IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

# Designed And Development Of Polyherbal Ointment Using Extract Of A.Vera, A. Indica, L. Esculentum And It's Characterization

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**Abstract**

The process of wound healing is a complex biological process and promotion of tissue recovery is the main objective of medical interventions. Skin lesions are caused due to different reasons such as burns, arterial diseases, surgery, and trauma. Wound healing is a dynamic process that takes place in three phases. The first phase is inflammation, congestion, and leukocyte infiltration. The second phase involves the removal of dead tissue and the third phase of proliferation includes epithelial regeneration and fibrous tissue formation. Several studies on Aloe vera have been conducted and shown to be effective in the prevention and healing of skin wounds The pH of formulations were found to be 6.4 to 6.8, it is near to skin pH so less sensitive to skin. It is clearly evident that formulation TSF-3 showed above 95.55 % drug release in 480 minutes while the formulation TSF-1 and TSF-2 showed 65.42 % and 68.21 % in 480 minutes. The water content and water uptake give the data in regards to the dependability of the preparation. The water content in transdermal patch formulations was found to be in range of 3.12 % ± 0.66 % to 3.91 % ± 0.61%. The stretch ability strength fallouts specify the strength of the patch and the possibility of patch cracking. The results were found to be in arrange of 0.083±0.08 kgcm-2 to 1.32±0.10 kg cm-2.

**Keywords:** A.Vera, Indica, L. Esculentum, Polyherbal Ointment, Phyto constitute**.**

## INTRODUCTION

Herbal medicine also called botanical medicine or phyto medicinal refers to the use of any plant seeds barriers roots leaves bark or flower for medicinal purpose .long practiced outside of convention medicine herbals is becoming more mainstream as up to date analysis and research shown their value in the treatment and prevention of diseases Ointment are greasy semisolid preparation and containing dissolved or dispersed medicament there are different type of ointment base like hydrocarbon bases usually consist of soft paraffin or its mixture with soft paraffin from a greasy film on skin inhibiting water loss thus hydrating skin because of their hydration effect on skin ointment are very effective in improving its hydration status absorption base these bases soak up water to form w/o emulsion while retaining their semisolid consistency .The maximum amount of water that can be added to 100gm of such a base at a given temperature is called as water number. World health organization estimated that 80 % of people worldwide rely on herbal medicines for some aspect of their primary health care for most herb contain a therapeutic effect is not known whole herb contain to produced decreased medicinal effect. Along with other dosage form herbal drugs are also formulated in form of ointment an ointment is a viscous semisolid preparation used topically on a variety of body surface these include the skin and the mucus membrane of the eye vagina anus and nose. An ointment may or may not be medicated ointment contain medicament dissolved suspended or emulsified in the base Ointment are used topically for several purpose e.g. as protectants antiseptic emollient Ointment base are almost always anhydrous and generally contain one or more medicaments in suspension or solution or dispersion ointment bases may be hydrocarbon oleaginous etc. In earlier study medicinal plants has been reported to be very beneficial in wound care and promoting the rate of wound healing with minimal path discomfort and sharing to the patient

**ALOE VERA**

The Aloe Vera look like a cactus but it isn ‘t. The botanical name of Aloe vera is Aloe Barbadensi similar. It belongs to (Liliaceae) family, an, perennial, xerophytic, succulent, and pea- green colour plant. Inside the leaf is a jelly like substance. The properties of Aloe vera were well accepted from China to India. Today, Aloe vera is cultivated throughout the world. Terms including, the potted physician and nature‘s medicine chest, attempted to describe the significant historical uses of Aloe vera. There are over 250 species of aloe grown around the world. However, only two species are grown today commercially, with Aloe Barbadensis Miller and Aloe aborescens being the most popular. The Aloe plant is grown in warm tropical areas and cannot survive freezing temperatures.

Three types of aloe are available in market depending upon the source plant viz.

i. Curacao aloe: a dark brown colored substance sourced from Aloe vera
ii. Cape aloe: greenish brown colored aloe sourced from Aloe ferox
iii. Socotrine aloe: reddish black colored aloe sourced from Aloe peyrii baker.
iv. TheoriginalcommercialuseoftheAloeplantwasintheproductionofalatexsubstancecalled Aloin, a yellow sap used for many years as a laxative ingredient.[3]

**Fig: 1. AloeVera**

1. Botanicalnames: Aloe barbadensis miller
2. Family: Liliaceae
3. Synonym: Aloe barbadensis, Aloe indica, Aloe arborescens, Aloe ferox

**ActiveConstitutes of AloeVera:**

TheAloeveraleafgelcontainsabout98%water6.ThetotalsolidcontentofAloeveragelis 0.66% and soluble solids are 0.56% with some seasonal fluctuation. On dry matter basisaloe gel consists of polysaccharides (53%), sugars (17%), minerals (16%), proteins (7%),lipids (5%)and phenol ccompounds (2%) Aloevera contains 200 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids, which are responsible for the multifunctional activity of Aloe [7 -9].

