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FORMULATION AND EVALUATION OF TOPICAL HERBAL OINTMENT FROM TRIDAX PROCUMBENS L.AND AZADIRACTHA INDICA LEAVES EXTRACT FOR WOUND HEALING ACTIVITY.

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ABSTRACT:

The main objective of the present work is to formulate and evaluate the formulations of wound healing ointment of Tridax procumbens and Azadiractha Indica extracts to give multipurpose effect and compare with marketed ointment. The ethanolic extracts contain phytoconstituents such as tannin, saponins, flavonoids, terpenoids and anthraquinones. After completion of formulations, it was evaluated for its physicochemical parameters like color, odour, pH, spreadability, extrudability, consistency, solubility and washability. It shows good antibacterial activity against E.coli and was determined by agar well diffusion method. Also the formulations were evaluated for their stability study at various test which shows no change in parameter test. Thus it could become a media to use the medicinal properties of Tridax procumbens and Neem effectively and easily as formulation forms like ointment for wound healing activity.

After the formulation of herbal ointment it was evaluated for their stability study of ointment on first day, after three months and after six months and also studied the comparitive study of marketed ointment preparation. The results showed that Herbal ointment passed all Parameters related to marketed ointment. Formulated herhal ointment is as good as marketed ointment.

Keywords: Tridax Procumbenes Linn, Azadirachta Indica, Leaves extract, Herbal onitment, Wound healing activity and Comparative study.

INTRODUCTION:

Herbal medicines are plant based medicines made from different combinations of plant parts. E.g roots, stems, leaves, bark, fruit, seeds, or flowers of various plants known or believed to have medicinal properties. The word "drug" comes from the French word drogue, meaning "dried herb", this drug is a purified form of the specific substance in the plant that is proven to have a beneficial medical effect.. Each part can have different medicinal uses and the many types of chemical constituents require different extraction methods. Herbal medicines which formed the basis of health care throughout the world since theearliest days of mankind are still widely used, and have considerable importance in international trade. Recognition of their clinical, pharmaceutical and economic value is still growing, although this varies broadly between countries. Plant is an important source of medicine and plays a key role in world health. Medicinal herbs or plants have been known to be an important potential source of therapeutics or curativeaids[1].

The use of medicinal plants has attained a commanding role in health system all over the world The reasonsfor this is because of their better cultural acceptability, better compatibility and adaptability with the human body and pose lesser side effects. Medicinal plants may be defined as those plants that are commonly usedin treating and preventing specific ailments and diseases and that are generally considered to be harmful to humans. Through recent researches on herbal plants or medicine, there have been great developments in the pharmacological evaluation of various plants used in traditional systems of medicine[2]. India is a birth placeof indigenous medicine such as Siddha, Ayurveda and Unani. Where many herbs have been used for treatment of human ailments. According to the World Health Organization (WHO) about 80% of developing countries depend on traditional medicines for their primary health care needs[3].

Advantage :-

- Lower risk of side effects
- These types of formulations are best for the people who are allergic to various types of drugs
- These types of medicines do not have any types of side effects and they are free
- low/minimum cost and effectiveness
- Potency and efficacy are very high

Disadvantage :-

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

Wound Healing Activity:

A wound is a physical trauma where the skin is torn, cut, or punctured. On exposure to air, microorganisms enter the wound which leads to wound contamination and finally the development of infection. It is a process that is fundamentally a connective tissue response. The initial stage of this process involves an acute inflammatory phase followed by the synthesis of collagen and other extracellular macromolecules that helps in the formation of a scar. This intricate process is initiated in response to an

injury that restores the function and integrity of damaged tissues. The widespread interest in drugs derived from plants is because of the plants are safe and dependable and with lesser side effects[4].

A review of the literature reveals that traditional plant drugs are beneficial for several skin-related problems and for wound healing. World Health Organization (WHO), as well as our country, has been promoting the use of traditional medicine because they are less expensive, easily available, andcomprehensive, especially in developing countries. Numerous studies have been conducted with the extracts of Tridax procumbens L. belongs to the family of Asteraceae and Azardiachta indica belongs tothe family of Meliaceae. Along with other dosage forms herbal drugs are also available in the form of ointment which is semisolid preparation used topically for several purposes e.g. as protectants, antiseptics, emollients, antipruritics, Keratolytics, and astringents.

Classification of Wounds

Wounds are classified as open wounds and closed wounds on the basis of the underlying cause of wound creation and as acute and chronic wounds on the basis of the physiology of wound healing.

A. Open wound:

Through the open wound, blood escapes the body and bleeding is clearly visible.

1) Incised Wounds:

It is an injury with no tissue loss and minimal tissue damage. It is caused by a sharp object such as aknife. Bleeding in such cases can be profuse, so immediate action should be taken.

2) Laceration wound or tears Wounds:

This is a nonsurgical injury in conjunction with some type of trauma, resulting in tissue injury and damage.

3) Puncture Wounds:

They are caused by some object puncturing the skin, such as a needle or nail. Chances of injection inthem are common because dirt can enter into the depth of the wound.

