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TO DESIGN AND CHARACTERIZATION OF HERBAL OINTMENT BY USING *MARTYNIA ANNUA, GOSSYPIUM HERBACEUM* WITH OTHER POLYHERBS FOR ITS WOUND HEALING POTENCY

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ABSTRACT
Wound healing is one of the most complex processes in the human body. It involves the spatial and temporal synchronization of a variety of cell types with distinct roles in the phases of haemostasis, inflammation, growth, re-epithelialization, and remodelling. With the evolution of single cell technologies, it has been possible to uncover phenotypic and functional heterogeneity within several of these cell types. There have also been discoveries of rare, stem cell subsets within the skin, which are unipotent in the uninjured state, but become multipotent following skin injury. Unravelling the roles of each of these cell types and their interactions with each other is important in understanding the mechanisms of normal wound closure. Changes in the microenvironment including alteration in mechanical forces, oxygen levels, chemokines, extracellular matrix and growth factor synthesis directly impact cellular recruitment and activation, leading to impaired states of wound healing. In conclusion. Wound healing is a multiphase process involving well- calibrated and synchronized responses to an injury to the skin.

KEYPOINT: Herbal Ointment, Wound Healing, *Martynia Annua, Tridax Procumbens, Gossypium Herbaceum, Curcuma Longa, Azadirachta Indica.*

1. INTRODUCTION
Herbal therapy predominates in traditional medicine as well as in alternative medicine practiced in the developing and the developed world. The widespread interest in drugs derived from plants is because of the belief that plants are safe and dependable, and with lesser side effects. Review of literature reveals that traditional plant drug is beneficial for several skin related problem and for wound healing.

A polyherbal formulation consist of than one herb. It is known that plants have different phytoconstituents which are responsible for the various activities that are attributed to them and when a combination of plants with these constituents are combined together it may show better activity when compared to the individual extract. But at the same time presence of many constituents may lead to chemical incompatibility which may result in instability. Hence it is a challenging task to formulate a stable polyherbal formulation. Thus, the main objective of the present study is to formulate and evaluate a polyherbal formulation which may be used as a potent wound healing agent. In the present study an attempt has been made to formulate and evaluate a polyherbal formulation consisting of the extract of *Martynia annua, Gossypium herbaceum, Tridax procumbence, Azadirachta indica and Curcuma longa* for its wound healing activity. These plants have been used traditionally for treating skin disease, wounds and inflammation; hence an ointment prepared from these plants could be an effective treatment for different types of wounds.[1]

Wound
A wound is defined as an injury to the body (as violence, accident, surgery) that typically involves laceration or breaking of a membrane such as the skin and usually damage to underlying tissues. There are two types of wounds:

1. Open Wounds

- Incisions or Incised Wound: - Caused by clean, sharp-edged object such as a knife, razor or glass splinter.
- Laceration: - A messy looking caused by tearing or crushing force. Lacerations and incisions may appear linear (regular) or stellate (irregular). The term laceration is commonly misused in reference to incisions.
- Abrasions (Grazes): - Superficial wounds in which the topmost layer of the skin is scraped off. Abrasions are often caused by a Sliding fall onto a rough surface such as asphalt, tree bark or concrete.
- Avulsions: - Injuries in which a body structure is forcibly detached from its normal point of insertion. A type of amputation where the extremity is pulled off rather than cut off.
- Punctured Wounds: - Caused by an object puncturing the skin, such as splinter, nail or needle.

2. Closed Wounds

- **Hematoma:** It is defined as a collection of blood outside of blood vessels. It occurs because the wall of the blood vessel (artery, vein or capillary) has been damaged and blood has been leaked into tissues. Hematoma is a term used to describe bleeding which more or less clotted, different from hemorrhage which signifies active ongoing bleeding. A bruise is an example of hematoma. During surgery, hematomas are caused by an injury to the wall of blood vessels, promoting blood to seep into the surrounding tissues.
- **Crush Injury:** Caused by a great or extreme amount of force applied over a long period of time. Wound healing is a complex and dynamic process of replacing devitalized and missing cellular structure and tissue layers. The human adult healing process can be divided into 4 phases that's are hemostasis, inflammatory, proliferation and remodeling. Wound healing involves an interaction between epidermal cells, dermal cells, extra cellular matrix, controlled angiosperms and plasma derived proteins which is all coordinated by an array of cytokines and growth factors.

