foam test	+	-
7	Resins	Acetic anhydride test	+	+
8	Phenol	Ferric chloride test	+	+
9	Tannins	Lead Acetate test	+	-
10	Terpenoids	Salkowski's test	+	+
11	Quinones	Con. HCL test	+	-
12	Proteins	Ninhydrin test	-	+

**Table no.09: Phytochemical screening**

### Thin layer chromatography:

The thin layer chromatography of *Thuja*

*Occidentalis* extract and *Euphorbia Caducifolia*

juice was found to be-

Sr.No.	Plants	RF Value
1	<i>Thuja Occidentalis</i>	0.69
2	<i>Euphorbia Caducifolia</i>	0.56

**Table no.10: TLC of *Thuja Occidentalis* extract and *Euphorbia Caducifolia* Juice**

### Evaluation of Herbal Ointment:



**Fig.no.08: Formulation F1, F2, F3**

- **pH Of Ointment:** The pH of *Thuja Occidentalis* and *Euphorbia Caducifolia* Ointment was found to be-

Sr. No.	Tests	Formulation		
		F1	F2	F3
1	pH	6.42	6.52	6.33

**Table No.11: pH of ointment**



**Fig. No.09: pH of Ointment**

- **Physical Appearance:** Color, odour, thickness was studied.

Sr. No.	Tests	Formulation		
		F1	F2	F3
1	Appearance	yellow	yellow	yellow
2	Odour	Characteristic	Characteristic	Characteristic
3	Consistency	Viscous, Greasy	Viscous, Greasy	Viscous, Greasy
4	Homogeneity	Good	Good	Good

**Table No.12: Physical Appearance**

- **Solubility:** Solubility of Herbal Ointment in water, alcohol, Glycerin and Propylene Glycol was found to be-

For Formulation F1, F2, F3			
water	Alcohol	Glycerin	Propylene Glycol
Insoluble	Soluble	Soluble	Soluble

**Table No. 13: solubility of Ointment**

- **Spreadability:-**The spread ability test of Herbal Ointment was found to be-

Sr. No.	Tests	Formulation		
		F1	F2	F3

1	Spreadability	8sec	10sec	7sec
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**Table No.14: Spreadability**

- **Skin irritation test:** The skin irritation test of given formulation of *Thuja Occidentalis* and *Euphorbia Caducifolia* show no reaction.

Sr. No.	Skin irritation test (Time)	Formulation		
		F1	F2	F3
1	10 min	No reaction	No reaction	No reaction
	1 hour	No reaction	No reaction	No reaction
	2 hour	No reaction	No reaction	No reaction

**Table No.15: Skin irritation test**

- **Viscosity:** viscosity of the ointment was found to be-

Sr. No.	Tests	Formulation		
		F1	F2	F3
1	Viscosity (cp)	11000cp	13000cp	14000cp

**Fig. No.16: Viscosity of Ointment**

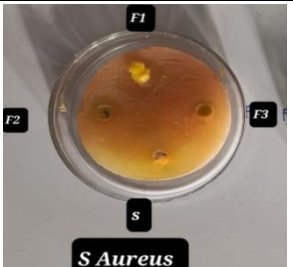
- **Washability:** Washability of the *Thuja Occidentalis* and *Euphorbia Caducifolia* ointment was found to be-

Tests	Formulation		
	F1	F2	F3
Washability	Slightly washable and Sticky	Slightly washable and Sticky	Slightly washable and Sticky

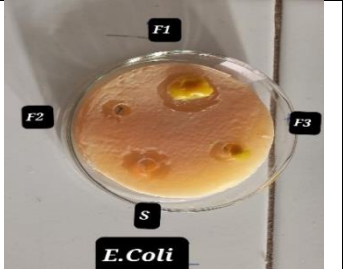
**Table No.17: Washability test of Ointment**

- **Anti-microbial test:** Anti -microbial test of formulation F1, F2 and F3 was performed against microbes such as *S Aureus* and *E Coli*.

For <i>S.Aureus</i>				
Conc.(µg)	Formulation			Standard (Ampicillin)
	F1	F2	F3	
	Zone of inhibition			
100	40mm	40mm	60mm	70mm



For <i>E.coli</i>				
Conc.(µg)	Formulation			Standard (Ampicillin)
	F1	F2	F3	
	Zone of inhibition			
100	30mm	30mm	50mm	70mm



**Table no.18: Antibacterial activity of herbal ointment**

- **Stability study:**



Evaluation	F1			F2		
	R.T.	Ref.	Oven	R.T.	Ref.	Oven
Temp	25.03±3.0°C	4.0±2.0°C	40.0±2.0°C	25.03±3.0°C	4.0±2.0°C	40.0±2.0°C
Colour	Yellow			Yellow		
Odour	Characteristic			Characteristic		
Spreadability	G	G	I	G	G	I
p.H.	6.42			6.52		

Evaluation	F3		
	R.T.	Ref.	Oven
Temp	25.03±3.0°C	4.0±2.0°C	40.0±2.0°C
Colour	Yellow		
Odour	Characteristic		
Spreadability	G	G	I
p.H.	6.33		

Table no.19: Stability study

- Comparison between Herbal Formulation and Marketed Formulation

Sr. No	Physicochemical Parameters	Prepared F1 Formulation	Prepared F2 Formulation	Prepared F3 Formulation	Marketed Formulation
1)	Color	yellow	Yellow	yellow	white
2)	Odor	Characteristic	Characteristic	Characteristic	Characteristic
3)	Viscosity	11000cp	13000cp	14000cp	12000cp
4)	pH	6.42	6.52	6.33	6.00
5)	homogeneity	Excellent	Excellent	Excellent	excellent
	Hard and sharp edged particles	No Hard and sharp edged particles	No Hard and sharp edged particles	No Hard and sharp edged particles	No Hard and sharp edged particles
	removability	Easily removable	Easily removable	Easily removable	Easily removable
6)	Thermal stability (35°C)	stable	Stable	stable	stable
7)	Irritation test	No irritation	No irritation	No irritation	No irritation
8)	Spreadability	8sec	10sec	7sec	9sec

Table no.20: Comparative studies of formulated preparation and marketed preparation.<sup>[7]</sup>

## DISCUSSION:

The colour of all the formulated Herbal Ointments yellow and all the Herbal Ointments were good in homogeneity. The pH of all the formulated Ointments were in the range of 6.3-6.6 matching with skin pH range. Viscosity of all the Herbal Ointments was ranging from 11000-15000 cp at 20 rpm measured with Brookfield viscometer. The batches was found to be stable at room temperature and refrigerator. The spreadability all Herbal Ointments was in the range of 7-10 sec. All the

formulated Herbal Ointments show good Anti-Bacterial activity.

## CONCLUSION:

From the present investigation it has been revealed that herbal ointment of plant *Thuja Occidentalis* leaves Extract and *Euphorbia Caducifolia* Juice can be formulated using simple ointment base with other ingredients and the evaluation of physical parameters show satisfactory observation. The prepared herbal ointment give potent action against tested pathogens i.e. *S. aureus* and *E.coli* which was comparable with



standard antibiotic i.e ampicillin. The formulations F1, F2 and F3 were prepared with the different proportions of extracts the study shows the F3 batch was found to be optimum. It consist of 1g of *thuja occidentalis* and 0.5g of *euphorbia caducifolia* juice. Also F3 batch was found to be stable at room temperature and refrigerator. This batch was found to be better and effective for the crack heels and gives optimum anti-bacterial action.

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