4) Penetration Wounds:

Penetration wounds are caused by an object such as a knife entering and coming out from the skin.

B. Closed wounds:

In closed wounds, blood escapes the circulating system but remains in the body. It includes contusion or bruises, hematomas or blood tumors, crush injury, etc.

1) Contusions or bruises:

Bruises are caused by blunt force trauma that damages tissue under the skin.

2) Hematomas or blood tumors:

They are caused by damage to a blood vessel that consequently causes blood to collect under the skin.

3) Crush injury:

Crush injury is caused when a great or extreme amount of force is applied to the skin over a long periodof time.

4) Acute Wounds:

An acute wound is a tissue injury that normally proceeds through an orderly and timely reparative process that results in sustained restoration of anatomic and functional integrity. It is usually caused by cuts or surgical incisions and complete the wound healing process within the expected time frame.

5) Chronic wounds:

Chronic wounds are wounds that have failed to progress through the normal stages of healing and therefore enter a state of pathologic inflammation. Local infection, hypoxia, trauma, foreign bodies, and systemic disorders such as diabetes mellitus, medications are the most frequent causes of chronic wounds. Chronic wounds may result from various causes, including naturopathic, pressure, arterial andvenous insufficiency, burns, and vasculitis.

Factors affecting wound healing :

Local factors	Systemic factors
Oxygenation	Disease: diabetes, jaundice, obesity
Infection	Medication: glucocorticoid steroids, NSAIDS, chemotherapy
Poor blood supply	Alcoholism, smoking,
Wound infection	Nutrition
Poor wound healing	Immune suppression

Table no: 1(FACTORS AFFECTING WOUND HEALING)

Literature review-

1.Lokesh Prasad MS et.al.(2017)[5]:

In these article the author studied about the formulation and evaluation of herbal formultions of ointment, cream and gel of tridax procumbens and area catechu extracts this also studied about physicochemical paramerets and stability study at various temperature conditions

2.R.A.Mutha et.al (2019)[6]:

The author discusses the introduction of Tridax Procumbens pharmacological structure, the chemical composition of different pharmacological activities are hepoprotective effect immunomodulating property, wound healing activity, antidiabetic activity, antimicrobial activity, anti-inflammatory and antioxidant activity

3.Amita Pandey et.al (2014)[7]:

In this article author briefly discussed pharmacognostic parameters of Tridax procumbens Linn. and also studied Macroscopical and microscopical character, Pre-phytochemical and Pharmacological studies. This review focus on the wide pharmacological activities of tridax procumbens.

4. Chris A. Alablor et.al.(2021)[18] :

In this article, the author studied the antibacterial potency of herbal ointment formulation with methanolic extract of Cassia alata and was evaluated. This study shows that cassia alata has antibacterial activity and also has high potential as an antibacterial agent when formulated as an ointment for topical use and explains the physical evaluation of formulated ointments of the plant in the treatment of common skin conditions.

5.Manimaran S et. al. (2014)[19]:

In this article author's work on prepared cream formulation was screened for their antibacterial and antifungal activity using gentamicin and amphotericin B as standard drugs for bacteria and fungi respectively. The the study was observed that the prepared topical herbal cream formulation showed significant antimicrobial and wound healing activities

6.V. Bharti et.al (2012)[22]:

Here in they have discussed the antibacterial activity of methanolic extract of tridax procumbens and examined against Escherichia coil, klebsiella pneumonia, salmonella typhi,bacillus cereas, and staphylococcus aureus .tridax procumbens showed effective inhibition against the staphylococcus aureusthan compared to other organisms

7.Anil saini et.al. (2018)[15]:

The author studied about classification of tridax procumbens. chemical constituents of plant, their extract processes and various pharmacological properties. This research work to extract any new or known chemical constituent of tridax procumbens.

8.Rupesh Thakur et. al. (2011)[23]:

Here in they have discussed the various plant products used in the treatment of wounds, herbal extracts, in vitro assays, Phyto constituent analysis, drug administration, evaluation of wound healing, and drug formulation.

AIM AND OBJECTIVES:

Aim

To develop and evaluate poly herbal ointment for wound healing activity.

Objectives

- Collection of the selected plants.
- Preparation of herbal extracts.
- Phytochemical analysis for the prepared extracts.
- Development of herbal formulations for the prepared extracts.
- Characterization of the developed formulations.
- In vitro pharmacological evaluation of the developed formulation.

PLAN OF WORK

- Review of Literature
- Selection of suitable drug and excipients
- Preparation of herbal extracts

Preformulation study of the plant

- Morphological study
- Microscopic study
- Preliminary test
- Phytochemical analysis for the prepared extracts

Experimental study

- Formulation and development of wound healing ointment containing tridax and neem extract
- Optimize the evaluation test of the developed formulations
- In vitro antimicrobial study
- Stability study
- Comparative study

PLANTS SELECTED FOR THE PRESENT STUDY:

In presence study the following plants which have been demonstrated for wound healing activity are selected and were used for the development of herbal formulations.