Pathophysiology of Wound Healing: It consist of 4 phases:

- Hemostasis
- Inflammation
- Proliferation
- Remodeling

Hemostasis phase: It is mechanism that leads to cessation of bleeding from a blood vessel. It is a process that involves formation of fibrin that clots the blood. This cascade culminates into the formation of a "plug" that close up the damage site of the blood vessel controlling the bleeding.

Inflammation phase: Inflammatory phase focus on destroying bacteria and removing debris that prepare the wound bed for the growth of new tissue. During this phase, Neutrophils enter the wound to destroy bacteria and remove debris. These often increase between 24 & 48 hours after injury. As the white blood cells called macrophages arrive to keep on cleaning debris. These cells also secrete growth factors and proteins that attract immune system cells to the wound to facilitate tissue repair. These phase lasts 4-6 days and is often associates with edema, erythema, heat and pain.

Proliferation phase: - These focuses on filling and covering the wound. The proliferative phase consists of the 3 distinct stages: filling the wound, contraction of the margins and covering the wound which is also known epithelization. This phase lasts from 4-24 days.[2]

Remodeling phase: During the remodeling phase the new tissue slowly gains strength and flexibility. In this phase, collagen fibers reorganize and increase in tensile strength of the tissue.[2]

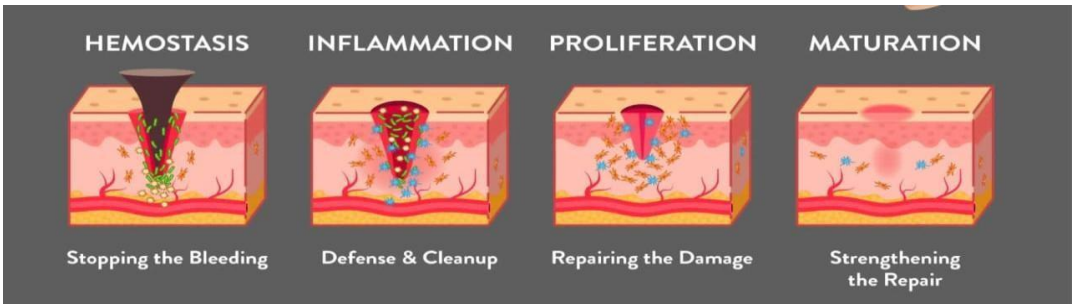


Fig. No. 01: Stage of wound healing.

2. Plant Profile

2.1 *Martynia annua*

- Botanical name- *Martynia annua*
- Kingdom- plantae
- Order- lamiales
- Family- martyniaceae
- Genus- martnia L,
- Species- martynia annua
- Popular name- Bhagnakhi, Devil's, Tiger's claw and Cats claw.
- Part use- leaves
- Habitat- It is native to Mexico, central America and the Caribbean.
- Chemical constitute- Alkaloids, glycosides, terpenoids, protein and amino acid, carbohydrate, flavonoids.

- Uses-
  - it is used to treat wound healing.
  - It used as antiseptic.
  - They are used for various skin condition, including aczema.



Fig. No: 02 *Martynia annua.*

2.2 *Tidax procumbens*

- Botanical name - *Tridax procumbens*
- Kingdom - plantae
- Order-Asteraceae
- Family -Asteraeae
- Genus -Tridax
- Species -procumbens
- Popular name-Kamar modi, coat button, tridax daisy.
- Part use- leaves
- Habitat- tridax are common weed found in sunny, disturbed habitats a across tropical and subtropical regions.
- Chemical constitute- Alkaloids (akuammidine), flavonoids (quercetin and catechin), terpenoids (oleanolic and lupeol), saponins.

