TO STUDY THE MADHUCA LONGIFOLIA LEAVES EXTRACT AND THE DEVELOPMENT OF THE FORMULATION

Mrs. Prachi Moon[1], Mr. Shubham Waghmare[2], Mrs. Harsha P. Sontakke[3], Ms. Pranjali Patil[5], Miss. Priyanka Sakhare[4]

[1] Research Scholar, Gurunanak College of Pharmacy, Nagpur

[2]Assistant Professor , Rasiklal Manikchand Dhariwal Institute of Pharmaceutical Education and Research, Pune.

[3] Assistant Professor, National College Of Pharmacy, Nagpur.

[4]Assistant Professor, Sushganga College of Pharmacy, wani-445304.

[5] Data Annotation Consultant, Smt. Kishoritai Bhoyar College of Pharmacy,nagpur

*** Corresponding author:**

Mrs. Prachi Moon

Research Scholar, Gurunanak College of Pharmacy, Nagpur, Maharashtra, India

shubham990207@gmail.com

7058707091.

Orcid Id- 0009-0008-4238-0685.

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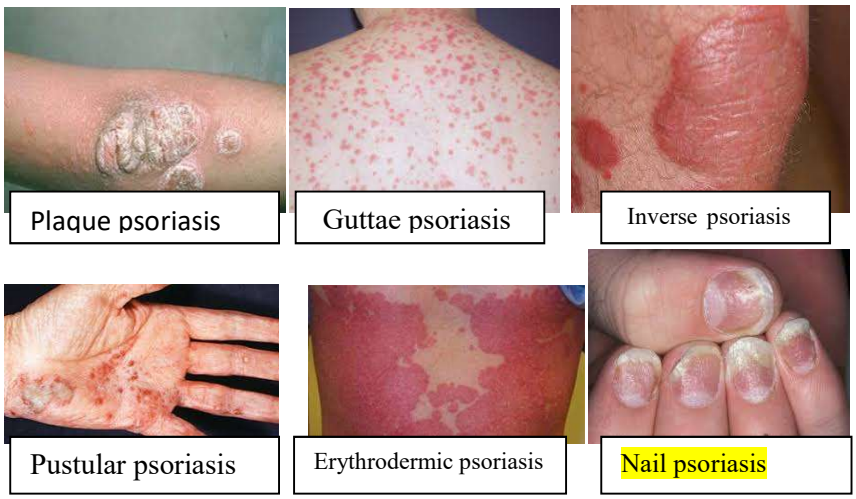
ABSTRACT

Psoriasis is a chronic inflammatory skin disorder requiring safer and cost-effective treatment options. This study evaluates the phytochemical profile of *Madhuca longifolia* leaves and develops a topical gel for antipsoriatic activity. Methanolic extract obtained via Soxhlet extraction showed the presence of flavonoids, alkaloids, glycosides, saponins, and carbohydrates. A Carbopol 934-based gel containing the extract was formulated and evaluated for physicochemical properties, showing acceptable pH, viscosity, spreadability, and homogeneity. Antipsoriatic activity was assessed using an imiquimod-induced psoriasis model in Swiss albino mice, where the formulation significantly reduced erythema, scaling, and skin thickening. Toxicity studies indicated safety at higher doses. The findings suggest that *Madhuca longifolia* gel may serve as a promising herbal alternative for psoriasis management.

1. INTRODUCTION

Psoriasis is a chronic, immune-mediated inflammatory skin disorder characterized by abnormal keratinocyte proliferation and differentiation, leading to erythematous, scaly plaques on the skin surface. The disease significantly affects the quality of life and is associated with systemic comorbidities such as arthritis, cardiovascular disorders, and metabolic syndrome. Globally, psoriasis affects approximately 2–3% of the population, with increasing prevalence in both developed and developing countries (Parisi et al., 2013; Boehncke & Schön, 2015). The pathogenesis involves a complex interplay between genetic predisposition, environmental triggers, and immune dysregulation, particularly mediated by T-helper (Th1 and Th17) cells and cytokines such as TNF-α, IL-17, and IL-23 (Lowes et al., 2014).