General Description of Tridax Procumbenes[5]:-

Tridax procumbens Linn are in the daisy family which is flowering plant species.. It is growing primarily during rainy season. Tridax procumbens L. belongs to the family of Asteraceae andtridax procumbens Linn. is popularly called as "coat buttons'' because of the appearance of its flowers. It is a wild herb distributed throughout India. As per traditional use and pharmacological consideration,it is well known for Immunomodulatory activity, Wound Healing activity, Antimicrobial Activity, anti- bacterial and Antioxidant activity..

Tridax procumbence Plant Profile [6]:

Fig no -1(Tridax procumbence)

Family : Asterceae

Synonyms: Ptiloatephium Kunth, Mandonia wedd, Bartolia adans, Batolina adans.

Chemical constituent: alkaloid,s steroids, flavonoids, carotenoids, fatty acids,phytosterol, tannin,and minerals.

Medicinal Uses : wound healing, antiinflammatory, antimicrobial, anticoagulant, and antifungal.

Scientifical classification[6,7]:

Kingdom – Plantae **Sub -kingdom** – Tracheobionta

Division – Magnoliophyta **Class** – Magnoliopsida.

Sub-class – Asteridae **Order** – Asteraceae.

Genus – Tridax **Species** – Procumbens.

Morphological structure of tridax procumbens [5,6].-

LEAVES:

Fig no :2(leaves)

STEM:

- Leaves are irregularly toothed &generally arrow head shaped.

- They are simple, ovate, opposite, exstipulate, lanceolate & they are 3-7 cm.Wedge shaped base lead, shortly petioles, hairy on both surfaces.

- The plant stem is ascending 30-50cm height, branched, sparsely, rooting at nodes.

Fig no :3(stem)

FLOWER :

Fig no: 4(flower)

- The plant flowers looking like daisy.
- The flower is tubular, yellow, centered white or yellow flowers with there- toothedray florets.
- Inflorescence capitulum.
- It has two types of flower.
- Ray florets & dies florets with basalplacentation.

FRUIT:

Fig no: 5(fruit)

- Fruit is hard achene covered with stiff hairs & having a feathery.
- At the one end It has plume like white pappus

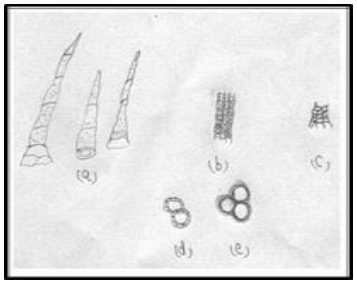
SEED:

Fig no: 6(seed)

- The plant seeds have produlous embryo endosperm is absent.

Microscopic study of tridax procumbence L.[7,8,9]:

Powder microscopy:



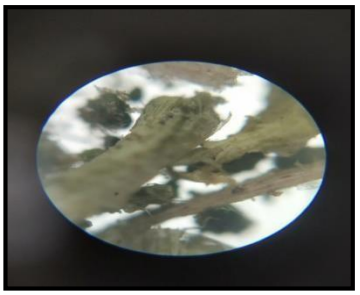


Fig no :7(powder microscopy)

- The powder appeared dark green, fine, odorless with a slightly bitter taste. The powder microscopy revealed the presence of different types of (glandular and non-glandular) Trichomes, trichome base, fibers, stone cells, and Laticifers with adjacent parenchyma. Spiral thickening vascular bundles were also present.

Petiole:

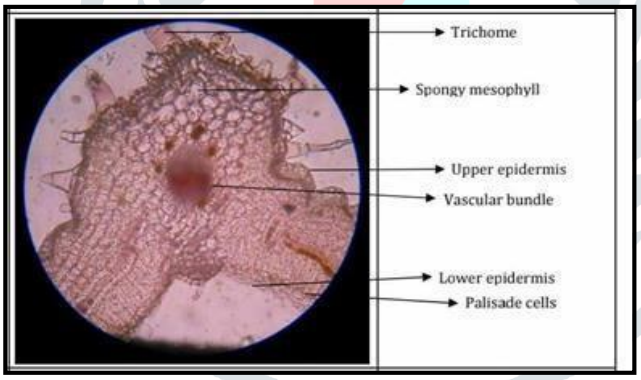


Fig no :8(T.S. of petiole)

Kidney shaped towards the distal end and crescent-shaped towards the laminal side. Single layered epidermis covered with cuticle and interrupted by simple, multicellular, 3-5 celled trichomes. Hypodermis 1-2 celled collenchymatous. Ground tissue parenchymatous; vascular bundles 5, the size of the vascular bundles varies from centre to margin i.e. Large too small. These are centripetal i.e. xylem surrounded by the phloem

Leaf:

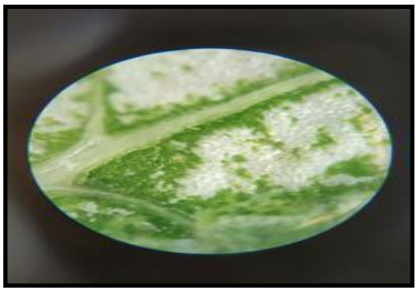


Fig no:9 (leaf)

T.S. leaf is dorsiventral, epidermis single-layered on both the surfaces and covered with thick cuticle. T.S. passing through the midrib region shows slight depression on the ventral side and slightly protuberated on dorsal size. Trichomes are simple, multicelled (3-6 celled), and more in number on the dorsal side. The basal cells of the Meristeel consists of a single centrally located collateral vascular bundle surrounded by some parenchymatous cells filled with dark content.

