



FORMULATION AND EVALUATION OF OINTMENT BY USING TRIDAX PROCUMBENS AND AZADIRACHTA INDICA LEAF

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Abstract : The Aim of the current research is to prepare and evaluate an ointment containing Tridax procumbens and Azardirchta indica that possesses wound healing activity. The use of herbal medicine has grown in importance on the global level in both medical and economic field. While there has been a surge in the use of these herbal medications, industrialized and developing nations have severe concern about their effectiveness, safety and quality. In this study, we used the maceration method to prepare the extract. Five different ointment formulations were prepared using the formed ointment based. Criteria such as PH, Colour, Spreadability, Washability etc. have been tested for each formulation.

IndexTerms - Azardirachta indica, Tridax procumbens, Stability, Formulation.

I. INTRODUCTION

In developed countries, the market for herbal products has increased in recent years. Neem and tridax Procumbens are two of the earliest known plants with therapeutic properties Neems effect on the skin is one of its most important benefits and Tridax procumbens is equally effective Herbal preparation are well recognized as effective treatments for the number of illness[3].

Neem

Azardirachta indica is a popular and fast growing plant .commonly found in region of India, Africa and America. Neem is consists of seed, stem, Leaves and other aerial parts. And shows many properties like Antiseptics, Anti-inflammatory as well as antiviral properties [2]

Tridax Procumbens

This plant is mostly used in the traditional Indian system of medicine. Tridax Procumbens is consisting of Leaves, Stem, Flower and other parts. And which is shown many properties Antiseptics, Insecticidal, etc. It is central American Annual or perennial weed that can be found all over India, but is most commonly found in Maharashtra, Madhya Pradesh, and Chhattisgarh region [4].

The most common reason for using traditional medicine are that it is more affordable, more closely correspond to patients ideology allays concern about the adverse effect of chemical (synthetic) medicines. The major use of herbal, medicines is for health promotion and therapy for chronic as opposed to life-threatening conditions.

World health organization has set precise guidelines for the evaluation of the safety, efficacy and quality of herbal medicines. Herbal drug is chief constituent in traditional; medicine and a common constituent in ayurvedic, Homeopathic, Neuropathic and other medicine system .Herb is usually considered as safe since they belong to natural source. The rapid use of the herbal drug due to toxicity and side effect of allopathic, medicines, has led to rapid increase in the number of herbal dug manufacture.

Advantages of Herbal Drug

- More protection
- Complete Accessibility
- Fewer side effects
- Enhanced tolerance
- Potency and efficacy is high

Disadvantages of Herbal Drug

- Risk with self-dosing
- Complexity in standardization
- Not able to cure rapid sickness and accidents

II. PLAN OF WORK

- Review of literature
- Gathering and authentication of the chosen plants
- preparation of herbal extract
- Development of herbal formulation for the prepared extract
- Evaluation of the developed formulation
- Results and discussion
- Conclusion

III. OBJECTIVES

- Collection of plant.
- Preparation of herbal extract.
- Development of herbal formulations for the prepared extract.
- Characterization of the developed formulation

IV. LITERATURE REVIEW

4.1 Lokesh Prasad Ms et al (2017):

In the study of that Article the author studied about the formulations of ointment, Cream and gel of tridax procumbens and area catechu extracts. This also studied about physicochemical parameter and stability study of various temperature conditions.

4.2 Arvinda Nalla et al (2017):

The author of this article researched the creation and testing of herbal ointments for antibacterial activity. They also specified several evaluation criteria as well as the process for making herbal ointment.

4.3 Wubante demiew et al (2018)

The author studied crude extract of A. Polystachyus leaves processes wound healing activities in their study.

4.4 Dr. Abhijeet S. kulkarani et al (2022)

In this article, the author discusses the creation of a herbal immune-booster that is based on jiggery. They also explain the process for a study on the stability of syrup.

4.5 Swati siddheshwar londhe (2020)

In this article the author studied formulation and evaluation of polyherbal ointment, they also defined the various evaluation parameters and how to performed.