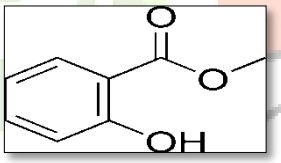


**Fig. 2. Salicylic acid**

**Vitamins:**

It contains Vitamins A (beta-carotene), C and E,which are antioxidants. It also contains Vitamin B12, folicacid, and choline. Antioxidant neutralizes free

**Enzymes:**

It contains 8 enzymes: aliiase, alkaline phosphatase, amylase, bradykinase,carboxy-peptidase, catalase, cellulase, lipase, and peroxidase. Brady kinase helps to reduce excessive inflammation when applied to the skin topically, while others help in the breakdown of sugars and fats.

**Minerals**

It provide scalcium,chromium,copper,selenium,magnesium,manganese,potassium,sodium and zinc. They are essential for the proper functioning of various enzyme systems in different metabolic pathways and few are antioxidants.

**Sugars:**

It provides monosaccharides (glucose and fructose) and polysaccharides: (glucomannans /polymannose). These are derived from the mucilage layer of the plant and are known asmuco polysaccharides. Recently, a glycoprotein with anti-allergic properties, called alprogen and novel anti-inflammatory compound, C-glucosyl chromone, has been isolated from Aloevera.

**Anthraquinones:**

It provides12anthraquinones, which are phenolic compound traditionally known as laxatives. Aloin and emodin act as analgesics, anti-bacterial and anti-viral.

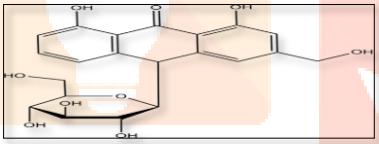

**Fig.3. Aloin**

**Fattyacids:**

It provides 4plant steroids; cholesterol, campesterol, β-sisosterol and lupeol. All the have anti-Inflammatory action and lupeol also possesses antiseptic and analgesic properties.

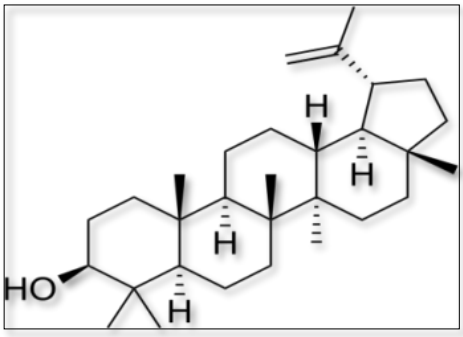


**Fig. 4.Lupeol**

**Hormones:** Auxins and gibberellins that help in wound healing and have anti-inflammatory action.

**Others:** It provides 20 of the 22-human required amino acids and 7 of the 8 essential aminoacids. It also contains salicylic acid that possesses anti-inflammatory and antibacterial properties. Lignin ,an inert substance, when included in topical preparations, enhances penetrative effect of the other ingredients in to the skin. Saponins that are the so substances form about 3%ofthegel and have cleansing and antiseptic properties.[9]

**Uses**

The plant Aloe vera is used in Ayurvedic, Homoeopathic and Allopathic streams of medicine, and not only tribal community but also most of the people for food and medicine. The plant leaves contain numerous vitamins, minerals, enzymes, amino acids, natural sugars and other bioactive compounds with emollient, purgative, antimicrobial, anti-inflammatory, anti-oxidant, aphrodisiac, anti-helminthic, antifungal, antiseptic and cosmetic values for healthcare. This plant has potential to cure sunburns, burns and minor cuts, and even skin cancer.

**Azadirachta Indica**

Azadirachta indica (A. indica) belongs to the botanic family Meliaceae, commonly known as Neem. It is used in traditional medicine as a source of many therapeutic agents. A. indica(leaf, bark and seeds) are known to contain antibacterial and antifungal activities against different microorganisms; in addition to antiviral activity against vaccinia, chikungunya,measles,and Coxsackie B viruses. Different parts of Neem (leaf, bark and seeds) have been shown to exhibit wide pharmacological activities such as antioxidant, antimalarial, antimutagenic, anticarcinogenic, anti-inflammatory, antihyperglycemic, antiulcer, and anti-diabetic properties.