Conventional therapies for psoriasis include topical corticosteroids, vitamin D analogs, systemic immunosuppressants, and biologics. Although effective, these treatments are often associated with limitations such as adverse effects, high cost, long-term toxicity, and reduced patient compliance (Armstrong & Read, 2020). These drawbacks have led to increased interest in alternative and complementary therapies, particularly plant-based medicines, which are considered safer and more economical.

Medicinal plants have been widely used in traditional systems of medicine such as Ayurveda for the treatment of skin disorders due to their anti-inflammatory, antioxidant, and immunomodulatory properties. Among these, *Madhuca longifolia* (Family: Sapotaceae), commonly known as Mahua, has gained attention due to its diverse pharmacological activities. It is widely distributed in India and has been traditionally used for treating various ailments including skin diseases, inflammation, diabetes, and infections (Jha & Mazumder, 2018).

The leaves of *Madhuca longifolia* are rich in bioactive phytoconstituents such as flavonoids (quercetin, myricetin), triterpenoids (oleanolic acid), sterols (β-sitosterol), and phenolic compounds, which contribute to its therapeutic potential. These compounds exhibit significant anti-inflammatory, antimicrobial, and antioxidant activities, making the plant a promising candidate for the treatment of psoriasis and other dermatological conditions (Verma et al., 2017; Khare, 2018).

Topical drug delivery systems are particularly advantageous in the treatment of psoriasis as they allow localized drug action, minimize systemic side effects, and improve patient compliance. Various formulations such as creams, gels, ointments, and films are commonly used for delivering therapeutic agents directly to the affected site. However, the effectiveness of topical formulations depends on the ability of the active constituents to penetrate the stratum corneum barrier (Prausnitz & Langer, 2008).

Extraction of bioactive compounds from plant materials is a critical step in the development of herbal formulations. Among various extraction techniques, Soxhlet extraction is widely used due to its efficiency, simplicity, and ability to achieve exhaustive extraction of phytoconstituents. It ensures continuous extraction using organic solvents under controlled conditions, thereby improving yield and reproducibility (Azwanida, 2015).

Therefore, the present study aims to investigate the phytochemical profile of *Madhuca longifolia* leaves extract and to develop a suitable topical formulation for the effective management of psoriasis. The study focuses on optimizing extraction methods, evaluating phytoconstituents, and formulating a stable and efficacious dosage form that can enhance therapeutic outcomes while minimizing adverse effects.

EXPERIMENTAL WORK:

The fresh Leaves of *MadhucaLongifolia* was collected from the Butibori, Nagpur, Maharashtra, India in the month of August 2020.

AUTHENTICATION:

The Leaves of *MadhucaLongifolia* was identified and authenticated by Dr. Nitin Dongarwar, Head of Department of Botany, Rashtrasant Tukdoji Maharaj, Nagpur University, Nagpur. Thevoucher number of authenticated specimen is10410

Fig.No.1: Authentication sheet of *Madhuca Longifolia*

EXTRACTION OF LEAVES OF *MADHUCA LONGIFOLIA*:

Soxhlet extraction of *Madhuca Longifolia*: Freshly collected leaves were cleaned and shade dried. Approximately 2 kg of leaves were powdered and successively defatted with petroleum ether at 40°C for about 30–35 cycles to remove chlorophyll. The defatted powder was further extracted with 88% methanol at 50°C for 90 minutes using a Soxhlet apparatus. The extract was then filtered through Whatman filter paper, concentrated under reduced pressure, dried, and stored in a desiccator for further studies **(Azwanida, 2015; Harborne, 1998; Handa et al., 2008)**.

Fig.No.2: Extraction of *madhuca Longifolia*

PRELIMINARY PHYTOCHEMICAL SCREENING:

Characterization of leaves extracts:

Organoleptic properties: The leaves extracts were analyzed for Colour, Odour and consistency.

Physical characterization: Obtained extracts were analyzed for Colour, Odour, taste and solubility. **(Kokate et al., 2010; Trease & Evans, 2009)**.