T.S. passing through the laminar region shows single-layered palisade cells just below the appearing epidermis followed by 5-7 celled mesophyll parenchyma mostly devoid of intercellular spaces.

General Description of Azadirachta indica[10,11]:

Azadirachta indica (AI) A. Juss (the neem tree) commomnly known as neem or nimtree, It belongs to the family Meliaceae and it is well known in India and neighboring countries.All parts of the neem tree have been used traditionally for the treatment of numerous ailments for instance bark as an analgesic, alternative, and curative of fever. Various chemical constituents, such as alkaloids,triterpenoids, glycosides, limonoids, flavonoids, fatty acids, and steroids from neem trees have been proven to exhibit anticarcinogenic, anti-inflammatory, antiulcer, antioxidant, immunomodulatory, antifungal, antibacterial, antiviral, antimalarial, antimutagenic, and antihyperglycemic properties.

Azadirachta indica Plant Profile[11,12] :

Fig no :10 (plant profile of azadirachta indica)

Family : meliaceae

Synonyms: Azadirachta indica, margosa ,roseship, melia azadirachta

Chemical constituent: Azadirachitin, salannin , meliantriol, nimbasterol, quercetin, glyceride,oleic acid and stearic acid

Medicinal Uses : Wound healing, treat acne , treat fungal infection,

Scientific Classification:

Kingdom : Plantae

Subkingdom: Tracheobionta

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Rosidae

Order : Sapindales

Family : Meliaceae

Genus : Azadirachta

Species : Indica

Morpholgical Study Of Azadiractha Indica[12]:

- **Leaves:**

i. They are imparipinnate, alternate, exstipulate, 3-6 cm long on long slender petioles; leaflets 7-17; alternate or opposite, very shortly stalked, 1-1.5 cm long.
ii. Colour: dark green.
v. Odour: typical.
vi. Taste: Bitter.

Fig no 11:(leaves)

- **Fruits:**

i. Shape: Ovoid, bluntly pointed, smooth drupe.
ii. Colour: Green (Young and unripe); Yellow to brown (Mature and ripe).
iii. Very scanty pulp and hard bony endocarp.
iv. Solitary with a thick Testa and embryo with foliaceous cotyledons in the axis of the scanty endocarp.

Fig no 12:(fruits)

- **Stem and Bark:**

Stem has a girth 1.8-2.4m and the bark is rough, hard, grey or dark grey, reddish brown inside with numerous oblique furrows and scattered tubercles.

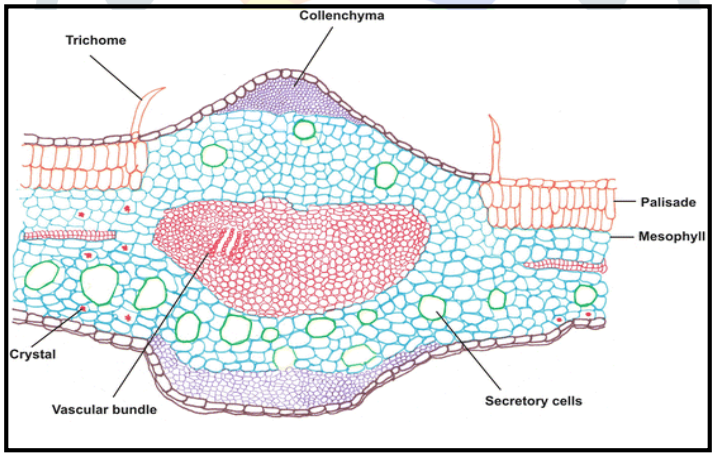
Fig no :13(stem &bark)

- **Flowers:**.

The flowers are hermaphrodite. White or pale yellow, small, scented and numerous. Flowers are very lax and in axillary panicles

Fig no: 14(flowers)

Microscopic study Of Azadirachta Indica:



TS. OF AZADIRACHTA INDICA

Fig no :15(T.S of azadirachta indica)

Neem lanina is isobilateral and shows two layer of palisade below upper and one below lower epidermis. Leaf bear unicellular covering trichomes, which are curved and with sharp apex on both the surfaces. The lower epidermis also shows the presence of few glandular trichomes with. multicellular head and unicellular stalk. Midrib region is biconvex in outline and shows 3-4 rows of collenchymatous cells beneath each epidermis.

Pharmacological Activities Of Tridax Procumbens L. and Azadirachta Indica :

Anti microbial activity[13]:

Whole plant has reported for its antimicrobial activity on various species of bacteria. A whole plant is squeezed between the palms of hands to obtain juice. Fresh plant juice is applied twice a day for 3-4 days to cure cuts and wounds. The extract of whole plant showed antibacterial activity .