**Fig:5. AzadirachtaIndica**

Botanical name: - Azadirachta Indica

Family: Mahogany

Synonyms: -margosa, arishth, , Melia Azadirachta, rosehip, witch-hazel melaleuca,

**Chemical Constituents:-**

Constituents: Leaf extracts: Active constituents of neem leaf extract include isomeldenin,nimbin, nimbinene, 6-desacetyllnimbinene, nimbandiol, immobile, nimocinol, quercetin, andbeta-sitosterol.Twoadditionaltetracyclictriterpenoidszafaral[24,25,26,27-tetranorapotirucalla-(apoeupha)-6alpha-methoxy-7alpha-acetoxy-1,14-dien-3,16-dione-21-al]and maleinanhydride [24,25,26,27-tetranorapotirucalla-(apoeupha)-6alpha-hydroxy,11alpha-methoxy-7alpha,12alpha-diacetoxy,1,14,20(22)-trien-3-one]havebeenisolatedfromthemethanolicextract of neem leaves.

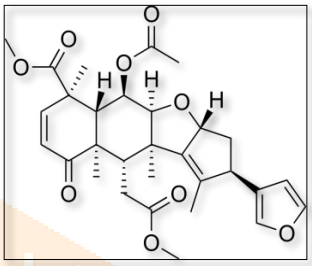

**Fig.5. Nimbin**

**Uses:-**

Neem is considered a boon for mankind by nature. Use of Neem has been recommended by Ayurveda for a wide range of diseases. Such usage is attributed to its purification effect on blood. Scientific research on Neem demonstrates it to be a Panacea. It is suggested to be anantibacterial, anthelmintic, antiviral, anticancer and more importantly immune modulatory agent.

**Lycopersicon Esculentum:-**

Solanum Lycopersicon, popularly known as tomato, originated in South America and now isused and cultivated in various parts of the world. This product is cultivated in warm climateregions, but can also be planted inside a greenhouse during winter. Tomatoes are full ofvitamins and antioxidants essential to a healthy body. Since the tomato doesn‘t need a lot oftending,it‘saneasyplanttogrow.Tomatoes can be consume several ways from salads up to sauces and easily harvested, making it the second most consumed vegetable of theAmerican diet and has China being the main country that producer tomatoes in 31% of thetotalproducein theworld.

**Fig:6.Lycopersiconesculentum**

- Binomialname:-Solanum lycopersicum
- Family:-Solanaceae
- Synonyms:-Solanum lycopersicum L., Lycopersicon lycopersicum
- Commonname:-

| Language | Name |
|---|---|
| English | Tomato |
| Tamil | Takkali |
| Hindi | Tamatar |
| Kashmiri | Ruvangum |

**Material and Method**

**1. COLLECTION OF PLANT MATERIAL**

The plant will collect from the local growing area and from market district.[24]

**2. AUTHENTICATION**

The collected specimens will botanically identified and authenticate

**CHEMICAL REQUIRED**

The chemicals required to the study: Dextrose Agar media, Dimethyl Sulfoxide, and Carbopol 934P. Emulsifying wax, white soft paraffin, liquid paraffin, and methanol etc.

**3. MACROSCOPIC EVALUATION**

The morphological feature of the plant will evaluated and record

## 4. MICROSCOPIC EVALUATION

### Fixation of leaf, stem and root

The whole plant was cut and fixed in FAA solution (Formalin 5ml + Acetic acid 5ml + 90ml of 70% Ethanol). The specimen was dehydrated after 24 hours of fixing. The whole plant was graded with series of tertiary butyl alcohol, as per the standard procedure. It was carried out by gradual addition of 58 – 60º C of melting pointed paraffin wax until Tertiary butyl alcohol (TBA) solution attained super saturation. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10 - 12µ. De-waxing of the sections was done by customary procedures. The sections were stained with haematoxilin. The stained sections were viewed under microscope.

### POWDER MICROSCOPY

The shade dried whole plant was powdered and used for powder microscopic analysis. The organoleptic characters were observed and to identify the different microscopical characteristic features various staining reagent were used. Powder was stained with 1% phloroglucinol in 90% ethanol, concentrated hydrochloric acid and observed under microscope. Powder analysis is used for the detection of characteristic structures and various cell components.

### PHYSIOCHEMICAL ANALYSIS

The shade dried powdered whole of lycopersicon esculantum was used for the analysis of various physiochemical parameters which is useful in the determination of quality and purity of crude drugs. Total ash, extractive values, loss on drying, foaming index, swelling index and foreign organic matters were determined as per the standard WHO guidelines which is very much useful in the determination of quality and purity of the crude drugs.

### DETERMINATION OF ASH VALUES

The residue remaining after incineration is the ash content of the drug, which simply represents the inorganic salts naturally occurring in the drug or adhering to it or deliberately added to it as a form of adulteration.

