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# Formulation and Evaluation of Antiarthritic Gel

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**Abstract**: *The objective of the study is to formulate and evaluate a topical herbal gel containing Limonia acidissima fruit extract for anti-arthritic. Herbal gel formulation was prepared using carbopol and it was evaluated for physical appearance, pH, viscosity, spreadability, extrudability, and primary skin irritation tests. Herbal Medicines are the major remedies for the traditional system of medicine have been used in medical Practices since ancient times. Limonia acidissima belongs to a monotypic genus, Limonia, and is classified under the family Rutaceae. It is an exotic deciduous tree found in India, Pakistan, Sri Lanka, and Southeast Asia. The Potential antimicrobial activity was examined by minimum concentration and zone of inhibition Analysis. Phytochemical screening results of the ethanol fraction of Limonia acidissima can be detected in the content of alkaloids, terpenoids. Anthraquinones, and saponins. Methanol extract showed good antibacterial activity with high inhibition zones. Arthritis is an inflammatory disorder that primarily affects the elderly and causes severe bone deterioration, inflammation, discomfort, and weakness. Allopathic treatment can only treat the symptoms. Phytochemical constituents of Limonia acidissima have been shown to have anti-inflammatory and antiarthritic effects.*

**Keywords**: Limonia acidissima, Inflammation, Antiarthritic, Carbopol

## I. INTRODUCTION

Limonia acidissima fruits play a significant role in nutrient nutrient-supplementary diet as well as in herbal medicine. They are the rich sources of vitamins, minerals, polyphenols, fibers, and antioxidants which provide various health benefits. In Ayurveda and siddha medicines, several edible fruits are used as medicines for various health disorders. is a moderate-sized deciduous tree confined to India, Pakistan, Sri Lanka, and Southeast Asia. It is also known as wood apple, elephant-apple, monkey fruit, curd fruit, kath bel, and kaitha. There are in excess of 100 various types of arthritis. Osteoarthritis and rheumatoid arthritis are two of the most regular structures. While both osteoarthritis and rheumatoid arthritis[1]

Arthritis is an auto immune disorder that affects about 0.5-1% of the population worldwide. The drugs commonly prescribed for Rheumatoid Arthritis are steroidal, non- steroidal anti- inflammatory, disease modifying anti- rheumatic and immunosuppressant drugs that are known to produce various side effects including gastrointestinal disorders, immunodeficiency and humoral disturbances.[2]

Anatomy of Skin

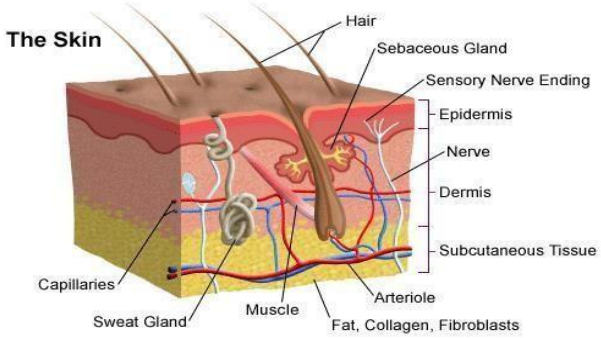


Fig. no. 1 Skin structure


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1. Epidermis: Skin layers consist of two different layers - the epidermis and the dermis. As shown in Figure there is a very clear wavy boundary between these two layers. The thickness of the skin layers differs quite a bit depending on gender, age, individual, body regions, etc.

a) Stratum spinosum (prickle cell layer): refers to the 10 to 20 layers that lie on top of the basal cell layer. Basal cells, through the process of turn-over, make their shape somewhat flatter (multi-sided) and form these layers. These cells are called prickle cells and have little spines on the outside of their membrane. The thickness of this sublayer is typically from 50 to 150 µm.

a) Stratum granulosum (granular cell layer):is composed of 2 to 4 granular cell layers. The typical thickness is 3 µm. In this sublayer, cornification called keratinization of keratinocytes begins. In this process, organelles such as nuclei and mitochondria start to resolve

b) Stratum corneum (horny cell layer): is the exterior sublayer of the epidermis. Its thickness ranges from 8 to 15 µm. This sublayer is composed of several layers of hexagonal-shaped flat and hard cells named horny cells or corneocytes.