6.4.2 Phyto-chemical screening:

The extracts were concentrated and subjected to phytochemical screening using standard procedures. The extracts were analysed for alkaloids, glycosides, flavonoids, tannins, carbohydrates, saponins, steroids and triterpenoids. **Harborne, 1998; Sofowora, 2008; Tiwari et al., 2011)**.

Test for Alkaloids:

Dragendroff's Test: To 2 ml Dragendorff's reagent was mixed with extract. Appearance of reddish brown colour indicates the presence of alkaloids.

Mayers test: To 2ml of extract few drops of the Mayer's reagent was added. Formation of white or pale yellow precipitate indicates the presence of alkaloids.

Hager's test: To 2 mg of the extract a few drops of Hager's reagent were added. Formation of yellow precipitate confirms the presence of alkaloids. **(Trease & Evans, 2009; Kokate et al., 2010)**

Test for Carbohydrate:

Benedicts test: To 0.5ml of extract, 5ml of Benedict s solution was added in a test tube and boiled for 5min. Formation of brick red coloured precipitate indicated the presence of carbohydrate.

Fehling's test: To 2ml of extract, 1ml mixture of equal parts ofFehling's solution A and B were added and boiled for few minutes. Formation of red or brick red coloured precipitate indicated the presence of reducing sugar. (Harborne, 1998; Tiwari et al., 2011)

Test for Flavonoid:

Hydrolyze 5ml of extracts with 10%v/v sulphuricacid and cooled. Then it was extracted with diethyl ether and divided into three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow colour demonstrated the presence of flavanoids.

Shinoda's -Test:

The extract was treated with magnesium powder and a few drops of sulphuricacid appearance of magenta colourindicate the presence of flavanoids.

Extract was mixed with lead acetate solution and observed for the formation of yellow precipitate that indicates the presence of flavonoid.

Alkaline reagent test:

To 2ml of test extract few ml of sodium hydroxide solution was added. At first intense yellow colour was appeared which turned colorless on addition of dil. HCl indicates the presence of flavonoid. **(Harborne, 1998; Sofowora, 2008)**.

Test for Glycosides

Borntragers test: Take 5ml of extract and boiled with equal amount of dil. Sulphuric acid in a test tube for 5min. While hot it was filtered and cooled. The filtrate was shaken with equal volume of chloroform. The lower layer of chloroform was separated. The layer was stunned with half of its volume of dil. Ammonia. Appearance of rose pink to red colour indicates the presence of glycoside. **(Trease & Evans, 2009)**.

Test for Saponin:

Froth formation test: 2ml of extract was shaken vigorously with water in a test tube and left for 3min. Formation of froth indicated the presence of saponin. **(Kokate et al., 2010)**.

Steroids and Triterpenoids

Salkowski reaction: Test extract was shaken with chloroform, to the chloroform layer, sulphuric acid was added slowly by the sides of test tube. Red colour indicated the presence of steroids.

Tannins

Ferric chloride test: To 1-2ml of the extract, few drops of 5% w/v $FeCl_3$ solution were added. Appearance of green colour indicated the presence of gallotannins, while brown colour indicated the presence of pseudo tannins. **(Sofowora, 2008)**.

PHYTOCHEMICAL STANDARDIZATION:

Estimation of total flavonoid content (Aluminium Chloride Colorimetric Method

Principle:

Aluminium chloride forms acid labile complexes with the ortho - dihydroxyl groups in the A- or B-ring of flavonoids. For building the calibration curve, quercetin is used as a standard material. Various concentrations of standard quercetin solution were used to make a standard calibration curve.

Procedure:

In this method, quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100 µg/ml. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λmax of quercetin) with a UV- Vis spectrophotometer (UV- 1800 Shimadzu). 1% Aluminium chloride and 1M potassium acetate solutions were used for assay.

Stock Solution of Extracts:

100 mg of the extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol.

Preparation of Test Solutions:

0.5ml of extract stock solution, 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Blank was prepared in similar way by replacing aluminium chloride with distilled water and absorbance was measured at 415 nm. All prepared solutions were filtered through whatmann filter paper before measuring.