- Uses-
  - The leaves of *T. procumbens* have antiseptic, insecticides properties.
  - It has been used for wound healing and as an anticoagulant, antifungal.



Fig. No: 03 *Tridax procumbens.*

2.3 *Gossypium herbaceum*

- Botanical name- *Gossypium herbecum*
- Kingdom- plantae
- Order- malvales
- Family- Malvaeae
- Genus- gossypium
- Species- G. herbaceum
- Popular name- levant cotton, kapas, Asiatic cotton.
- Part use- leaves
- Habitat-Gossypium herbecum is native to the semi-arid regions of sub-saharab Africa and arabia.
- Chemical constitute- Carbohydrate (raffinose and starch), flavonoids (quercetin and gossypritin), steroids (sitosterol and ergosterol), terpenoids (caryophyllene).

- Uses-
  - It can help in managing itching and irritation in sensitive area,
  - Acts as an analgesic and supports wound healing.
  - The herbs is known for its anti0inflammatory.



Fig. No: 04 *Gossypium herbecum*.

2.4 *Curcuma longa*

- Botanical name- *Curcuma longa*
- Kingdom- plantae
- Order- Zingiberlas
- Family- zingiberaeae
- Genus- curcuma
- Species- C. longa
- Popular name- Haldi, curcuma valeton and curcuma tinctoria.
- Part use- Rhizome (underground stem)
- Habitat- Curcuma longa, or turmeric, is a cultivated plant native to south and southeast asia that thrives in warm, humid tropical.
- Chemical constitute- curcumin demethoxycurcumin and bisdemethoxycurcumin as curcuminiods (3-6%) are major polyphenolic in turmeric rhizomes.
- Uses-
  - It is used chronic inflammation leads to destruction of normal tissue injury.
  - It Can help with skin healing and condition like eczema.
  - It Can help reduce pain and swelling.



Fig. No. 05: *Curcuma longa.*

2.5 *Azadirachta indica*

- Botanical name- *Azadirachta indica.*
- Kingdom- plantae
- Order- sapindals
- Family- meliaceae
- Genus- Azadirachtra
- Species- A. indica
- Popular name- Neem, margosa and Indian lilac.
- Part use- leaves
- Habitat- Its natural habitat includes dry, deciduous and mixed forests.
- Chemical constitute- Carbohydrate (quercetin), Fatty acids (glycerides of oleic acid about 50% and stearic about 20% acids).

- Uses-
  - Neem has an anti-inflammatory property which help to reduce fungal infections.
  - It used as antioxidant.
  - It used as antibacterial properties can help with eczema and wound healing.



Fig. No. 06: *Azadirachtra indica.*

3. MATERIAL AND METHOD

LIST OF DRUG AND EXCIPIENTS

Table No. 01: List of drug and materials.

Sr.NO.	MATERIALS	SOURCE
1	*Martynia annua*	Local area of Gondia
2	*Tridax procumbens*	MIBP, Gondia
3	*Gossypium herbaceum*	Local area of Gondia
4	*Curcuma longa*	Local area of Gondia
5	*Azadiractha indica*	Local area of Gondia
6	Wool fat	MIBP, Gondia
7	Hard paraffin	MIBP, Gondia
8	Cetostearyl alcohol	MIBP, Gondia
9	White soft paraffin	MIBP, Gondia
10	camphor	MIBP, Gondia
11	Rose water	MIBP, Gondia

INSTRUMENTS

Table No. 02: list of Instrument.