V. WOUND HEALING

The particular biological process of tissue formation and growth or wound healing, is connected to this more general phenomena. A range of cellular and matrix components work together during the course of wound healing to restore the integrity of damaged tissue and replace lost tissue. These stages are interdependent and overlap. [5]

Factors Affecting Wound Healing

Factors affecting wound healing [6]

Local Factors	Systemic Factors
Oxygenation	Age and gender
Infection	Sex hormones
Foreign body	Stress
Venous	Ischemia

Phases of Wound healing

Inflammatory phase

The lesioned blood vessels constrict in a licking blood coagulates as a vascular inflammatory reaction, helping to maintain its integrity. Leucocyte inflow in the area of the wound characterizes a cell response during the inflammatory stages. [7]

Proliferative phase

The aim of the proliferative stage is to diminish the lesioned tissue area by contraction and fibroplasia, establishing a viable epithelial barrier to activate keratinocytes. [7] When ongoing injury has cased homeostasis has been achieved and immune response successfully set in place, the acute wound shifts toward tissue repaired [8].

Remodeling phase

The third phase of healing consists of remodeling, which begins two to three weeks after the onset of the lesion and can last for one year or more. [7]

As the final phase of wound healing, the remodeling phase is responsible for the development of new epithelial and final scar tissue formation [8].

VI. MATERIAL AND METHOD

Collection of material

The leaves of Tridax Procumbens collected from the surrounding area of Ashokrao mane institute of pharmaceutical sciences and research save, and authenticated from Yashwantrao chavan warna Mahavidyalaya, Warnanagar, The Reference no. is AMIPSRs

/1048/2022-23. Kept it for shade drying. Crushed to a coarse powder. The powder was stored in an air tight container, until further use.

The leaves of *Azadirachta Indica* collected from the surrounding area of Ashokrao mane institute of pharmaceutical sciences and research save, and Authenticated by Yashwantrao chavan warna Mahavidyalaya, Warnanagar. The Reference no. is AMIPSRs/1048/2022-23. The leaves were cleaned, dried in shade and crushed to coarse powder. and stored in container until further use. [9]

Maceration process

The maceration technique was used to prepare the extraction. As part of the maceration extraction process, finely powdered drug material, such as leaves, stem bark, or root bark, is placed within a container. Menstrual fluid is then poured on top of the drug material until it is completely covered. After that, the container is shut and maintained for at least seven days. To achieve thorough extraction, the substance is periodically mixed and, if placed inside a bottle, shaken. Filtration is used to separate the micelle from the mark following extraction. [10].

Preparation of extraction

Extraction was prepared by using maceration process. 150 gm of powder of *Tridax Procumbens* was weighed and soaked in 1500ml of distilled water with 15ml chloroform. 40gm powder of *Azadirachta indica* was weighed and soaked in 500ml of distilled water with 5ml chloroform. Then kept for 7 days with occasional shaking. After 7 days filtrate by using filter paper. Then the filtrate was evaporated by using electric water bath. Within the help of extract, we prepared ointment [11].



Fig 1- Maceration Process

VII. PLANT SELECTED FOR THE PRESENT STUDY

1. *Tridax procumbens*



Fig-2 *Tridax procumbens*

Tridax Procumbens Linn a widespread plant that grows largely during the rainy Season and is found tropical region of all nations. Its popular name in English is "Coat button". It is member of asteraceae family. [4]

Medicinal use: Wound healing, antimicrobial, anti-inflammatory.

Scientific classification

Kingdom: Plantae
 Subkingdom: Tracheobionta
 Division; Mangleliopsida
 Subclass: Asteridae
 Order: Asterales
 Family: Asteraceae
 Genus: *Tridax*

Pharmacognostic characteristics

- Parts: Leaves
- Arrangement: Opposite
- Size: 3-7cm long, 1-4 cm wide
- Colour: Green
- Odour: characteristics
- Taste: Acrid
- Base: wedge shaped
- Apex: Acute

2. Azadirachta indica**Fig: 3 Azadirachta indica**

The plant *Azadirachta indica* is also referred to as Neem. It is a member of the Meliaceae family. Tropical and subtropical regions are typically where it is grown. [2]