PREPARATION OF TOPICAL GEL

PreparationofTopicalgel usingCarbopol934: Das, S., Haldar

Accurately weighed Carbopol 934 powder was taken in a beaker and dispersed in 50 ml of distilled

water. The beaker was kept aside to swell the Carbopol for half an hour and then stirring was done using mechanical lab stirrer at 1200 rpm for 30 min. 5ml propylene glycol was taken in a beaker and weighed quantity of propyl paraben and methyl paraben were added to it and stirred properly. Further 1 g extract of *Madhuca Longifolia* leaves was mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. Preservatives solutions were added to the swollen Carbopol gel with constant stirring. Triethanolamine was added drop wise to the formulations for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency. **(Aulton, 2018; Allen, 2013)**.

Table No. 1: Composition of Topical Gel (Trial formulation)

Sr. No.	**Ingredients**	**G1**	**G2**	**G3**
1	Carbopol 934	0.5g	1g	1.5g
2	Propylene glycol	10ml	10ml	10ml
3	Methyl paraben	0.2g	0.2g	0.2g
4	Propyl paraben	0.5g	0.5g	0.5g
5	Triethanolamine	Dropwise	Dropwise	Dropwise
6	Distilled water	Q.S	Q.S	Q.S

Gel formulation 2 was good in appearance and consistency i.e. selected for formulation of topical gel containing extract.

Table No. 2: Composition of Topical Gel containing extract (Final formulation)

Sr. No.	**Ingredients**	**Formulation**
1	Carbopol 934	1g
2	Propylene glycol	10ml
3	Methyl paraben	0.2g
4	Propyl paraben	0.5g
5	Triethanolamine	Dropwise
6	Distilled water	Q.S
7	Extract of *MadhucaLongifolia*	1%

Final Gel formulations were used for Evaluation and Antipsoriatic activity

6.6.2 Evaluation of Gel

Physical appearance:

The prepared gel formulations containing *MadhucaLongifolia leaves* extract were inspected visually for their color, homogenicity, consistency and phase separation.

pH:

The pH of developed gel formulations was determined using digital pH meter. 1gm of gel was dissolved in 100 ml distilled water and kept aside for two hrs. The measurement of pH of each formulation was done in triplicate and average values are calculated.

Spreadability: Laxmi, R.J., Karthikeyan

The spreadability of the formulation was determined using glass slides, where gel (1g) was applied within the two slides, which was retained for one minute. The spreadability of the formulation was then determined by measuring the circumference of the spreaded gel. Spreadability was calculated using the formula:

S=M × L /T

Where, S= Spreadability,

M=Weight on upper slide

L=Length of glass slide

T= Time taken

Viscosity:

The viscosity of the developed gel formulations was studied using Brookfield viscometer with spindle No. 96.

Homogeneity:

After the gels have been set in the container, all developed gels were tested for homogeneity by visual inspection. They were tested for their appearance and presence of any aggregates.

Drug Content:

About 1g of gel was accurately weighed and transferred to 100 ml volumetric flask methanol was added to dissolve the gel. After mixing the volume was made upto remaining amount of methanol and dissolved for 20 min, then filter. From filtrate 1ml sample was withdrawn and dilute with methanol. The resultant solution was then estimated for the total flavonoid content.

Skin irritation test:

Hairs were removed from the dorsal site of mice and skin was cleaned. The gel formulations (0.5%, 1%, 2% w/w) were then applied to three different groups of animals and fourth group was kept as normal. The skin of each animal was perceived for symptoms of any inflammatory reactions, erythema, irritation and development of oedema following 4 hrs and then upto 48 hrs of application of Gel. **(Sinko, 2011; Indian Pharmacopoeia, 2018)**.

6.7 IN-VIVO STUDY:

EXPERIMENTAL ANIMALS

Healthy Swiss Albino mice of either sex weighing between 20 and 30 g and 8-10 weeks of age were obtained from the Animal House of the Gurunanak college of Pharmacy, Nagpur, Maharashtra, India. The pathogen free animals were maintained under standard conditions (12h light and dark cycle at an ambient temperature of 25± 1 ^{0}C). They were fed with commercially available mice feed and water was given at libitum. The animals were allowed to acclimatize to the environment for 7 days before the commencement of experiments. All experimental protocols were performed after approval from the Institutional Animal Ethical Committee (Reg. No: 675/02/C/CPCSEA, Dated: 13-09-2007) **(van der Fits et al., 2009; OECD, 2008)**.