Anti-oxidant Activity:

The production of free radicals at or around the wound may contribute to delay in wound healing through the destruction of lipids, proteins, collagen, proteoglycan and hyaluronic acidAgents that demonstrate a significant antioxidant activity may, therefore, preserve viable tissue and facilitate wound healing.

Anti-inflammatory activity :

Wounds in persistent inflammatory phase may delay the healing process. Preventing prolonged inflammatory phase hasten the healing process.

MATERIAL AND METHODS[14,15]:

Collection Of Plant Material:

First, we identified and collect the plant leaves 0f tridax procumbens and azadirachta indica from the different localities of nesari and its nearby areas and washed them thoroughly with distilled water. The cleaned plant parts are then allowed for the complete shade drying and then made to a fine powder with a mechanical grinder and stored in an air-tight container

Apparatus and chemicals used:

Soxhlet extractor, forceps, clamp stand, heating mantle, three round-bottomed flasks, reflux condenser, heating mantle and chiller, TLC plate, distillation apparatus, iodine chamber, and test tubes.Ethanol, Ethyl acetate, Silica gel and distilled water.

Prepration of Tridax Procumbens L. extract:

Fig no :16 (prepration of tridax procumbance l.extract)

The collected fresh leaves of tridax procumbens were dried in shade for 7 days. After drying plant material was coarsely powdered and kept in a well-closed container. About 100gm of powder of plant leaf was taken in soxhlet apparatus for 72 hours and extracted with [45 to 55°c] ethanol. The extracts were collected and concentrated using a rotary vacuum evaporator. The crude semisolid extract was collected and stored in a small vial. The extract was stored at 4°c until further use for formulation.

Prepration of azadirachta indica extract :

Fig no :17(preparation of azadirachta indica extract)

Leaves of the plant were collected and washed thoroughly with distilled water and shade dry for 10 days .Dried leaves were ground into powdered form. 100gm powder was imbibed with 350ml of 90% ethanol for 3 hours and transferred to a percolator with the addition of 150 ml of 90% ehanol

for maceration for 7 days with occasional stirring. Finally, the ethanolic extract was collected and concentrated to get blackish-green residue. The extract was stored in an airtight container in a cool and dark place.

Chromatographic evaluation of the extract:

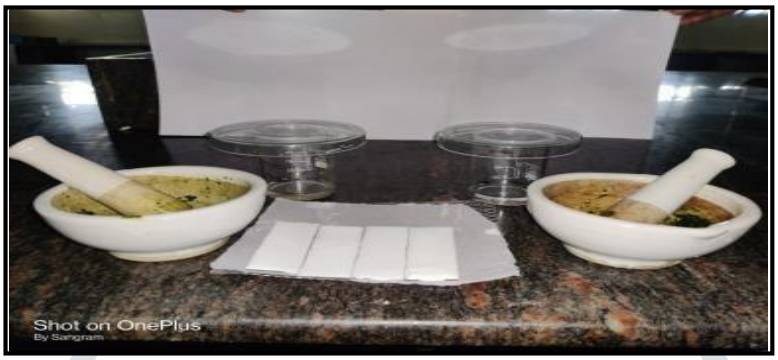


Fig. no.18: (Preparation of mobile phase and stationary phase)

Solvent system: Hexane: Ethyl acetate=3:1

Under sunlight **Under UV**

Fig . no.19 (TLC Observation)

PRELIMINARY PHYTOCHEMICAL SCREENING OF TRIDAX PROCUMBENS[16] :

Sr no.	Test	Observation	Inference
1	**Test for flavonoids-** **Alkaline reagent test** Extract+ 10% NaOH.	Formation of intense yellow colour.	Flavonoids is present
2	**Test for tanins –** A] 2ml alc. Extract +1% lead acetate B]4ml alc. Extract+4ml $Fecl_3$	A]yellowish ppt B]green colour indicates	A] presence of tannins B]presence ofcondensed tannins
3	**Test for saponin-** 5ml alc. Extract+20 ml distilled water then agitated in graduated cylinder for 15 minutes	Formation of foam	Saponin is present
4	**Test for Alkaloids-** 3ml conc.extract +1ml HCL heat for20min cooled and filter **Wagner test –** Filterate treated with wagner reagent.	Formation of brown reddishppt	Alkaloid is present
5	**Test for amino acid-** **Ninhydrin Test-** 2ml alc. Extract +2ml ninhydrin reagent,boil for 2min.	Formation of blue colour indicates	Amino acid is present
6	**Test for phenol-** **Ferric chloride test** Test extract+ 4 drops alc. Fecl3 Sol.	Formation of bluish black colour.	Phenol is present

Table no 2: (PRELIMINARY PHYTOCHEMICAL SCREENING OF TRIDAX PROCUMBENS)

PRELIMINARY PHYTOCHEMICAL SCREENING OF AZADIRACHTA INDICA[17]:

Sr no.	Test	Observation	Infer ence
1	**Test for Tannin-** 0.5gm of plant extract+2ml of water+heat on water bath,filter+1ml 10%$Fecl_3$	Formation of blue black solution	Tannin is present
2	**Test for flavonoids-** 5ml distilled water+0.2gm extract mixed+1ml of 1%$Alcl_3$ added	Formation of Light yellow ppt	Flavonoids is present
3	**Test for Glycoside-** 0.2gm extract &2.5ml dil.sulphuric acid mix ,boiled for 15 min,cooled,neutralize with 5ml each of fehling solution	Formation of brick red ppt	Glycoside is present
4	**Test for Amino Acid-** 0.2gm extract+5ml dist.water mixed, left for 3 hours then filter.2ml filtrate+0.1ml million reagent	Formation of yellow ppt	Amino acid is present

TABLE NO 3: (PRELIMINARY PHYTOCHEMICAL SCREENING OF NEEM)

Fig no.20: (Phytochemical test of Tridax Procumbens)

Fig no.21: (Phytochemical test of Azadirachta Indica)

Role of action of ingredients for ointments formulation :

Sr. no.	Name of ingredient	Role
1	Wool fat	Emollient.
2	White soft paraffin	Moisturizes the skin.
3	Cetostearyl alcohol	Emulsifier,surfactant,foam booster,viscosity increasingagent.
4	Stearic acid	Emulsifier,emollient,lubricant.

Table no 4 :(ROLE OF ACTION OF INGREDIENTS FOR OINTMENTS FORMULATION)

FORMULATION DEVELOPMENT :

Formulation Of Herabal Ointment:

Sr no	Name of ingredients	F1 (gm)	F2 (gm)	F3 (gm)	F4 (gm)
1	Wool Fat	1	1.5	1.5	1.5
2	Cetostearyl Alcohol	1	2	3	4
3	Stearic Acid	1	2	3	4
4	White Soft Paraffin	4.5	4	4	4

Table no 5:(FORMULATION OF HERBAL ONITMENT)

Sr no	Name of ingredients	F1 (gm)	F2 (gm)	F3 (gm)	F4 (gm)
1	Tridax Procumbens Extract	1	1	1	1
2	Neem Extract	1	1	1	1
3	Ointment base	7.5	9.5	11.5	13.5

Table no 6:(FORMULATION OF HERBAL ONITMENT)

PROCEDURE OF PREPERATION OF HERBAL OINTMENT[18,19]:

1.Initially ointment base was prepared by using wool fat, white soft paraffin, cetostearyl alcohol and steric acid weighing accurately and which was placed in evaporating dish on water bath. After the melting stirred gently to form homogeneous mixture and then cooling the ointment base.

2.Herbal ointment was prepared by mixing accurately weighed Neem and Tridax procumbence extract to the ointment base to prepare a smooth paste with 2 or 3 times its weight of base, gradually incorporating more base until to form homogeneous ointment, finally transferred in a suitable container.

Fig. no. 22(formulation batches of herbal ointment)

Evaluation test:-

1) **P^H:** P^H of prepared herbal ointment was measured by using a digital PH meter. The solution as ointment was prepared by using 10 ml of distilled water and set aside for 5 min.

2) **Colour and Odour:** Physical parameters like color and odor were examined by visual examination.

3) **Consistency**: Smooth and no greediness is observed.

4) **Spreadability**: The spreadability was determined by placing an excess of sample in between two slides which were compressed to uniform thickness by placing a definite weight for a definite time.Thetime required to separate the two slides was measured as spreadability. Lesser the time taken for the separation of two slides results in better spreadability.

Spreadability was calculated by the following formula:

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

Where, S- spreadability

M- weight of sample in gramT-

Time taken in seconds.

5) Extrudability: The formation were filled in a collapsible tube container. The Extrudability was determined in terms of the weight of formulation required to extrude o.5 cm of ribbon of ointment in10 sec.

6) Loss on drying: Loss was determined by placing the formulation in a petri dish on an oil bath and drying for the temperature at 105°C.

7) Solubility: Soluble in boiling water, miscible with alcohol, ether, and chloroform

8) Washability: Formulation was applied on the skin and then ease extend of washing with water and checked.

9) Non-irritancy: The formulation prepared was applied to the skin of a human being and observed forthe effect.

10) Stability study: Physical stability study tests of the formulation were carried out for first day,after three month and after six month at temperature 37^0 C for various physicochemical parameters .The formulation was found to be physically stable at different physicochemical parameter for six months.

11) Viscosity: The measurement of viscosity of prepared ointment was carried out with a Brook field viscometer .

Anti-microbial Study[22,23]:

- **Protocol:**

The nutrient agar media was used for antimicrobial study. E.coli microorganism culture was used andthe Incubation time was set up for 24hrs.

- **Method:**

Agar bore well diffusion method.

- **Procedure:**

E.coli suspension was introduced to each plate and 40ml of sterile nutrient agar media was poured into each sterilized plate. The plates were agitated carefully to allow homogeneous mixing of the agar with the text organism the plates were left on the flat solid surface and allow to harden. In each plate a 10ml of media was poured and was bored in the medium with a cork bore. The disc of the agar bore was removed by a sterilized dissecting needle while being careful not be damage the cups. In each plate, an equal amount of ointment formulation having the same strength was placed in the cup, and we are incubator at 37°C_+ 2°C for 24 hrs. in the incubator the entire operation was carried out under aseptic conditions and calculated the zone of inhibition obtained for prepare formulation shown in the figure.