Scientific classification

Kingdom: Plantae
 Subkingdom: Tracheobionta
 Division: Mangelopsida
 Subclass: Rosidae
 Order: Sapindales
 Family: Meliaceae
 Genus: *Azadirachta*
 Species: *indica*

Pharmacognostic characteristics

Parts: Leaves
 Arrangement: Opposite
 Size: 2-6cm long, 1-3 cm wide
 Colour: Dark Green
 Odour: characteristics
 Taste: Bitter
 Base: Oblique
 Apex: Acute

VIII. PROCEDURE FOR PREPARATION OF HERBAL OINTMENT

a) Prepare the ointment foundation first by properly weighing the grated hard paraffin that will be placed in an evaporating dish on a water bath. After the hard paraffin has melted, add the remaining components and gently stir to help the mixture melt and blend evenly. Next, let the ointment base cool.

b) The herbal ointment is made by using a levigation process to combine precisely weighed Neem and then *tridax procumbens* extract with the ointment base to create a smooth paste that is 2 or 3 times the base's weight. Additional base is then progressively added until the ointment is homogeneous and is then transferred to an appropriate container. [12]

Formulation of ointment base

Sr. No.	Name of ingredient	F1	F2	F3	F4	F5
1.	Wool fat	1.25gm	1.25gm	1.25gm	1.25gm	1.25gm
2.	Cetostearyl alcohol	1.25gm	1.25gm	1.25gm	1.25gm	1.25gm
3.	Hard paraffin	1.25gm	1.25gm	1.25gm	1.25gm	1.25gm
4.	Yellow soft paraffin	21.25gm	21.25gm	21.25gm	21.25gm	21.25gm

Formulation of herbal ointment

Sr. No.	Name of ingredient	F1	F2	F3	F4	F5
1.	Neem extract	0.15gm	0.30gm	0.45gm	0.60gm	0.75gm
2.	Tridax procumbens extract	0.25gm	0.50gm	0.75gm	1gm	1.25gm
3.	Ointment base	25gm	25gm	25gm	25gm	25gm



Fig-4 formulations of herbal ointment

IX. OINTMENT BASES

Ideal characteristics

- They must possess affinity for skin and should be miscible with both aqueous and oily secretion of the skin.
- It should be stable
- It should be non-irritating and non-sensitizing properties.
- It should be possessing property of smoothness.
- It must be able to release easily its incorporated medicaments.

9.1 Lanoline

Lanoline or wool fat is a refined, decolorized, and deodorized material obtained from sheep wool. It is available as pale yellow, waxy material with a characteristic odor. Lanoline is typically mixed with vegetable oils or paraffin to produce an ointment base that can absorb approximately twice its own weight of water to produce water in oil emulsion. [13]

Uses- Protectant, Emollient

9.2 Hard Paraffin

It is a mixture of solid saturated hydrocarbon which are derived from petroleum or shale oil. Hard paraffin is a colorless or white wax like material that is physically composed of a mixture of microcrystals. The melting point of hard paraffin is between 47 °C and 65 °C. It is used to enhance the rheological properties of ointment bases. [13]

9.3 Yellow soft paraffin

It is a purified mixture of semisolid hydrocarbons (containing branched, linear and cyclic chains) that are derived from petroleum. Yellow soft paraffin consists of microcrystal embedded in a gel composed of liquid and amorphous hydrocarbon that are themselves dispersed in gel phase obtaining liquid and amorphous hydrocarbon. [13]

9.4 Cetostearyl alcohol

In topical pharmaceutical formulation cetostearyl alcohol will increase the viscosity and act as emulsifier in both w/o and o/w emulsion. Cetostearyl alcohol is generally regarded as a non-toxic material. Although it is essentially non-irritating, sensitization reaction to cetostearyl, cetyl, and stearyl alcohol have been reported.