1. Dermis: The dermis is the second layer of skin, beneath the epidermal layer. This layer is much thicker than the epidermis. The main components of the dermis are collagen and elastin fibers.

2.

3. Subcutaneous: Subcutis, or hypodermis in histology, is the third layer beneath the dermis. It is important to note that it is not categorized as another skin layer. Subcutis is an elastic layer and includes a large amount of fat cells that work as a shock absorber for blood vessels and nerve endings3

Function of Skin

Protection: Skin is the body's first physical barrier against the environment, protecting against mechanical damage, UV light, microorganisms, and dehydration. It also has anti- oxidant and antimicrobial functions.

Sensation: Skin is the sense of touch organ that detects pain, temperature, and deep pressure.

Regulation: Skin regulates body temperature and the amount of water released into the environment.

Storage: Skin stores fats and water in its tissues, which provide extra insulation. Endocrine activity: Skin initiates the biochemical processes involved in Vitamin D production, which is essential for calcium absorption and normal bone metabolism.[4]

**ANTI-ARTHRITICS –**

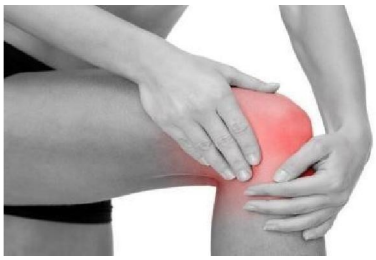

Fig. no.2 Joint pain

Arthritics – arthritic is an extremely common and chronic disease, especially in people older than 50. Arthritis is the swelling and friction of one or more joints. It causes joint pain, stiffness, and inflammation.

Arthritics can affect any joint, but is most common in people:

- Hands and wrists
- Knees
- Hips
- Feet and ankles
- Shoulders
- Lower back [5]

Types of arthritis: There are more than 100 different types of arthritis. Some of the most common types include,

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1. Osteoarthritis: Osteoarthritis (OA) is caused by the wear and tear damage to the joint's cartilage. Osteoarthritis is more common in women and usually affects people from the age of 45 onwards. The parts of the body most commonly affected are the knees, hands, hips, and back.[6]

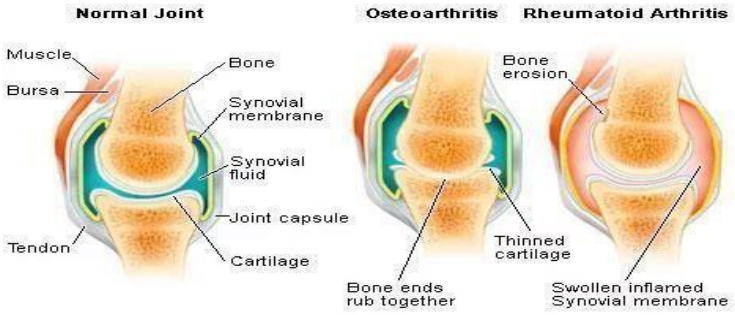


Fig. No. 3 Osteoarthritic

2. Rheumatoid arthritis: Arthritis that happens when your immune system mistakenly damages your joints. Rheumatoid Arthritis (RA) is an inflammatory disease that's caused by an autoimmune condition. The condition occurs when bodily cells begin to attack and target their own healthy joint tissues resulting in redness, inflammation, and pain. Patients with RA may be given antiarthritics that are used to block inflammation and help prevent joint damage. Rheumatoid arthritic can affect adults of any age. It most commonly starts among people between the ages 40-60.