### TOTAL ASH

Silica crucible was heated to red hot for 30 minutes and it was allowed to cool in desiccators. About 2gm of powdered sample was weighed accurately and evenly distributed in the crucible. Dried at 100 – 105ºC for 1 hour and ignited to constant weight in a muffle furnace at 600±25ºC. The crucible was allowed to cool in a desiccator. The percentage yield of ash with reference to the air dried substance was then calculated by the formula

**Water soluble ash**

The total ash was boiled for 5min with 25ml of water. The insoluble matter was then collected in an ash less filter paper. It was washed with hot water and ignited for 15min at a temperature not exceeding 450ºC. The weight of the insoluble matter was subtracted from the weight of the ash and the difference in weight represented the water soluble ash, the percentage of water soluble ash with reference to the air dried substances was calculated with reference to the air dried material.

**Acid insoluble ash**

Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth Procedure To the total ash obtained previously, 25ml of dilute hydrochloric acid was added, covered with a watch glass and boiled gently for 5min on a burner. The watch glass was rinsed with 5ml of hot water and these washings were added to the crucible. The insoluble matter was collected on an ash less filter paper by filtration and the filter paper was rinsed repeatedly with hot water until the filtrate is neutral and free from acid. Filter paper containing the insoluble matter was transferred to the crucible, dried on a hot plate and ignited to a constant weight in the muffle furnace at 450- 500ºC. The silica crucible was removed from the muffle furnace and allowed to cool in a desiccator for 30min, and then weighed without delay. The content of acid insoluble ash was calculated.

**Sulphated ash**

About 3gm of air-dried substance was ignited gently at first in a crucible, until the substance was thoroughly charred. Then the residue was cooled, moistened with 1ml of sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800 ± 25ºC, until all the black particles were disappeared. The crucible was allowed to cool, a few drops of sulphuric acid was added and heated. Then it was ignited as before, cooled and weighed. The percentage of sulphated ash with reference to the air- dried substance was then calculated.

**DETERMINATION OF EXTRACTIVE VALUES**

This method is used to determine the amount of active constituents in a given amount of plant material when extracted with solvents. Extractive values are useful for the evaluation of phytoconstituents especially when the constituents of a drug cannot be readily estimated by any other means. Further these values indicate the nature of the active constituents present in a crude drug.

**Determination of water soluble extractive**

About 5gm of the powder was weighed and macerated with 100ml of chloroform water (95ml distilled water and 5ml chloroform) in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of thefiltrate was evaporated to dryness in a tarred flat bottomed shallow dish. 2 ml of alcohol was added to the residue and it was dried at 105ºCfor 1 hour in the hot air oven and cooled in desiccators for 30min and

weighed. The process was repeated till a constant weight was obtained; the percentage of water soluble extractive value with reference to the air dried drug was calculated. Determination of alcohol soluble extractive The alcohol soluble extractive value is also indicative for the same purpose as water soluble extractive value. The solvent strength of alcohol varies from 20- 90%v/v. The solvent strength has to be chosen depending upon the strength of alcohol used for the extraction of powdered drug. Procedure About 5gm of the powder was weighed and macerated with 100ml 90% ethanol in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. It was dried at 105ºC for 1hour in a hot air oven. The dish was cooled in desiccator and weighed. The process was repeated till the constant weight was obtained. The percentage of alcohol soluble extractive value with reference to the air dried drug was calculated.

**Loss on Drying** Accurately weighed quantity of the substances was taken in a previously ignited and cooled silica crucible and the substance was evenly distributed by gentle side wisesubstance was heated for a specified period of time to a constant weight. The crucible was covered with the lid and allowed to cool in a desiccator at room temperature before weighing. Finally the crucible was weighed to calculate the loss on drying with reference to the air dried substance.

**Determination of Foaming Index** About 1gm of the coarsely powdered drug was weighed and transferred to 500ml conical flask containing 100ml boiling water. The flask was maintained at temperature 80-90ºC for about 30min. It was then cooled and filtered into a volumetric flask and sufficient water was added through the filtrate to make up the volume to 100ml. The decoction was poured into 10 stopper test tube (height 16cm, diameter 16mm) in successive portions of 1ml, 2ml, 3ml, 4ml up to 10ml and the volume of the liquid in each tube was adjusted with water to 10ml. The tubes were stoppered and shaked in a length wise motion for 15 seconds, two shakes per second. Allowed to stand for 15min and the height of the foam were measured. The results are assessed as follows: if the height of the foam in every tube is less than 1cm, the foaming index is less than 100. If a height of 1cm is measured in any tube, the volume of the plant material decoction in the tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result. The height of the foam is more than 1cm in every tube the foaming index is over 1000. In this case repeat the determination using a new series of dilution of the decoction in order to obtain a result. Calculate the foaming index using the following formula

Foaming index = 1000/a Where, a is the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed.