Studies on Anti psoriatic activity of extract:

Imiquimod induced psoriatic activity (prmaritankar)

Experimental animals were shaved along their backs using hair remover cream (Olivia, Herbs De Olivia Pvt. Ltd, India) and randomly divided into 6 groups of 6

animals and received treatment as shown in the table below. To induce psoriasis, commercially available IQM 5%w/w cream (Glenmark Pharmaceuticals Pvt. Ltd, Goa, India) was applied to mice at a daily dose of 80 mg on the back skin for 15 consecutive days in all groups except normal group. Treatment was started after 7 days and continued for 15 days after induction of psoriasis.

Animal groups and Treatment

Group	**Treatment**
Normal	Gel base
Control	Only 5% IMQ cream

Standard	IMQ + Clobetop-S6 ointment (Clobetasol propionate 0.05%w/w+ Salicylic acid 6%w/w,JVJ Pharmaceuticals Pvt. Ltd. Mumbai, India.)
Test group-1	IMQ plus 0.5% Gel
Test group-2	IMQ plus 1% Gel
Test group-3	IMQ plus 2% Gel

Scoring severity of skin inflammation:

To score the severity of inflammation of the back skin, an objective scoring system was developed based on the clinical Psoriasis Area and Severity Index (PASI). Erythema, scaling, and thickening were scored independently on a scale from 0 to 4: where 0 corresponds to none; 1 corresponds to slight; 2 corresponds to moderate; 3 corresponds to marked and 4 corresponds to very marked.

Statistical Analysis:

The experimental results were expressed as mean±SD, with six animals in each group. One-way Analysis of variance (ANOVA) and Dunnett's test were used to test significance differences between groups. P values <0.05 were considered to be significant.

RESULTS AND DISCUSSION:

7.1 RESULT:

7.1.1 Physical properties of extract:

Maheswari Chinnadhurai*et al.(2019)*extracted*MadhucaLongifolia*leaves with methanol by soxhletation. The obtained extract showed 7.7% w/w extractive yield with Brownish green colour, solid consistency. In preliminary phytochemical screening Methanol extract showed the presence of alkaloids, terpenoids, carbohydrates, flavonoids, saponins, proteins, and glycosides.

TableNo.4:Physical properties of Extract

Extract	**Characteristics**
Colour	Brownish green
Odour	Pleasant

Physical nature	solid

7.1.3 Phytochemical screening of extracts:

Table No.5: Phytochemical screening of extract

Chemical Test	**Observation**	**Inference**
Test for Alkaloids a) Dragendroff's Test	reddish brown colour	+
b) Mayerstest	pale yellow precipitate	
c)Hagers test	yellow precipitate	
TestforCarbohydrate a)Fehlings test	brick red coloured precipitate	+
b)Benedicts test	brick red coloured precipitate	
TestforFlavonoids a)Shinoda's –Test	magenta colour	+
b) lead acetate solution	yellow precipitate	
c)Alkaline reagent test	Yellow colour not turn to colourless	
TestforGlycosides a)Borntragers test	Appear rose pink colour	+
TestforSaponin a)Froth formation test	Foam persist	+
TestforSteroidandtriterpenoids a)Salkowski reaction	Red colour	-
TestforTannins a)Ferric chloride test	Not appear any colour	-

+ indicates presence of Phytoconstituents, - indicates absence of Phytoconstituents.

7.1.4 UV Spectrum of *MadhucaLongifolia*:

Vinti Singh et al. (2016) investigated the total flavonoid content *MadhucaLongifolia* of using the aluminium chloride colorimetric method. The extract sample was diluted with methanol to 100 mg/ml. The calibration curve was prepared by diluting quercetin in methanol (0–100 mg/ml). The diluted extract or quercetin (2.0 ml) was mixed with 0.1 ml of 10% (w/v) aluminium chloride solution and 0.1 ml of 0.1 mM potassium acetate solution. The mixture was kept at room temperature for 30 min. The maximum absorbance of the mixture was then measured at 415 nm using a UV–Vis spectrophotometer. Total flavonoids content was expressed as miligrams of quercetin equivalent per gram mahua (mg QCE/g).