Sr.No.	INSTRUMENTS	MANUFACTURE
1	Soxhlet Apparatus	Labline
2	Ph Meter	Systronics Globe Instrument
3	Brookfield Viscometer	Brookfield Engineering Laboratories
4	Digital Balance	Kerro P3
5	Digital Autoclave	ASI-125
6	Mixer Grinder	Jyoti
7	B.O.D Incubator	HMG Gondia
8	Hot Air Oven	Metalab Scientific Industries

Preparations of plant extracts

The dried plant materials (leaves) are 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 polyherbal plant done by hot-continuous method/Soxhlet extraction method, in which 50gm 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 takes 24 hours to complete extraction process and to collect extract in a boiling flask. Collected extract evaporate on water bath for 2 hours and we get concentrated extract.[10]

% Yield = Weight of extract/weight of ground powder materials*100.[3]

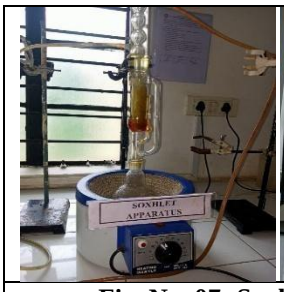




Fig. No. 07: Soxhlet extraction.

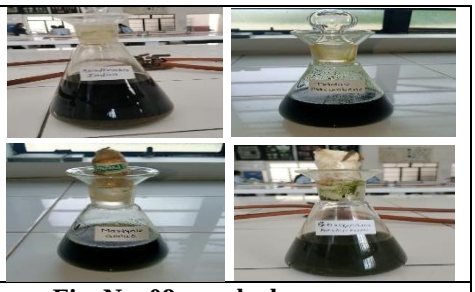


Fig. No. 08; crude drug extract.

- **Method of preparation**

- Clean all the glassware and dry them properly as per SOP.
- Weight all the ingredients properly.
- Take hard paraffin and cetostearyl alcohol. Melt them in porcelain dish and kept on water bath.
- To above melted mixture adds wool fat, white soft paraffin, camphor and extract then stir well.
- Add turmeric and maintain 70-75 ℃. Stir continuously until a uniform ointment is formed.
- After melting all ingredients, removed it from a porcelain dish.
- Pour it into an air tight container and place it in a cool and dry place.[7]

- **FORMULA**

Table No. 03: Control batch formulation of herbal ointment.

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

- **BATCHES PREPARED**

Table No. 04: formulation of preparation of herbal ointment.[5]

Sr. No.	Ingredients	Uses	F1 (20g)	F2 (20g)	F3 (20g)
1	*Martynia annua extract*	Faster epithelialization	0.5gm	0.75gm	1gm
2	*Tridax procumbens extract*	Collagen formation	0.5gm	0.75gm	1gm
3	*Gossypium herbaceum extract*	Promoting tissue formation	0.5gm	0.75gm	1gm
4	*Curcuma longa extract*	Antioxidant property	0.5gm	0.75gm	1gm
5	*Azadirachta indica extract*	Antibacterial property	0.5gm	0.75gm	1gm
6	Cetostearyl alcohol	Ointment base	1gm	1gm	1gm
7	White soft paraffin	Ointment base	1gm	1gm	1gm
8	Wool fat	Ointment base	1gm	1gm	1gm
9	Hard paraffin	Ointment base	0.25gm	0.25gm	0.25gm
10	camphor	Cooling effect	0.7gm	0.7gm	0.7gm



4. PRELIMINARY PHYTOCHEMICAL SCREENING[9]

A) TESTS FOR CARBOHYDRATES

a) General test

Molisch's test (General test): To 2-3 ml aqueous extract, add few drops of alpha-naphthol solution in alcohol, shake and add conc. H_2SO_4 from sides of the test tube. Violet rings formed at the junction of two liquids.

b) Tests for reducing sugars

1. **Fehling's test:** Mix 1ml Fehling's A and 1ml Fehling's B solution, boil for one minute. Add equal volume of test solution. Heat in boiling water bath for 5-10 min. First yellow, then brick red ppt.is observed.
2. **Benedict's test:** Mix equal volume of benedict's reagent and test solution in test tube. Heat in boiling water bath for 5 min. solution appears green, yellow or red depending on amount of reducing sugar present in in test solution.

c) Test for non- reducing polysaccharide (starch)

1. **Iodine** test: Mix 3ml test solution and few drops of dilute iodine solution. Blue colour appears, it disappears on boiling and reappears on cooling.
2. **Tannic acid test for starch:** With 20% Tannic acid, test solution gives ppt.