**DETERMINATION OF SWELLING INDEX**

The swelling index is the volume in ml occupied by the swelling of 1gm of plant material under specified conditions. Procedure A specified quantity of the plant material was accurately weighed and transferred into a 25ml glass stoppered measuring cylinder. The internal diameter of the cylinder should be about 16mm, the

length of the graduated portion about 125mm, marked in 0.2ml divisions from 0 to 25ml in an upward direction. Unless otherwise indicated in the test procedure, add 25ml of water and shake the mixture thoroughly every 10min for 1hour, allowed to stand for 3 hours at room temperature. The volume in ml occupied by the plant material was measured including any sticky mucilage. Calculate the mean value of the individual determination, related to 1gm of plant material. The coarse powder of the leaves (50 g) was extracted by a continuous hot percolation process.[2,11] The powder was first defatted with n-hexane and then allowed to dry. The marc thus obtained was extracted for 72 hours with methanol as a solvent. The resulting solvent was then removed under reduced pressure, and thus formed a semisolid, which was vacuum dried using a rotary flask evaporator, to get a solid residue. This residue was the Methanol Extract of *azadirachta indica*). The dried extract thus obtained will used for the formulation.

**PHYTOCONSTITUENT EVALUATION**

The dried methanolic extract of azadirachta indica and aloe vera and licopersicon esculantum will analyzed for various phytoconstituents like alkaloids, proteins, steroids, saponins, flavonoids, phenolic compounds and tannins, and gums and mucilages.[13]

1. TEST FOR SAPONINS Foam test A small amount of extract taken in a test tube with little quantity of water. Shake vigorously. Appearance of foam persisting for 10 minutes indicates presence of Saponin.

2. TEST FOR ALKALOIDS 1. Dragendroff's test: Dissolve extract of the herbal drug in chloroform. Evaporate chloroform and acidify the residue by adding few drops of Dragendroff's reagent (Potassium Bismuth Iodide). Appearance of orange red precipitate indicates presence of alkaloids. 2. Mayer's test: 2-3 ml of filtrate with few drops of Mayer's reagent gives ppt. 3. Wagner's test: 2-3 ml of filtrate with few drops of Wagner's reagent gives reddish brown colour. 4. Murexide test for purine alkaloid: To 3-4 ml. test solution add 3-4 drops of conc.HNO3. Evaporate to dryness. Cool and add 2 drops of NH4OH. Purple

3. 3 TEST FOR CARBOHYDRATES. Fehling's test: Mix 1ml. Fehling's A and 1ml. Fehling's B solutions boil for one minute. Add equal volume of test extract solution. Heat in boiling water bath for 5-10 min. Appearance of orange red precipitate indicates presence of carbohydrates. ii. Benedict's test: Mix equal volume of Benedict's reagent and test extract 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 test solution.

4. TEST FOR FLAVANOIDS : i. Ferric chloride test: To the alcoholic solution of the extract add few drops of neutral ferric chloride solution. Appearance of green colour indicates presence of flavanoids. ii. Shinoda Test: To dry extract, add 5 ml. of 95% ethanol, few drops conc. HCL and 0.5 g magnesium turnings. Pink colour observed. iii. Zinc-hydrochloric acid-reduction test: Test solution with zinc dust and few drops of HCL shows magneta red colour iv. Alkaline reagent test: Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour which becomes

colourless on addition of few drops of dilute acid. v. Lead acetate solution test: Test solution with few drops of lead acetate solution (10%) gives yellow precipitate. Mohsin J. Jamadar et al. / SGVU Journal of Pharmaceutical Research & Education, 2017, 1( 2), 201-224

5. TEST FOR PROTEINS : i.Biuret test: a) Add 2ml of Biuret reagent to 2ml of extract. Shake well and warm it on water bath. Appearance of red or violet colour indicates presence of proteins. b) To 3 ml. extract add 4% NaOH and few drops of 1% CuSO4 solution. Violate or pink colour appears. ii.Million's test: Test solution treated with million's reagent and heated on a water bath, ii. Xanthoprotein test: Test solution treated with conc. nitric acid and on boiling gives yellow precipitate. iv. Ninhydrine test: Test solution treated with Ninhydrine reagent gives blue colour.

6. TEST FOR GLYCOSIDES i. Baljets test: Treat the extract with sodium picrate solution. Appearance of yellow to orange colour indicates presence of glycoside with lactone ring. ii. Keller-killiani test: The test solution with few drops of glacial acetic acid in 2 ml of ferric chloride solution and conc. sulphuric acid is added from the sides of test tube which shows the separation between two layers, lower layer shows reddish brown and upper layer turns bluish green. iii. Bromine water test:Test solution dissolved in Bromine water gives yellow precipitate. iv. Legal's test: Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gives pink to red colour.