Calibration curve of *MadhucaLongifolia* Extract

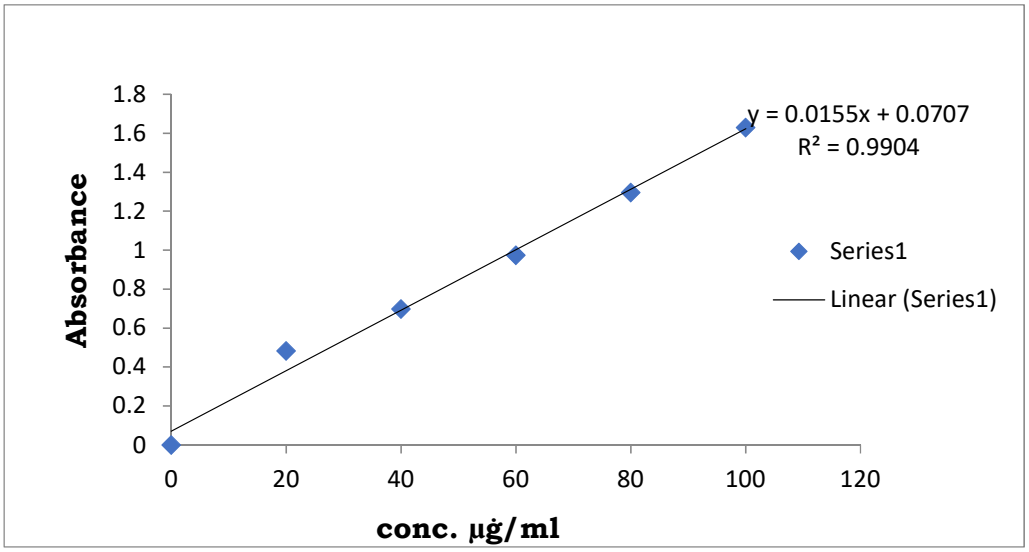


Fig 7:- Calibration curve of *MadhucaLongifolia* extract

Spectrophotometric Analysis of Quercetin

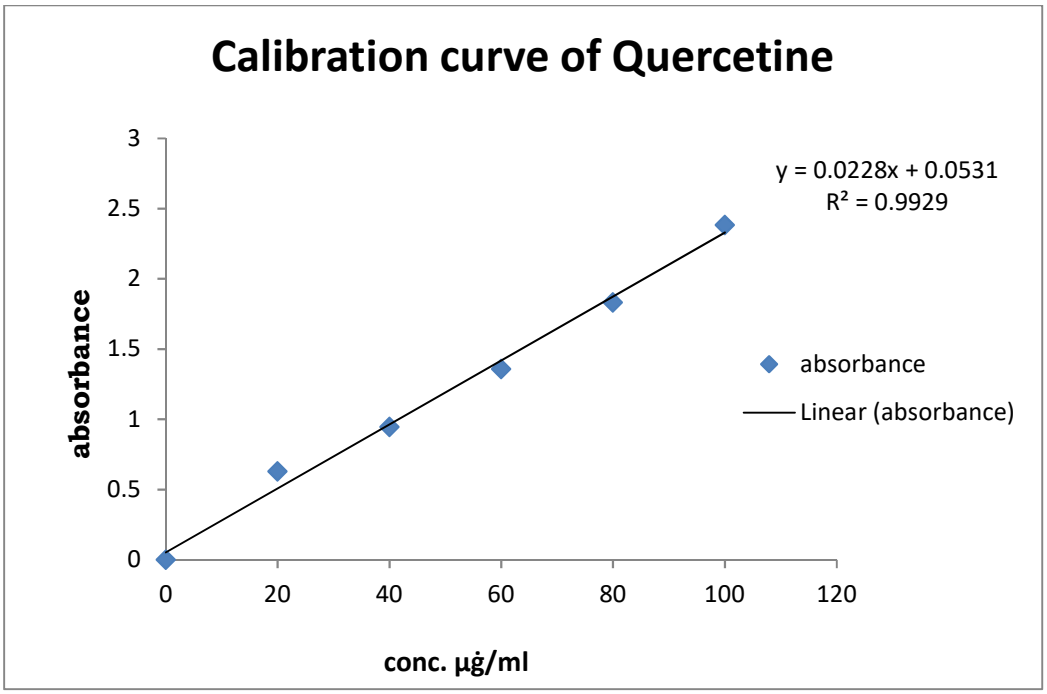


Fig 5:-Spectrometric Analysis of Quercetin

Table no -6: Flavonoid content in Madhuca Longifolia extract expressed in term of quercetine equivalent (mg of QE/g of extract).

Extract	Mg of QE/g of extract
MaducaLongifolia	3.63

7.1.7 Physicochemical evaluation of gel:

evaluated topical gel containing *Madhuca Longifolia* extract on following parameter

Table No.7: Evaluation of Gel

Parameter	**Formulation**
colour	Light yellow
pH	6.4
Viscosity	2200
Spreadability (g.cm/sec)	19.78
Consistency	Semisolid
Homogeneity	No gritty particles and lumps in formulation
Drug content	74.97%

7.1.8 Toxicity study:

N. Devi *et al*. (2019)evaluated hydroalcoholic leaf extract of *MadhucaLongifolia* for its in vivo acute and sub-acute toxicity as per the OECD guidelines in albino Wistar rats. Acute toxicity studies were performed by administering 2000 mg per kg body weight extract orally. In Sub- acute toxicity study 200 and 400 mg per kg body weight extract were administered orally for 28 d. the LD_{50} of the extracts was greater than 2000 mg per kg body weight. In repeated dose sub-acute toxicity study, the results did not reveal any treatment-related abnormalities in terms of body weight, relative organ weight and biochemical parameters.