X. EVALUATION PARAMETERS

1. Colour and odour
2. Consistency
3. Non irritancy
4. Washability
5. Solubility
6. Spreadability test
7. Loss on drying
8. PH
9. Stability study

10.1 Colour and odour

Physical parameters like colour and odour were examined by visual examination. [14]

10.2 Consistency

After applying the cream, the kind of film or smear that developed on the skin was examined. [15]

10.3 Non irritancy

Mark an area (1sq.cm) on the left hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24 hrs. and reported.[16]

10.4 Washability

After applying the formulation to the skin, the ease of water washing was evaluated. [12]

10.5 Solubility

Solubility of ointment can be checked by dissolving 1 gm of sample in both alcohol and water.

10.6 Spreadability test

By placing much more than sample between 2 slides that were compressed to uniform thickness by placing an explicit weight for a definite time. As spread capacity, the time required to separate the 2 slides was measured. Less time required for the separation of 2 slides results in greater potential for spread. [15]

The ability to spread was determined by the formula

$$S = M \times L / T$$

Wherever,

S= Spreadability

M= Weight tide to the higher slide

L= Glass slide length

T= Time taken to make the slides separate.

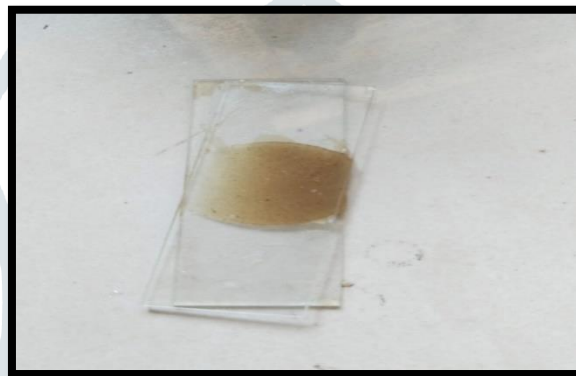


Fig-5 Spreadability test

7. PH

pH of prepared herbal ointment was measured by using digital pH meter. The solution of ointment was prepared by using 100ml of distilled water and set aside for 2hrs. pH was determined in triplicate for the solution and average value was calculated.[12]



Fig-6 PH Test

8. Loss on drying

LOD was determined by placing the formulation in petri dish on hot air oven and dried for 105 °C temperature. [12]

Loss on drying is determined by following formula

The following is LOD of Formulation 1

Weight of empty petri dish = 79.92 gm

Weight of drug taken = 2 gm

Weight of empty petri dish + Weight of drug taken = 81.92 gm

Weight of empty petri dish + Weight of drug taken after drying =81.78 gm

Moisture content = Weight of empty petri dish + Weight of drug taken -

Weight of empty petri dish + Weight of drug taken after drying

= 0.14 gm

Percentage of moisture content of 2 gm of drug = 0.14 gm
Therefore 100 gm of crude drug content = $0.14/2 \times 100 = 7\%$
Loss on drying = 7%



Fig-7 loss on drying

XI. STABILITY STUDY

The prepared herbal ointment was tested for stability by exposing the samples to accelerated temperature conditions. The samples of ointment are stored in petri dishes by wrapping it by aluminum foil and stored at 4°C accelerated temperature Room temperature and 47 degrees Celsius, respectively. The all of the samples was keep through the Spreadability, PH, Consistency and colour and odour at 24 hr., and 48 hr. intervals It takes 72 hours to notice any changes.[17]



Fig-8 formulations after 24 hrs. (F1, F2, F4 & F5)



Fig-9 Formulations after 48 hrs. (F1, F2, F4, F5)



Fig-10 Formulation after 72 hrs. (F1)

XII. RESULTS AND DISCUSSION

The current study was conducted to organize and evaluate the herbal ointment. To do this, the herbal extracts were made using a straightforward maceration procedure, which caused no harm to the chemical components or their functioning. The levigation

procedure is used to prepare ointment that would remain stable throughout storage, in increasing order of quantity of extract in formulation to prepare about 5 formulations. These formulations are studied for various evaluation parameters like spreading ability, extrudability, washing ability, solubility, drying loss, non-irritancy ,PH etc. are adequate enough to have an impact on chemical research. Then formulation was jointly placed for a stability investigation for 16 days by different environmental conditions by the cycle of three temperature conditions 24hr, 48hr, 72hr the resulting results are noted and the effective ointment formulation is defined to F1. The F1 Formula doesn't make any change in any physicochemical properties in formulation so it is found effective.