7. TEST FOR AMINO ACIDS i. Ninhydrine test: Heat 3 ml extract and 3 drops of 5% Ninhydrine solution in boiling water bath 10 min. Purple or bluish color appears. ii. Test for Tyrosine: Heat 3 ml extract and 3 drops of Million's reagent. Solution shows dark red color. iii. Test for cysteine: To 5 ml of extract add few drops of 40% NaOH and 10% lead acetate solution. Boil. Black ppt. of lead sulfate is formed.

8. TEST FOR STEROIDS i. Salkowski reaction: To 2 ml of extract, add 2 ml chloroform and 2 ml of conc. H2SO4. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence. ii. Liebermann's reaction: Mix 3 ml extract with 3 ml acetic anhydride. Heat and cool. Add few drops of conc. H2SO4. Blue color appear

**Formulation of ointment**

Required quantities of emulsifying wax, white soft paraffin, and liquid paraffin, accurately weighed, were heated up to 70 – 75°C and stirred until a uniform mass was obtained The extracts were incorporated into the ointment base and the composition of the ointment base and the ointment formulation

**Formulation of ointment**

Ointment will prepared by dispersing the polymer in a mixture of water and glycerol with methyl paraben as the preservative. The extract was incorporated into it. The dispersion was then neutralized and made viscous by the addition of triethanolamine.[17] The composition of ointment formulations are carried out.

**Table 1 Composition of herbal ointment**

**Formulation of Ointment**

| S. No | Name of Ingredient | Quantity to be taken |
|---|---|---|
| 1 | Wool fat | 1.5gm |
| 2 | Cetostearyl alcohol | 1.5gm |
| 3 | Hard paraffin | 1.5gm |
| 4 | Yellow soft paraffin | 1.5gm |

**Table 2 Formulation of ointment**

| S. No. | Name of Ingredients | FORMULATION CODE | | | |
|---|---|---|---|---|---|
| | | **F1 10 gm** | **F2 10 gm** | **F3 10 gm** | **F4 10 gm** |
| 1 | Prepared Neem extract | 2ml | 1.5 ml | 1.5 ml | 1 ml |
| 2 | Prepared aloe Vera | 1gm | 1 gm | 1.5 gm | 2 gm |
| 3 | Lycopersicon Esculentum Seed Powder | 1 gm | 1.5gm | 1 gm | 1 gm |
| 4 | Ointment base | 6 gm | 6 gm | 6 gm | 6 gm |

**Physical evaluation of the formulation**

The formulations will inspect visually for their color, homogeneity, consistency, and phase separation.

**Measurement of pH**

The pH was measured using a pH meter, which was calibrated before each use with standard buffer solutions at pH 4, 7, 9. The electrode get inserted into the sample for 10 minutes prior to taking the reading at room temperature.

**Viscosity**

The viscosity of the formulations will checked using a Brookfield Viscometer. The ointment were rotated at 3,6,11 rotations per minute. The viscosity of the ointment was obtained by multiplying the corresponding dial reading with the factor given in the Brookfield Viscometer catalog.

**Spreadability**

Spread ability is expressed in terms of time in seconds taken by two slides to slip off from the gel when placed in between the slides under the direction of a certain load. The excess amount of sample was placed between the two glass slides and a definite amount of weight was placed on these glass slides to compress the glass slides of uniform thickness. A weight of 70 g was added and the time required to separate the two slides was noted. Separability was calculated using the formula

$S = M.L / T$

Where, M = wt tied to upper slide, L = length of glass slides, T = time taken to separate the slides.

**Extrudability**

The formulations were filled in collapsible tubes after the ointments were set in the container. Extrudability of the different ointment formulations was determined in terms of weight in grams required to extrude a 0.5 cm ribbon of ointment in 10 seconds.

**Microbial contamination**

Microbial contamination of ointment with bacteria and moulds, respectively, was determined by spreading a thin loopful of the material withdrawn from the depth of the bulk product on a nutrient and Sabouraud agars, and incubating for 24 to 48 hours, at 37°C.

In order to assess the degree of contamination, 1 g of material was dispersed in 4 ml of sterile Ringer solution, containing 0.25% Tween 80. Appropriate dilutions were made in the same dispersion vehicle and 0.5 ml was plated out on the appropriate solid medium using the surface viable method. Emergent colonies were counted after necessary incubation.

**Antimicrobial evaluation**

**In-vitro antibacterial activity**

*Staphylococcus aureus* and *Pseudomonas aeruginosa* are the commonly occurring gram-negative and gram-positive bacteria causing skin infection, hence, will selected for the study.

**Test organism**

Clinical microbial extracts of gram-negative (*Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus*) will used as antibacterial agents.