7.1.8 IN-VIVO STUDY:

KamleshWadher*et al.*2021, Evaluated antipsoriatic activity of gel containing Pongamiapinnata extract on Imiquimod-induced psoriasis. Psoriasis was induced in rat within period of 7 days. The grading was given to each rat before and after treatment with test, and psoriatic control along with naive control.

Table No. 8: PASI grading of psoriasis animal before and after treatment

Group	**Initial grading after induction of psoriasis using imiquimod [PASI]**	**Final grading after treatment of 7 days [PASI]**
1.Control	None	None
2. drug	Very Marked	None
3.Marketed formulation	Very Marked	None

Results were analyzed by two-way ANNOVA followed by post hoc Bonferroni multiple comparison test. Result reveals that, there was significant increase in psoriasis after applying IMQ (topically). Daily treatment with extract starting from post day-8 following IMQ, progressively reduced the psoriasis score in rats as compared to the standard tested animals stastical analysis revealed that significant recovery of psoriasis on post psoriasis days 12 ($P < 0.001$) of the protocol.

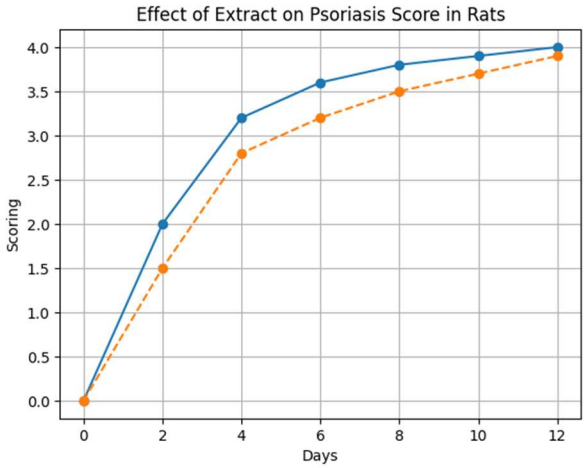


Figure represents the effect of topical treatment of Ethanolicextract on PASI grading on IQM-induced psoriatic mice. (Table --).