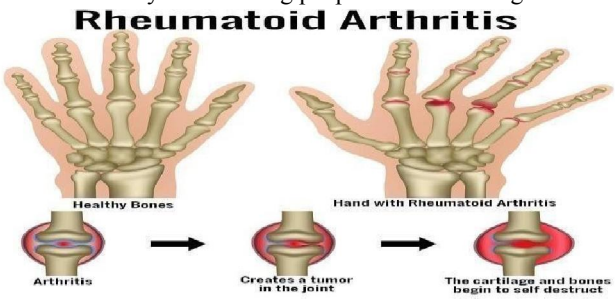


Fig no. 4 rheumatoid arthritic

Symptoms of rheumatoid arthritic :

- Swollen and tender joint
- Swelling and stiffness in joints
- Severe tiredness also called fatigue

3. Gout: Arthritis that causes sharp uric acid crystals to form in your joints. Gout is the type of inflammatory arthritic that can causes painful swelling in joints. It typically affect the big toe, but it can also affect other joints in the body. There are also conditions that causes calcium crystals to form in and around joints.

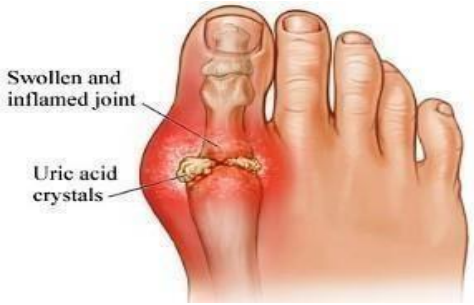


Fig no. 5 Gout


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3. Juvenile arthritis: Arthritis in kids and teens younger than 16.
They are auto-immune condition and the immune system can cause pain and swelling in joints. Juvenile rheumatoid arthritis, the most common type of childhood (under age of 16) arthritis, can cause permanent physical damage to joints.
4. Ankylosing spondylitis: Arthritis that affects joints near your lower back.
5. Psoriatic arthritis: Arthritis that affects people who have psoriasis.[7]

Symptoms and sign:
• Joint pain
• Swelling
• Loss of function
• Deformity
• weakness
• instability
• Stiffness and reduced range of motion swelling
• Tenderness and sensitivity to touch around a joint
• Feeling of heat or warmth near your joints.

Causes: Causes of arthritic is depending on their type of arthritic condition:
• Wear and tear of joint due to erosion and overuse
• Auto-immune attack
• Abnormal metabolisum
• Infection
• Degeneration of joints
• Muscle weakness
• Overweight

Risk factors:
• Age
• Obesity
• Genetics
• Heredity or genes
• Environmental factors
• Autoimmune disorders
• Injuries
• Infection[8]

LITERATURE REVIEW
1) A. Sharma et.al (2008)
The Research work consists of Limonia acidissima has nutritious fruits that are used in a variety of food preparations. The fruit as well as different parts of L. acidissima are also used in traditional systems of medicine to treat numerous ailments and reportedly possess different pharmacological activities. Keeping this in mind, an attempt has been made to do a qualitative analysis of phytochemical screening of fruit pulp. The results revealed that the aqueous, methanolic, and ethanolic extracts of fruit pulp have diverse secondary metabolites like tannin, saponin, flavonoids, phenolic compounds, reducing sugars, alkaloids, etc.
2) Soniya Choudhary et.al (2013)
This study conducted to analyze the preliminary phytochentical characters of an important medicinal plant Limonioacidissima Linn. Methods: Studies were carried out in terms of sequential extraction in petroleum ether,

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ethanol, methanol and water, total extractive values, powder behavior analysis of aerial parts and qualitative and quantitative estimation of phytochemicals. The preliminary phytochemical analysis showed the presence of carbohydrates, proteins, lipids, alkaloids, flavonoids, terpenoids and tannins.

3) Pratima Vijay vargia et.al (2014)
In Present work, Plants provide a major resource for a large number of traditional medicines that have been in existence for thousands of years in country like India. Ayurveda, one of the oldest medicinal systems in the world, provides leads for a vast number of therapeutically useful compounds. The combination of traditional and modern knowledge can produce better source of the active constituents for the treatment of diseases with fewer side effects. With the ever increasing interest of today's population towards natural products, Limoniaacidissima L. emerged out to be one of the most eyes catching plant bearing multiple medicinal properties, belonging to family Rutaceae.