**Antibacterial activity**

*In-vitro* antibacterial activity will be evaluated using the agar well diffusion technique. Muller-Hinton agar was used as the medium. The sterile agar was inoculated with the bacteria culture (*S. aureus, P. aeruginosa*) for 48 hours, at 37°C. Wells bored by using a sterile borer, and standard formulations (will prepared by

dissolving the test sample in DMSO) were placed into them. Plates were kept for two hours in the refrigerator to enable pre diffusion of the extracts into the agar. Next, the plates were incubated overnight (24 hours) at 37°C. The spectrum of activities of the extracts were compared with the marketed formulation, betadine ointment.[22–26]

**In-vitro antifungal activity**

A preliminary study of the *in-vitro* antifungal activity of the methanolic extract of *Samadera indica* against various fungal species like *Candida albicans, Aspergillus niger*, and *Aspergillus fumigates* showed that *Samadera indica* showed activity only against *Candida albicans* at a minimum inhibitory concentration of 250 µg / ml. Therefore, in the present study *Candida albicans* was used for the study.[2]

**Test organism**

This study employed Candida albicans

**Antifungal activity**

Sterile Sabouraud Dextrose Agar plates were prepared and 0.1 ml of inoculums from the standardized culture of the test organism was spread uniformly. Wells were prepared by using a sterile borer of diameter 10 mm. The test substance of 100 µl, prepared by dissolving in dimethyl sulfoxide (1000 µg / ml) and the solvent control (DMSO) were added in each well separately. The plates were placed for 1 hour, at 4°C, to allow the diffusion of the test solution into the medium; and the plates were incubated at 28°C for 48 hours, a period of time sufficient for the growth of at least 10 to 15 generations. The zone of inhibition around the well was measured in millimeters and compared with clotrimazole cream (marketed formulation).[25,26]

**Result & Discussion**

**Table 1. Macroscopical features of plant materials**

| Sr.no. | Parameters | *Aloe Vera* | *Azadiracta Indica leaf* | *Lycopersicon esculentum. leaf* |
|---|---|---|---|---|
| 1 | Colour | Depends on variety dark brown, Brownish Black or black | Greenish brown color Powder | Red uneven with light inclusion |
| 2 | Odour | Characteristic | Typical | Typical |
| 3 | Taste | Intensely bitter and Nauseating | Bitter | A bit sour with a smack of Over ripe tomatoes |
| 4 | Size | Masses of various sizes | 20-40 cm long | 1 – 10 cm long |
| 5 | Shape | N/A | Oblong | Oval |

**Table 2. Total phenolic content in extracts of Fruits of *Aloe Vera*, leaves of *Azadiracta Indica* and Lycopersicon Esculentum**

| Plant | Intensity absorbance | Dilution factor | Concentration µg / ml | Final concentration |
|---|---|---|---|---|
| *Aloe Vera* | 0.068 | 10.00 | 2.052 | 50.14 % |
| *Azadiracta Indica* | 0.089 | 10.00 | 3.195 | 65.81 % |
| *Lycopersicon Esculentum* | 0.082 | 10.00 | 3.111 | 60.41 % |

**Table 3. Percentage yield of extracts of *Lagenaria siceraria, Azadiracta Indica,* and *Lycopersicon Esculentum extracts***

| Sr.no. | Plant name | Extracts | % yield w/w | Physical state of extract |
|---|---|---|---|---|
| 1 | *Aloe Vera* | Aqueous (200g) | 9.22 % | Semisolid viscous |
| 2 | | Methanol (200 g) | 11.2 % | Semisolid viscous |
| 3 | *Azadiracta Indica* | Aqueous (200g) | 12.25 % | Semi solid viscous |
| 4 | | Methanol (200 g) | 7.15 % | Semi solid viscous |
| 5 | *Lycospercon Esculentum* | Aqueous (200g) | 25.60 % | Semi solid viscous |
| 6 | | Methanol (200 g) | 9.8 % | Semi solid viscous |

**Table 4.** ***Aloe Vera*** **extracts qualitative phytochemical study**

| Sr.No. | Phytoconstituents | *Aloe Vera* | |
|---|---|---|---|
| | | Methanolic extract | Aqueous extract |
| 1 | Flavonoids | + | + |
| 2 | Alkaloids | + | + |
| 3 | Glycoside | + | + |
| 4 | Saponins | + | + |
| 5 | Tannins and phenolic compounds | + | + |
| 6 | Steroids and terpenoids | + | + |
| 7 | Carbohydrates | + | + |
| 8 | Proteins and Aminoacids | + | + |
| 9 | Vitamins | + | + |

**Table 5.** ***Azadiracta Indica*** **extracts qualitative phytochemical study**

| Sr.No. | Phytoconstituents | *Azadiracta Indica* | |
|---|---|---|---|
| | | Methanolic extract | Aqueous extract |
| 1 | Flavonoids | + | + |
| 2 | Alkaloids | + | - |
| 3 | Glycoside | + | + |
| 4 | Saponins | - | + |
| 5 | Tannins and phenolic compounds | + | + |
| 6 | Steroids and terpenoids | + | + |
| 7 | Carbohydrates | - | - |
| 8 | Proteins and aminoacids | + | + |
| 9 | Vitamins | + | + |

