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RESEARCH ARTICLE

Phytochemical Characterization, Antioxidant Profiling, and Wound Healing Potential of Aqueous and Ethanolic Extracts of *Michelia champaca* Linn. (Magnoliaceae): An Integrative In Vitro and In Vivo Study

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ABSTRACT-

Background: Chronic and acute wounds impose a formidable global health burden. *Michelia champaca* Linn. (Magnoliaceae), widely distributed across tropical Asia, is ethnomedicinally valorised for wound management and anti-inflammatory applications, yet rigorous pharmacological substantiation remains incomplete.

Objective: To perform comprehensive phytochemical characterization, Total phenolic content (TPC), Total flavonoid content (TFC), DPPH antioxidant activity, TLC, and HPTLC fingerprinting of aqueous (AEMC) and ethanolic (EEMC) leaf extracts of *M. champaca*, and to evaluate wound healing efficacy of their formulated herbal ointments in a validated in vivo model.

Methods: Leaf powder was extracted via Soxhlet apparatus (distilled water, 95% ethanol). Physicochemical standardization followed WHO guidelines. TPC was quantified by Folin–Ciocalteu assay (mg GAE/g), TFC by aluminium chloride colorimetry (mg QE/g), and antioxidant capacity by DPPH radical scavenging. HPTLC fingerprinting employed a Toluene:EthylAcetate:FormicAcid:Methanol (3:6:1.6:0.4) system. Herbal ointments (2% w/w, fusion method) were evaluated in a 10 mm biopsy punch excision wound model in male Albino Wistar rats (n=5/group) over 14 days, benchmarked against Povidone-Iodine (10% w/w). Data were analysed by two-way ANOVA with Tukey's post-hoc test (p<0.01).

Results: EEMC exhibited superior TPC (494.85±18.40 mg GAE/g) and DPPH inhibition (72.36±6.41%) versus AEMC (86.39±3.43 mg GAE/g; 51.14±8.22%). HPTLC confirmed rutin (0.175–0.225%) and caffeic acid (0.086–0.093%) in both extracts. EEOMC achieved 100% wound closure by Day 12, comparable to the standard, while AEOMC completed closure by Day 14 (p<0.0001 vs. control).

Conclusion: *M.champaca* leaf extracts demonstrate potent wound healing activity attributable to their phenolic–flavonoid constituents. The ethanolic formulation represents a promising botanical wound care candidate warranting further mechanistic and histopathological validation.

KEYWORDS:

Michelia champaca, wound healing, excision wound model, Total phenolic content, Total flavonoid content, DPPH radical scavenging,

1. INTRODUCTION

Mainstream clinical care of wounds is focused on synthetic antiseptics, antibiotics, and high-tech wound dressings, and these products are of major importance in resource limited environments where infectious complications are a major driver of morbidity and mortality. *Michelia champaca* Linn. is native to Southeast Asian countries and is well known for its use in folk medicine for wound healing. *Michelia champaca* Linn. is a native plant of Southeast Asian countries, which possess many folk uses in wound healing due to its well documented antioxidant, anti-inflammatory, antimicrobial and collagen stimulating properties all of which are relevant to the wound healing cascade. The medium-to-large sized evergreen tree (synonym: *Magnolia champaca*) of Magnoliaceae is a tree with special importance in the traditional medicine systems of South and Southeast Asia. The tree is distributed widely through the Sub-Himalayan tracts, West Bengal, Assam, South India, Sri Lanka, China, and Myanmar, growing at elevations up to 2,100 m above sea level[1],[2]. Known by diverse vernacular designations as Swarna Champa (Sanskrit), Champak (Hindi), Sambhagam (Tamil), and Joy Champa (Bengali) the plant has been a cornerstone of Ayurvedic, Siddha, and folk medicine traditions for centuries[3]. Ethnobotanical records document the use of various parts of *M. champaca* in managing a broad spectrum of ailments. The genus *Michelia* is also mentioned in formulations from classical Ayurvedic texts for inflammatory dermatoses and scorpion envenomation as both bark and root are used as febrifuges, anti-inflammatory agents, and the flowers and seeds as nervine stimulants and diuretics, respectively. Phytochemical studies of *M. champaca* have revealed a plethora of structurally diverse bioactive secondary metabolites with potential applications in medicine. The leaves contain sesquiterpenes (β -caryophyllene, α -humulene), sesquiterpene lactones (parthenolide, guaianolides), steroidal alkaloid liridenine and phytosterol β -sitosterol. The stem and bark yield magnograndiolide, N-docosanic acid, michampanolide, and assorted resinous constituents[4],[2]. Notably, flavonoids such as rutin, and Phenolics such as Caffeic acid, detected in the present study's TLC and HPTLC analyses have been individually demonstrated to accelerate wound closure through stimulation of fibroblast proliferation, collagen deposition, and inhibition of pro-inflammatory mediators including TNF- α and IL-6[5],[6]. Among the phenolic acids, caffeic acid and among Flavonoids Rutin has been quantified by HPTLC in the present work has been demonstrated to attenuate oxidative stress in wound microenvironments by scavenging reactive oxygen species (ROS), suppressing lipid peroxidation, and modulating the Nrf2/HO-1 antioxidant signalling axis.[7] Similarly, rutin, the predominant flavonoid glycoside identified in both extracts, exerts pronounced anti-inflammatory activity via cyclooxygenase (COX) and lipoxygenase (LOX) inhibition, and promotes angiogenesis - a critical determinant of wound bed vascularization and granulation tissue formation[8]. Despite this substantial ethnopharmacological and phytochemical backdrop, systematic evaluation of *M. champaca* leaf extracts under validated in vivo wound models remains fragmentary. The previous excision wound study by Shanbhag et al[9] used floral extracts, thereby neglecting to comprehensively explore the therapeutic potential of the leaf, the plant organ that is most accessible and is harvested more than any other organ. Moreover, no study has been reported so far which has correlated the quantitative HPTLC fingerprinting and in vivo wound contraction kinetics for this species. Another important