Fig. no. 23 (Sterilization of apparatus and agar media)

RESULT AND DISSCUSION :

A) Preliminary Phytochemical Screening Of Tridax Procumbens L. And Azadirachta Indica :

Fig. no.24 :(Phytochemical test of Tridax Procumbens)

Fig No.25: (Phytochemical test of Azadirachta Indica)

B) PHYSICOCHEMICAL EVALUATION OF ONITMENT:

Sr No	Physicochemi-cal parameters	F1	F2	F3	F4
1	Appearance	Dark green	Dark green	Pale green	Pale green
2	Odour	Characteristics	Characteristics	Characteristics	Characteristics
3	Consistency	Smooth	Smooth	Smooth	Smooth
4	Spreadability (second)	7	7	6.8	6
5	P^H	5.78	5.91	6.31	6.51
6	Solubility in : Boiling Water Alcohol Ether chloroform	Soluble	Soluble	Soluble	Soluble
7	Washability	Very Good	Very Good	Good	Avarage
8	Non-irritancy	Non irritant	Non irritant	Non irritant	Non irritant
9	Viscosity	1258 cps	1125 cps	970 cps	950cps
10	Extrudablity	0.4gm	0.4gm	0.3gm	0.4gm

Table no 7:(PHYSICOCHEMICAL EVALUATION OF ONITMENT)

C) Stability study (Evaluation tests for initial day, after three month and after six month):

The stability study was carried out for the prepared ointment at temperature of 37 ℃ for six months .

Time period	Formulation	Colour	Consistency	Spreadability	pH	Washability	Irritancy	Stability	Viscosity
Day 1	F1	Dark green	Smooth	7mm	5.78	Very good	Non irritant	Stable	1258cps
	F2	Dark green	Smooth	7mm	5.91	Very good	Non irritant	Stable	1125cps
	F3	Pale green	Smooth	6.8 mm	6.31	Good	Non irritant	Stable	970cps
	F4	Pale green	Smooth	6mm	6.51	Average	Non irritant	Stable	950cps
After 3 month	F1	Dark green	Smooth	7 mm	5.78	Very Good	Non irritant	Stable	1258cps
	F2	Dark green	Smooth	7mm	5.91	Very Good	Non irritant	Stable	1125cps
	F3	Pale green	Smooth	6.8 mm	6.31	Good	Non irritant	Stable	970cps
	F4	Pale green	Smooth	6 mm	6.51	Average	Non irritant	Stable	950cps
After 6 month	F1	Dark green	Smooth	7 mm	5.78	Very Good	Non irritant	Stable	1250cps
	F2	Dark green	Smooth	7 mm	5.91	Very Good	Non irritant	Stable	1123cps
	F3	Pale green	Smooth	6.8mm	6.31	Good	Non irrtant	Stable	965cps
	F4	Pale green	Smooth	6 mm	6.51	Average	Non irritant	Stable	939cps

Table no 8:(Stability Study for initial day, after three month and after six month))

Evaluation parameters :

1)P^H:

Formulation Batch	1st day	After 3 monthes	After 6 monthes
F1 F2 F3 F4			

Table no. 9:(pH meter reading)

2}Spreadability :

Formulation batch	1st day	After 3 month	After 6 month
F1 F2 F3 F4			

Table no. 10:(Spreadibility)

D) Comparative Study Of Herbal And Marketed Preparation :

Sr.no.	Physiochemical parameters	Herbal ointment formulations (batch no. 1)	Herbal ointment formulations (batch no .2)	Marketed preparation (Puradine ointment)	Marketed preparation (fusiwal ointment)
1	Appearance (colour)	Dark green	Pale green	Brownish	White
2	Odour	Charactertics	Charactertics	Slightly aromatic	Slightly aromatic
3	Consistency	Smooth	Smooth	Smooth	Smooth
4	Excrudability	0.4	0.4	0.5	0.3
5	Spreadability	7mm	7mm	6.5mm	6.8mm
6	P^H	5.78	5.91	6.87	6.54
7	Solubility	Soluble	Soluble	Soluble	Soluble
8	Washability	Easily Washable	Easily Washable	Easily Washable	Easily washable
9	Irritancy	Non irritant	Non irritant	Non irritant	Non irritant
10	Stability	Stable	Stable	Stable	Stable
11	Viscosity	1258 cps	1125 cps	1263cps	1269cps

Table no 11 :(COMPARATIVE STUDY OF HERBAL AND MARKETED PREPRATION)

1) $P^{H:}$

Fig. no. 26:(Comparative study of herbal and marketed preparation)

2) spreadability;

Fig. no. 27:(Comparative study of herbal and marketed preparation)

Zone Of Inhibition [22,23];