4) Dr.G.S.Bhoyar et.al (2019)
The aim of present investigation was to evaluate anti-rheumatic activity of herbs Limoniaacidissimaand to formulate a topical gel dosage form. Boswellia serrata dry extract 65% was collected from the Konark Herbals and Health Care and Commiphoramukul dry resin was collected from Local Markey,Nagpur. The evalution of crude drug and the re-successive solvent extraction of resin was carried out. Development of gel was done by using carbapol 934 and PEG 400. From the result of preliminary phytochemical screening of extract, it was observed that the fractional product of Commiphoramukul resin contained triterpenoids and sterols.

5) Rohitas Deshmukh et.al (2023)
The Research work consists of Rheumatoid arthritis (RA) is a debilitating inflammatory disorder that primarily affects the elderly and causes severe bone deterioration, inflammation, discomfort, and weakness. Allopathic treatment can only treat the symptoms. Zingiberofficinale, often known as turmeric or Boswellia, is a plant that has long been used in the alternative medicine of many cultures to treat rheumatoid arthritis. The rhizomes contain many phytochemicals with medicinal advantages, including RA alleviation. The purpose of this review is to compile a list of these phytochemical components alongside the stated processes by which they work. It is believed that these phytochemicals can serve as the foundation for the development of new medications that not only alleviate symptoms but also potentially cure RA by halting the disease's effect.

6) Megha. S.Shah et.al (2020)
Current research on drug development has emphasized on traditional uses of herbal constituents which were practiced in ancient times. Ayurveda, one of the oldest medicinal systems in the world, provides leads for a vast number of therapeutically useful compounds. The combination of traditional and modern knowledge can produce better source of the active constituents for the treatment of diseases with fewer side effects. India is a rich source of many underutilized medicinal plants that possess the potential to treat diverse human aliments and diseases.

AIM AND OBJECTIVE
Aim: To Formulate and Evaluate herbal antiarthritic gel from Limmonia acidissima.

Objective:
a. To carry out extraction of selected herbs such as Limonia acidissima.
b. To developing a gel formula that provides effective pain relief for arthritis patients.
c. To formulate suitable stable gel dosage form of the herbal extract.
d. To perform evaluation parameter.

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PLAN OF WORK

The objective of the present research work is to develop Formulation and evaluation of antiarthritic gel. In order to achieve the above referral objectives, the research work was planned in the following:

1. Selection of materials
• Procurement of drug and polymers which are required for study:
• Drug and Excipient Profile

2. Experimental Study
• Phytochemical screening
• Preparation of extract
• Preparation of formulation

3. Preparation of gel
4. Evaluation of gel
• Physical Examination
• Determination of pH
• Spreadability
• Viscosity
• Extrudability
• Washability
• Irritancy Test
• Stability studies

PLANT PROFILE

Limonia accidisima:

Those plants used were called as medicinal plants. The medicinal plants are referred to
plants that are used for their therapeutic or medicinal values. The presence of various life sustaining constituents in plants made scientists to investigate these plants for their uses in treating certain infective diseases and management of chronic wounds.

The whole plant or its different parts may be valued for its therapeutic, medicinal, aromatic or savoury qualities. Medicinal plants are cheaper, more accessible to the most of the population in the world. Thus, there is need to encourage the use of medicinal plants as potential sources of new drugs. There has been as highly increased interest for herbal remedies in several parts of the world.[9]

Fig. No 6 Limonia acidissima fruit

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TAXONOMY

Kingdom: Plantae Subkingdom : Tracheobionta Superdivision : Spermatophyta Division : Magnoliophyta Class : Magnoliostida Subclass: Rosidae

Order: Sapindales Family : Rutaceae Genus : Limonia L. Species : L acidissima10

Indication and uses

The pulp and powdered rind of the fruit is applied as a poultice for insect bites and stings; the unripe fruit is used in the treatment of gum diseases, sore throat, coughs, dysentery and diarrhoea. The powdered fruit is mixed with honey and used to treat dysentery in children.[11]

## MATERIAL AND METHOD

Material: Material and their role used in this study are listed in table 1.