XIII. STABILITY STUDY

Parameter	F1	F2	F3	F4	F5
Colour	White	Pale Green	Bright Green	Green	Dark Green
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Consistency	smooth	Smooth	Smooth	Smooth	Smooth
PH	6.76	6.81	6.93	6.97	7.21
Spreadability	5 Sec	5 Sec	5 Sec	5 Sec	5 Sec
LOD	7%	6%	8%	6%	11%
Solubility	Insoluble in alcohol and water	Insoluble in alcohol and water	Insoluble in alcohol and water	Insoluble in alcohol and water	Insoluble in alcohol and water
Washability	Good	Good	Good	Good	Good
Non irritancy	Non Irritant	Non Irritant	Non Irritant	Non Irritant	Non Irritant

1. Formulation (F1)

Time Duration (hrs.)	Temperature	Physicochemical Parameters			
		Colour	Odour	Spreadability	PH
24	4 °C	No change	No change	No change	6.76
	Room temp	No change	No change	No change	6.76
	47 °C	No change	No change	No change	6.78
48	4 °C	No change	No change	No change	6.78
	Room temp	No change	No change	No change	6.78
	47°C	No change	No change	No change	6.56
72	4°C	No change	No change	No change	6.79
	Room temp	No change	No change	No change	6.69
	47 °C	No change	No change	No change	6.71

2. Formulation (F2)

Time Duration (hrs.)	Temperature	Physicochemical Parameters			
		Colour	Odour	Spreadability	PH
24	4 °C	No change	No change	No change	6.81
	Room temp.	No change	No change	No change	6.83
	47 °C	No change	No change	No change	6.89
48	4 °C	No change	No change	No change	6.93
	Room temp	No change	No change	No change	7.41
	47°C	No change	No change	No change	7.73
72	4°C	No change	No change	No change	-
	Room temp	No change	No change	No change	-
	47 °C	No change	No change	No change	-

3. Formulation (F3)

Time Duration (hrs.)	Temperature	Physicochemical Parameters			
		Colour	Odour	Spreadability	PH
24	4 °C	No change	No change	No change	6.93
	Room temp.	No change	No change	No change	6.93
	47 °C	No change	No change	No change	7.21
48	4 °C	No change	No change	No change	7.42
	Room temp	No change	No change	No change	7.43
	47°C	No change	No change	No change	8.12
72	4°C	No change	No change	No change	-
	Room temp	No change	No change	No change	-
	47 °C	No change	No change	No change	-

4. Formulation (F4)

Time Duration (hrs.)	Temperature	Physicochemical Parameters			
		Colour	Odour	Spreadability	PH
24	4 °C	No change	No change	No change	6.97
	Room temp.	No change	No change	No change	7.31
	47 °C	No change	No change	No change	7.81
48	4 °C	No change	No change	No change	7.84
	Room temp	No change	No change	No change	8.12
	47°C	No change	No change	No change	8.34
72	4°C	No change	No change	No change	-
	Room temp	No change	No change	No change	-
	47 °C	No change	No change	No change	-

5. Formulation (F5)

Time Duration (hrs.)	Temperature	Physicochemical Parameters			
		Colour	Odour	Spreadability	PH
24	4 °C	No change	No change	No change	7.21
	Room temp.	No change	No change	No change	7.24
	47 °C	No change	No change	No change	7.34
48	4 °C	No change	No change	No change	7.23
	Room temp	No change	No change	No change	7.34
	47°C	No change	No change	No change	7.36
72	4°C	No change	No change	No change	7.9
	Room temp	No change	No change	No change	8.2
	47 °C	No change	No change	No change	8.3

XIV. CONCLUSION

In Ayurveda tridax procumbens and Neem were used for various medicinal properties like antibacterial, anti-inflammatory, wound healing. By using these properties of plant the formulation was prepared known as ointment. For the formulation of an ointment by maceration process initially the extract of these plants was taken. About five formulations were prepared by changing the concentrations of extract in each formulation. The F1 shows the good Spreadability, good consistency & physicochemical

parameters during study period and appeared better results. The final results can be concluded by using the stability study and it defines the F1 is stable. So, herbal ointment which is non-toxic, safe; effective by using herbal extract would be highly acceptable.