The antibacterial activity was performed by the agar well diffusion method. The plates were evaluated after incubation at 37°C for 24 hours after which the zone of inhibition around each was measured by using a scale in millimeters (mm). The ratio between the diameter of the inhibition zone (mm) produced by plant extracts and the inhibition zone around the well with formulation was used to express antibacterial activity

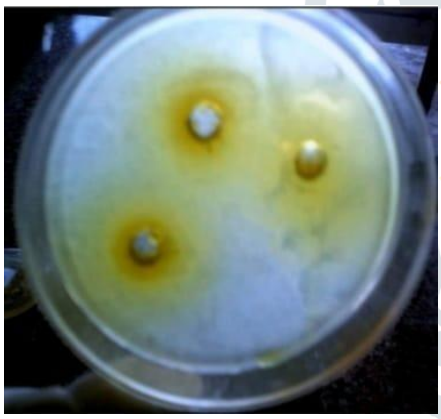


Fig. no.28(Zone of Inhibition Of F1, F2 and Marketed Preparation)

fig no. 29 :(Zone Of Inhibition Of Herbal Extraction)

Microorganism	F(1)	F(2)	Marketed
E.coli	14.37	16.52	23.35

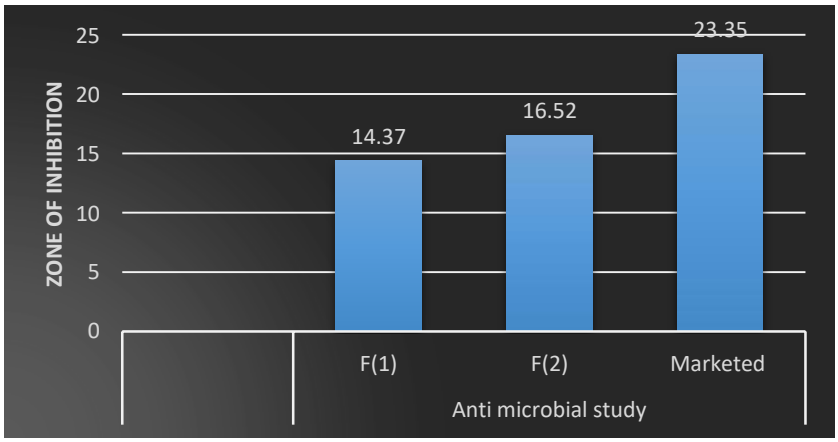


Fig No.30:Various Formulation Antibacterial Activity Of Ointment

The present study was done to prepare and evaluate the herbal ointment.For this the herbal extract were prepared . The ointment formulation were subjected to various physicochemical evaluations and all the parameters were found to be within the limits.All the formulations were greenish in colour and had characteristics odour . The pH of all the ointments ranges from 5.78-6.51found in the range which is good for skin pH. The formulations showed pH nearer to skin as required i.e. pH of F1-5.78 and F2-5.91.The spreadability of the ointment was found to be in the range of 6-7mm, confirming that these ointment may spread smoothly and uniformly . The homogeneity and tube extrudability of all formulations was good.The ointment formulations of F1 and F2 showed good physicochemical properties as well as good drug content compared to other formulations . Hence, theses formulations were further selected for anti microbial studies. The results of anti microbial studies showed that formulation of F1 and F2 showed a maximum zone of inhibition against e.coli.The formulation F1 and F2 showed no redness, edema, inflammation and irritation during irritancy studies. These formulations were safe to use for skin. All formulations produced uniform distribution of extracts in ointment This was confirmed by visual appearance and by touch.When formulation were kept for six month stability study , it found that no change in evaluation parameter of ointment. Emolliency, slipperiness and certain amount of residue left after the application of fixed amount of ointment was found. The test was conducted to evaluate the irritationcaused by the formulated ointment . The results showed that the formulation F1 and F2 do not showed any skin reaction. The above table shows that formulated herbal ointment is having and equal or near about and engrossing over the marketed preparation. However,the marketed preparation shows significant activity when compare to tested ointment containing ethanolic extractof neem and tridax leaves.

CONCLUSIONS:

In Ayurveda Tridax procumbens and Neem were used for their various medicinal properties like antibacterial, antifungal, anti-inflammatory, wound healing, etc. Thus, these formulations could become a medium to use these medicinal properties effectively and easily as a formulations dosage form like Ointments using locally available plants. Based on antimicrobial efficacy, two different local plants were taken and their ethanolic extracts were incorporated in the most effective ratio inappropriate base. The phytochemical constituents such as alkaloids, flavonoids, glycosides, tannins, carbohydrates, sterols, saponins, proteins, and other miscellaneous phenolic components are believed to play a pivotal role in the healing of the wound by significantly increasing the rate of wound closure and epithelisation. The F-1and F-2 product readily spread on the skin surface, showed no irritant effect, diffused well, and was stable at different evaluation parameters. The ointment was found to have antimicrobial activity againstE.Coli .It was concluded that the fomulations was found to be better physicochemical characteristics and higher pharmacological activity compared to marketed formulation.The formulation developed from tridax and neem showed significant results so it can be further used commercially to develop wound healing ointment after conducting clinical trials

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