Table no.1 list of ingredients and its role

| Sr no | Ingredients | Role |
|---|---|---|
| 1 | Limonia acidissima | Anti-arthritic |
| 2 | Carbapol 934 | Gelling agent |
| 3 | Polyethylene glycol | Humectant |
| 4 | Isopropyl alcohol | Cooling agent |
| 5 | Ethanol | Solvent |
| 6 | Methylparaben | preservatives |
| 7 | Triethanolamine | pH adjuster |
| 8 | Water | Vehicle |

Instruments: The instruments are used in this study there are listed in table.

Table no. 2 List of Instruments

| Sr.no. | Instruments | Manufacturer |
|---|---|---|
| 1 | Heating Mantle | Science and Surgical House |
| 2 | pH Meter | Equiptronic |
| 3 | Magnetic Stirrer | Science and Surgical House |
| 4 | Brook Field Viscometer | DV-E Viscometer |
| 5 | Electronic Balance | Wensar |

Methods:

Chemicals: carbapol 934, polyethylene glycol, Isopropyl alcohol, ethanol ,methyl paraben, triethanolamine.

Collection of palnt:

The following limonia acidisssima was collected from the plant by Local Market.

Preparation of herb powder:

Collect the fresh fruits pulp and dried under the sunlight. Then make a powder of the sample by using grinding mixer.

Preparation of plant extract:

- The fresh fruits was collected from the local market.
- fruit was dried shade to avoid degradation of phytoconstituents.
- After drying material was coarsely powered with grinding mill and kept in well closed container about 30g powder respectively was defatted with ethanol in maceration followed by extraction with ethanol.
- The collected extract
- kept in a vacuum filteration until used.[12]

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Fig no.8 maceration method

Apparatus and chemicals are used in extraction process:
Apparatus: heating mantle, round bottom flask, weighing balance,

Chemicals: Ethanol
Phytochemical screening: [13]
1) TEST FOR ALKALOIDS
• Mayers test: 1 ml of the filtrate of fruit pulp extract was taken and added 3 to 4 drops of mayers reagent. The formation of white or creamy precipitate confirmed the presence of alkaloids.

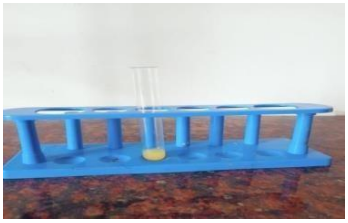

Fig no. 9 Mayers Test

2) TEST FOR FLAVONOIDS:
• Lead acetate test: 1ml of fruit pulp extract and add few drops of lead acetate solution. A bulky white lead precipitate are form it indicate the test are present.

Fig.no.12 Lead acetate Test

3) TEST FOR CARBOHYDTRATE:
• Fehling test: To 2ml of filtrate, 2 drops of alcoholic solution of a naphthol were added, the mixture was shaken well and 1ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A reddish brown color indicated the absence of carbohydrates.

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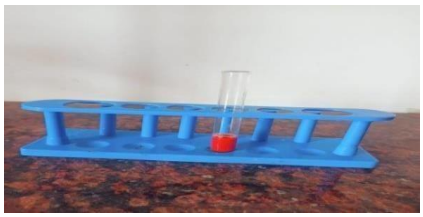

Fig.no.13 Fehlings Test

• Benedicts test: 1ml of fruit pulp extract and add 0.5 ml benedict reagent .Then these mixture was heated on boiling on water bath for 2min.coloured precipitate shows the presence of carbohydrate test.

Fig.no.14 Benedicts test

4) TEST FOR PHENOLIC:

• Lead acetate test: 1 ml of fruit pulp extract and add 3ml lead acetate solution. Then these mixture was shows the bulky white lead precipitate confirm the presence of phenolic test.

Fig.no.15 Lead Acetate test

5) TEST FOR GLYCOSIDE:

• Borntrager test:

To 2ml of filtered hydrolysate, 3ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Green colour indicate the presence of glycosides.