B) TESTS FOR GLYCOSIDES

1. TEST FOR CARDIAC GLYCOSIDES

a. **Balijit's test:** A section shows yellow to orange colour with sodium picrate.

b. **Liebermann's test (test for bufadenoloids): T**wo millimeters of the plant extracts was added to2.0ml of acetic anhydride and cooled in ice, sulfuric acid was added carefully along the side of the test tube. A colours change from violet to blue green indicates the presences of steroids nucleus (i.e. glycone portion of the cardiac glycoside).

2. TESTS FOR ANTHRAQUINONE GLYCOSIDES

i. **Borntrager's test for anthraquinone glycoside:** To 3ml extract, add dil.H_2SO_4 Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well. separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.

3. TESTS FOR SAPONIN GLYCOSIDE

a. **Foam test:** Shake the drug extract or dry powder vigorously with water. Persistent stable foam

observed.

4. TESTS FOR CYANOGENETIC GLYCOSIDE

i. To dry drug powder or extract, add 3% aqueous mercurous nitrate solution. Metallic mercury forms.

5. TESTS FOR PHENOLS [FERRIC CHLORIDE TEST]

To 1ml of alcoholic solution of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green color indicated the presence of phenols.

6. TESTS FOR ALKALOIDS [MAYER'S TEST]

1.36gm of mercuric chloride dissolve in 60ml and 5gm of potassium iodide were dissolved in 10ml of distilled water respectively. These two solvents were mixed and diluted to 100ml using distilled water. To 1ml of acidic aqueous solution of samples few drops of reagent was added. Formation of white or pale precipitate showed the presence of alkaloids.

7. TEST FOR FLAVONOIDS

In test tube containing 0.5ml of alcoholic extract of the samples, 5 to 10 drops of diluted HCl and small amount of Zn or Mg were added and the solution was boiled for few minutes. Appearance of reddish pink or dirty brown colour indicated the presence of flavonoids.

8. TEST FOR TANNINS

Lead acetate test: In a test tube containing about 5ml of aqueous extract, a few drops of 1% solution of lead acetate were added. Formulation of yellow or red precipitation indicated the presence of tannins.[12]

9. TEST FOR TERPONOIDS

2ml of chloroform and 1ml of conc. H_2SO_4 was added to 1 mg of extract and observed for reddish brown color that indicated the presence of quinone.

10. TEST FOR STEROIDS [SALKOWASKI'S TEST]

About 100mg of dried extract was dissolved in 2ml of chloroform Sulphuric acid was carefully added to form a lower layer. A reddish-brown color at the interface was an indicative of the presence of steroidal ring.

11. TEST FOR SAPONINS

A drop of sodium bicarbonate was added in a test tube containing about 50ml of an aqueous extract of sample. The mixture was shaken vigorously and kept for 3min. A honey comb like froth was formed and it showed the presence of saponins.



Fig. No. 09: Preliminary phytochemical screening.

PRELIMINARY PHYTOCHEMICAL SCREENING OF PLANT EXTRACT

Preliminary phytochemical test for identification of phytoconstituents of polyherbal drugs.

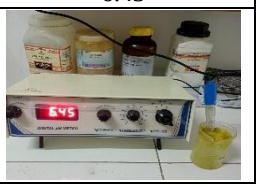
Table No. 05: Phytochemical Screening.