**Table 6. *Lycopersicon Esculentum* extracts qualitative phytochemical study**

| Sr.No. | Phytoconstituents | *L. Esculentum* | |
|---|---|---|---|
| | | Methanolic extract | Aqueous extract |
| 1 | Flavonoids | + | + |
| 2 | Alkaloids | + | + |
| 3 | Glycoside | + | + |
| 4 | Saponins | + | + |
| 5 | phenolic compounds and Tannins | + | + |
| 6 | Steroids and terpenoids | + | + |
| 7 | Carbohydrates | + | + |
| 8 | Proteins and Aminoacids | + | + |
| 9 | Vitamins | + | + |

**Table 7. Evaluation of pH, spreadability and viscosity of conventional polyherbal formulations**

| Formulation | pH (1 g / 100 ml) | Spread ability (g / sec) | Viscosity in cps | | | |
|---|---|---|---|---|---|---|
| | | | 2.5 rpm | 5 rpm | 7.5 rpm | 10 rpm |
| TSF-1 | 6.5 | 38.81 ± 0.29 | 66920 ± 20.24 | 34561 ± 18.30 | 24174± 33.12 | 14310 ± 14.32 |
| TSF-2 | 6.4 | 13.38 ± 0.078 | 65665 ± 1.26 | 34747 ± 20.36 | 26152 ± 26.53 | 14645 ± 20.12 |
| TSF-3 | 6.8 | 18.28 ± 0.126 | 63825 ± 16.23 | 34970 ± 24.36 | 26433 ± 27.91 | 14256 ± 20.48 |
| TSF-4 | 6.7 | 38.31 ± 0.048 | 62825 ± 27.31 | 35465 ± 21.22 | 26899 ± 29.78 | 14565 ± 22.10 |

**Conventional Formulations:**

The drug release data of prepared polyherbal conventional formulations were subjected to drug release kinetic study to check the best fit model and to check the release pattern of the drug from the preparations. The fallouts of kinetic modeling are exposed in table8.

**Table. 8. Drug releases kinetic data of conventional poly herbal formulations**

| MODEL | | TSF-1 | TSF-2 | TSF-3 | TSF-4 |
|---|---|---|---|---|---|
| Zero order | *$r^2$ | 0.993 | 0.990 | 0.988 | 0.940 |
| | Slope | 4.641 | 5.288 | 6.655 | 0.838 |
| | Intercept | 3.479 | -1.190 | 10.47 | -0.535 |
| First order | *$r^2$ | 0.826 | 0.883 | 0.751 | 0.988 |
| | Slope | 0.092 | 0.105 | 0.088 | 0.077 |
| | Intercept | 0.794 | 0.663 | 1.015 | 0.075 |
| Higuchi model | *$r^2$ | 0.962 | 0.941 | 0.971 | 0.865 |
| | Slope | 0.051 | 0.042 | 0.034 | 0.241 |
| | Intercept | 0.631 | 0.902 | 0.424 | 1.164 |
| Hixoncrowell | *$r^2$ | 0.934 | 0.963 | 0.904 | 0.956 |
| | Slope | 0.478 | 0.544 | 0.548 | 0.211 |
| | Intercept | 2.305 | 1.745 | 3.338 | 0.648 |
| Korsmeyer–Peppas | *$r^2$ | 0.164 | 0.285 | 0.74 | -0.068 |
| | Slope | 0.729 | 0.978 | 0.576 | 0.824 |
| | Intercept | -1.51 | -1.74 | -1.188 | -2.465 |

*$r^2$ = Correlation coefficient

This result showed that the release of the drug from the polyherbal formulation TSF- 1, TSf-2 and TSF-3 followed zero order kinetics as best fit model. Whereas TSF-4 shows zero order kinetics based on the $r^2$ values.

**Stability**

The stability studies were carried out for all the formulations. The formulations were kept at two different temperature 4 ± 2°C and 30 ± 2°C, 65 RH, for three months. The pH and the viscosity of the formulations, which were determined after three months, were compared with the initial pH and viscosity.

**Statistical analysis**

All experimental measurements were carried out in triplicate and were expressed as an average of three analyses ± standard deviation. Statistical analyzes was performed by the t-test. Spreadability The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as spreadability. Lesser the time taken for separation of two slides results better spreadability.

Spreadability was calculated by following formula S=M×L/T Where, S= Spreadability M= Weight tide to the upper slide L= Length of glass slide T= Time taken to separate the slides.

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