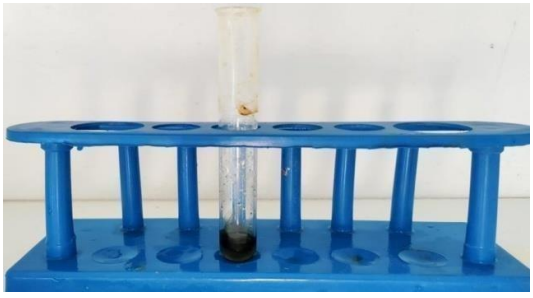

Fig.no.16 Borntrager Test

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• Molisch test:

Add few ml of extract in chloroform then add alpha naphthol and ethanol and shake it. Then add few ml of sulphuric acid and Reddish brown colour is present it indicates the absence of glycosides.

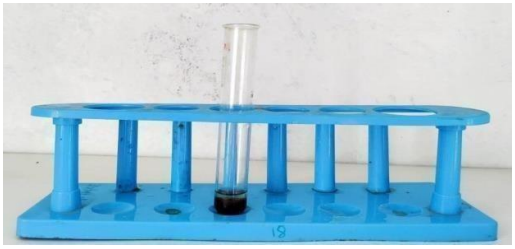

Fig.no. 17 Molisch Test

Preparation of gel base:

• The gel prepared first the Required quantity of water was taken in a beaker
• Then weighed quantity of carbapol934 0.5 gm dispersed in distilled water and stirring on laboratory stirrer. (HOMOGENIZER )
• In another beaker, iso-propyl alcohol dissolve in polyethylene glycol and add slowly methyl paraben .
• This solution is added into the above gel solution and stirred it.
• triturated wel and at last, triethanolamine was added to adjust the pH.[14]

Preparation of gel formulation:

• The gel prepared first the Required quantity of water was taken in a beaker
• Then weighed quantity of carbapol 934 0.5 gm dispersed in distilled water and stirring on laboratory stirrer. (HOMOGENIZER )
• In another beaker, limmonia accidisimal extract was dissolved in isopropyl alcohol then polyethylene glycol ,ethanol
• and then add slowly methyl paraben.
• This solution is added into the above gel solution and stirred it.
• triturated wel and at last, triethanolamine was added to adjust the pH 7.08[15]

Evaluation of gel:

• Physical examination
• Determination of ph
• Spreadability
• Viscosity
• Extrudability
• Washability
• Irritancy test
• Stability test

1. Physical examination-

a) Colour:

it can be inspected visually colour of the gel.

B) Clarity and grittiness:

The evaluation of the developed formulations for clarity observed visually with naked eye. Smears of gels wereprepared on glass slides and observed under a compound microscope for the presence of any insoluble particles or grittiness.

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b) Homogeneity and Phase separation:
It can be asseseed by Visual inspection.

Consistency:
It can also assessed by Visual Inapection.[16]
Extrudability:
A closed collapsible tube containing about 20 g of gel was pressed firmly at the crimped end and a clamp was applied to prevent any roll back. The cap was removed and the gel was extruded. The amount of the extruded gel was collected and weighed. The percentage of the extruded gel was calculated by using the following formula
Extrudability = weight applied to extrude gel from tube (in gm ) / area (in cm2)[17]

2. pH measurement :
pH measurement of the gel was carried out using a digital pH meter by dipping the glass electrode completely into the gel system to cover the electrode, The measurement was carried out in triplicate and the average of the three readings was recorded.
3. Spreadability:
Spreadability of gel is measured in terms of diameter of gel circle produced when gel is placed between two glass plates of definite weight. A weighed quantity (350 mg) of gel is taken on one glass plate and another glass plate is dropped from a distance of 5cm. The diameter of the circle of spread is measured. It is calculated by using the formula:
Formula: S- M.L / T
Where, S –Spreadability
M-Weight tied to upper slides L- Length of the glass slide T- Time taken in sec.[18]
3. Viscosity:
The Viscosity of anti arthritic gel was determined using Brookfield viscometer with spindle no.18 at 100 rpm at 25°C.[19]

4. Washability:
Gel were applied on the skin then ease and extent of washing with water were checked manually.
5. Irritancy Test:
Mark an area of 1 sq. cm on the left-hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema was checked, if any, for regular interval up to 24 hrs.[20]
6. Stability studies:
The stability study was performed by the formulation was packed in collapse tubes and stored for one month's at room temperature. The formulations were analyzed after every one month for physical properties, spreadability, pH, and drug content.[21]