REFERENCES

1. Chatterjee Arjit: Neem seed extract may be used as Topical ointment to treat wound infections caused by pseudomonas aeruginosa: International journal of herbal medicine; 2016.
2. Mayuri Kale , Anilkumar aher, Shraddha dhanokar: Authentication of Azadirachta indica (Neem) Plant by Pharmacognostic, physicochemical and phytochemical evaluation; Current trend and pharmaceutical chemistry; 2020 2(3).
3. Lamia Abdalla Gadien, Abdel Karim M., Abdel Karim, Eltayeb Suliman Elamin: Antimicrobial and wound healing activity of Neem fruits extract, ointment and gel formulas; International journal of innovative Pharmaceutical sciences and research; 2015 3 (8).
4. Shahnawaz ahamad M, Zubair J, Shafia M et al. A Concise Review on Biological activity of *Tridax procumbens* Linn; Organic chemistry current research; 2017.6 (1)
5. Joshua S. Boateng, Kerr H. Matthews, Howard N.E. Stevens, Gillian M. Eccleston: Wound healing Dressing and drug delivery system: Journal of pharmaceutical science 2007; 97(8)
6. S. Guo and L.A. DiPietro: Factors Affecting Wound Healing. 89(3)
7. Ana cristina de oliveira gonzalez, Tila Fortuna Costa, Zilton de Araujo Anaraea; Wound healing- A literature Review: 91(5) . 2016.
8. T velnar, T Bailey and V Smrkolj; The Wond Healing Process: An Overview Of The Cellular and Molecule Mechanism; 37 (5) ;The Journal of international Medical Research
9. Venugopalan S, Visweswaran N. Neem (*Azadirachta indica*): Prehistory to contemporary medicinal uses to human kind 7th edition . Asian Pacific Journal of Tropical Biomedicine,
10. .Abdullahi R. Abubakar and Mainul Haque: Preparation of medicinal plants: Basic extraction Fractionation procedures for experimental purposes ;Journal of Pharmacy and Bioallied Sciences; 2020.
11. Wubante Demilew, Getnet Mequanint Adinew, and Seyfe Asrade; Evaluation of wound healing activity of the crude extract of leaves of *Acanthus polystachyus* Delile (*Acanthaceae*). 2018.
12. Aravinda Nalla, Krishna Mohan Chinnala. Formulation and evaluation of herbal ointment for antimicrobial activity. World journal of pharmaceutical and medical research; 2017.
13. Dr. Ashok A. Hajare, Dr. Durgacharan A. Bhagvat : A Textbook of Pharmaceutics ĩ Nirali Prakashan.
14. Samanta Krishanu. Formulation and evaluation of herbal ointment using leaves of *Acacia nilotica* (L.) Delile extract. 1st edition. International journal of advances in pharmacy and biotechnology; 2020.
- [15] Swati Siddheshwar Londhe, Mangesh Gautam Bhosale, Amol. Arun Joshi, Geeta Sapkale , Mohini Khandagale, Amitkumar Jadhav. Formulation and Evaluation of Polyherbal Ointment. 3rd edition. International Journal of Advances in Pharmacy and Biotechnology; 2017.
16. Himaja. N Formulation and evaluation of herbal cream from *Azadirachta Indica* ethanolic extract. 1st edition. International journal of research in drug and pharmaceutical science; 2017.
17. Dr. Abhijeet S. Kulkarni, Umesh D. Jirole, Vivek S. Zade, Niraj B. Nikam Pallavi R. Magdum. FORMULATION AND EVALUATION OF HERBAL JAGGERY BASED IMMUNE-BOOSTER. 11th edition. World Journal of Pharmaceutical Research; 2022.