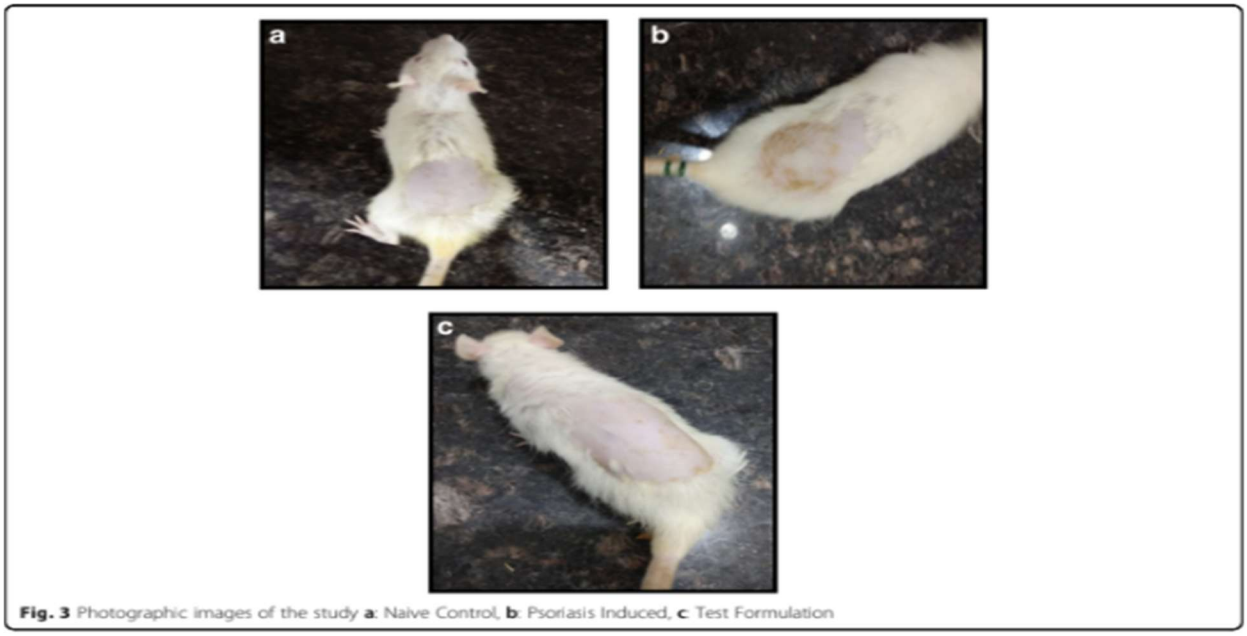


Fig. 3 Photographic images of the study **a**: Naive Control, **b**: Psoriasis Induced, **c**: Test Formulation

Vijayalakshmi A*et al.* (2021) assessed anti-psoriatic activity of the methanol extract and the isolated flavonoid quercetin from the rhizome of Smilax china (S. china). Methanol extract (100 and 200 mg/kg b.w.) and isolated flavonoid quercetin (25 and 50 mg/kg b.w.) were tested in Swiss albino mice. The flavonoid quercetin shows significant orthokeratosis, anti-inflammatory and maximum anti-proliferantive activities in the mouse tail model.

Table No.9: Effect of methanol extract and isolated flavonoid quercetin on the degree of orthokeratosis, epidermal thickness and the 'drug activity' in the mouse tail test (mean ±SEM) (n=6)

Treatments	Orthokeratosis (%)	Activity (%)	Change in epidermal thickness (%)
Saline	17.07± 3.20	-	-
Retinoic acid (0.1 mg/kg)	65.06±1.09**	57**	5
Methanol extract (100 mg/kg)	19.06±2.90	2	0
Methanol extract (200 mg/kg)	26.86 ±3.10	11*	2
Isolated flavonoid quercetin (25 mg/kg)	32.18±3.10**	18**	2
Isolated flavonoid quercetin (50 mg/kg)	39.80±1.60**	27**	3

Result were analyzed by ANOVA, followed by Dunnet's 't' test (n=6). **: P <0.01, *: P <0.05 when compared with control

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