RESULT AND DISCUSSION :
1) Phytochemical screening:

Table NO 4 -Phytochemical screening

| SR. NO | TEST | OBSERVATION | INFERANCE |
|---|---|---|---|
| A)<br>1. | Test for alkaloids Mayers test<br>Wagners test Hagers test | White or creamy ppt Reddish –<br>brown ppt Yellow ppt | Alkaloids present Alkaloides<br>present Alkaloides present |
| B) | Test for flavonoids<br>Lead acetate test | Bulky white lead ppt | Flavonoids present |
| C) | Test for carbohydrate Fehling test<br>Benedict test | Red ppt Coloured ppt | Carbohydrate present<br>Carbohydrate present |
| D) | Test for phenolic | | |

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| | | | |
|---|---|---|---|
| | Lead acetate test | Bulky white ppt | Phenols are present |
| E) | Test for glycoside Borntrager test Molisch test | Green ppt Reddish brown test | Glycoside present Glycoside present |

Formula : Composition of Gel:

Table no. 4 Formula of Gel

| Sr. no | Ingredient | Quantity |
|---|---|---|
| 1 | Limonia acidissima | 0.5 |
| 2 | Carbapol 934 | 1g |
| 3 | Polyethylene glycol | 10ml |
| 4 | Isopropyl alcohol | 10ml |
| 5 | Ethanol | 5ml |
| 6 | Methylparaben | 0.15g |
| 7 | Triethanolamine | 2-3 g |
| 8 | Water | Upto 100ml |

Fig No.18 Preparation of Gel Base Fig No.19 Prepared Gel Base

Fig no.20 prepared gel formulation



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1) Results of Evaluation parameters:

a. Physical Examination

Table NO5 Evaluation physical appearance

| Sr. no | Parameter | Observation |
|---|---|---|
| 1 | Colour | Brown |
| 2 | Odour | Characteristic |
| 3 | Clearity | Clear |
| 4 | Gritiness | Smooth |
| 5 | Consistency | Smooth |
| 6 | Phase separation | No |

a) Determination of pH

Table no 6. Determination of pH

| Sr. no | pH |
|---|---|
| 1 | 7.08 |

Fig no.21 pH test

1. Spreadability: The value of Spreadability indicate that the gel is Easily spreadable by small amount of shear. The spredability shown in Table (7).

Table no.7. Determination of spreadability

| Sr. No. | Diameter | Spreadability |
|---|---|---|
| 1 | 1.4 | 50 |

S = M.L/T

S = 10*7.5/1.5

S = 50 gm.cm/sec

Fig no.22 Spreadability test

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d) Viscosity:
The viscosity of gel was found to be 12653 cps.

e) Washability:
The gel was easily washable and good washability.

f) Extrudability:
Weight applied to extrude gel from tube (in gm) / (in cm2)
= 50 gm / 4 cm2
= 12.5

g) Irritancy test:
No any irritation or rashes seen on skin.

h) Stability study:
The gel were found to be stable after one month at room temperature ,no change was recorded in parameter like colour, odour, pH, spreadability, the results shown in table

Table no 8. Stability study

| Sr.no | Test | After one month |
|---|---|---|
| 1 | Physical appearance | Semisolid |
| 2 | Colour | Brown |
| 3 | Odour | Characteristic |
| 4 | Ph value | 7.08 |
| 5 | Spreadability | 50 |

## II. CONCLUSION

Nowadays, there is a lot of demand for herbal formulations in the market due to their cost effectivity and absence of any side effects. From the above experimental data, it is clear that a gel formulation with herbal ingredients such as Limonia acidissima has good characteristics, viscosity activity which is necessary in the management of skin permeation. In this study the formulation were subjected to various evaluation parameters such as physical appearance, pH, evaluation, spreadability, Rheological studies, were found to be within the limits. Limonia acidissima was successfully formulated in a gel drug delivery with propylene glycol as penetration enhancer.

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