Sr. No.	Plant constituents	Test/ reagents	*Martynia annua*	*Tridax procumbens*	*Gossypium herbaceum*	*Curcuma longa*	*Azadira-chtaindica*
1.	Alkaloids	Mayer's test	+	+	+	+	+
2.	Flavonoids	Shinoda test	+	+	+	+	+
3.	Glycoside	Aqueous NaOH test	+	+	+	-	+
4.	Steroids	Salkowaski's test	+	+	+	+	-
5.	Cardiac glycosides	Kellar Kellani's test	-	+	+	+	+
6.	Saponins	Foam test	+	-	+	+	+
7.	Phenol	Ferric chloride test	+	+	+	-	+
8.	Tannins	Lead acetate test	+	+	+	+	+
9.	Terpenoids	Salkowaski's test	+	+	-	+	+

5. EVALUATION OF HERBAL OINTMENT[8]

pH of ointment: The pH of polyherbal ointment was found to be –

Table No. 06: & Fig No. 10: pH of ointment.

Sr. No.	TESTS	FORMULATION		
1.	pH	F1	F2	F3
		6.20	6.39	6.45
				

PHYSICLAL APPEARANCE: Color, odor, thickness was studies.

Table No. 07: Physical Appearances.

Sr. No.	TESTS	FORMULATION		
		F1	F2	F3
1.	Appearances	Yellowish- green	Yellowish- green	Yellowish-green
2.	Odour	characteristic	characteristic	characteristic
3.	Consistency	viscous	viscous	viscous
4.	Homogeneity	Good	Good	Good

SOLUBILITY: Solubility of herbal ointment in water, alcohol, ether, chloroform and 5% NaOH was found to be-

Table No: 08 & Fig. No. 11: Solubility of ointment.

FOR FORMULATION F1, F2, F3					
Water	Alcohol	Ether	chloroform	Glycerin	ropylene glycol
Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
					

SKIN IRRITATION TEST: The skin irritation test of given formulation of polyherbal ointment show no reaction.

Table No. 09: Skin Irritation test.

Sr. No.	Skin irritation Test (time)	Formulation		
		F1	F2	F3
1.	10min	No reaction	No reaction	No reaction
	1 hour	No reaction	No reaction	No reaction
	2 hours	No reaction	No reaction	No reaction
	3 hours	No reaction	No reaction	No reaction

SPREADABILITY: The spread ability test of herbal ointments was found to be-

Table No. 10: Spreadability.

Sr. No.	Tests	Formulation		
1.	Spread ability	F1	F2	F3
		8sec	10sec	6sec

VISCOSITY: viscosity of the ointment was found to be-

Table No. 11 & Fig. No. 12: Viscosity of ointment.

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

WASHABILITY: Washability of the polyherbal ointment was found to be –

Table No: 12: Washability of ointment.

Sr. No.	Tests	Formulation		
1.	Washability	F1	F2	F3
		Slightly washable and sticky	Slightly washable and sticky	Slightly washable and sticky

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

Table No. 13: & Fig. No. 13: Antibacterial activity or herbal ointment.[6]

Anti-microbial test of E. coil					
Conc.(ug)	F1	F2	F3	Standard	
	Zone of inhibition			(Neomycin)	
100	3mm	3mm	5mm	7mm	
Anti-microbial test for S. Aureus					
Conc.(ug)	F1	F2	F3	Standard	
	Zone of inhibition			(Neomycin)	
100	4mm	4mm	6mm	7mm	

6. CONCULSION

From the present investigation it has been revealed that Polyherbal ointment of plant Martynia annua, Tridax procumbens, Gossypium herbaceum, Azadirachta indica & Curcuma longa can be formulated using simple ointment base with other ingredients and the evaluation of Physical parameter show satisfactory observation. From the different evaluation and antibacterial test by cup plate method suggest that the prepared herbal ointment give potent action against wound healing containing pathogens i.e. S. aureus and E. coli which was comparable with standard antibiotic i.e. neomycin, and it responsible for the faster tissue growth and collagen formation. The formulation F1, F2 and F3 were prepared with the different proportions of extracts the study shows F3 batch was found to be optimum. It consists of 1gm of all polyherbs. This batch was found to be better and effective for the wound healing and gives optimum wound healing potency.

7. Future Scope

These polyherbal ointment prepares by us that should be used in future for the eczema and fungal infection.

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