translational gap between lab and clinic is the lack of standardized formulation of ointments. To address these lacunae the present study involved rigorous physicochemical standardization of *M. champaca* leaf powder according to WHO Pharmacopoeial guidelines, comparative phytochemical profiling of aqueous and ethanolic extracts of *M. champaca* based on TPC, TFC, DPPH, TLC, and HPTLC, formulation and physicochemical characterization of herbal ointments at 2% w/w and evaluation of the wound-healing activity in the IAEC approved excision wound model in Wistar rats using Povidone-Iodine as standard. The integrated design provides, for the first time, a phytochemically-informed, mechanistic basis for the traditional application of the tree to wound healing, an application that is ecologically important.

1.1. Chemical Constituents

Plant of *Michelia champaca* possess various chemical constituents such as flower contains α -Ionon, Methyl benzoate, Dihydro- β -Ionone, β -Ionone, Linalool, Benzyl acetate, Methyl anthranilate in and leaves contain β -Caryophyllene, α -Humulene, Liridenine, Parthenolide, Sesquiterpene lactones, β -Sitosterol, Guaianolides and the stem contain Magnograndiolide, N-Docosanic acid, Michampanolide and the bark contains volatile oil, fixed oil, resin, mucilage, sugars; branches contains Anonaine, Asimndoline, Romerine, Anolobinelinan-Syringaresinol, Vanillic acid and many more[3], [10], [11].

2. METHODOLOGY

2.1. Plant material collection and Authentication

The Fresh leaves of *Michelia champaca* were collected in the month of december from Lucknow region, India.

The Plant of *Michelia champaca* was authenticated by Dr. J C Arya , Incharge of Department Botany, Central Ayurvedic Research Institue (CARI) with the Accession no. 28813, Collected leaves were cleaned with water, shade dried and coarsely powdered and used for the study.

2.2. % Loss On Drying

% Loss On Drying (% Moisture content) determined gravimetrically, 6gm of air dried plant material was placed in earlier tared and dried 6 crucibles, 1gm for each crucible. The crucibles were all placed in the hot air oven at 120 °C and weigh at an interval of 15min. until two continue weighings did not vary by more than 5mg[12],[13].

% loss on drying has been calculated with reference to initial weight –

$$\% \text{ Loss on drying (LOD)} = \frac{Fw - Pw}{W} * 100$$

F_w = Final constant weight of drug along with Crucible

P_w=Pre-weight of Crucible

W=Total weight of drug taken

2.3.Ash Value

The calculation of Ash value has been achieved by the gravimetrically, 6gm of air dried plant was kept in a earlier tared and dried 6 crucibles, 1gm for each crucible. The crucibles were placed in heating furnance and temprature set to 500-600°C for 1hr until it turned white, mentioning the unavailability of carbon. The ash was let cooled and weighed. The total ash value has been elaborated in mg/g of plant material[12],[13].

Total Ash value has been calculated using the formula-

$$\% \text{ Total Ash} = \frac{Fw - Pw}{W} * 100$$

F_w = Final weight of crucible with total ash

P_w = Pre-weight of crucible

W = Total weight of powdered plant material

2.4. Acid Insoluble Ash

Acid insoluble ash has been calculated gravimetrically, To the crucible holding ash 2.5ml of hydrochloric acid added to each crucible and filtered with ashless filter paper, the insoluble substance that was collected on an ashless filter paper shifted to the initial crucible, and ignite it in the heating furnace to a constant weight. The residue was let cooled and weighed. The residue of acid insoluble ash was elaborated in mg/g of air dried material[12],[13].

Acid Insoluble ash has been calculated using the formula-

$$\% \text{ Acid Insoluble Ash} = \frac{Fw - Pw}{W} * 100$$

F_w = Final weight of crucible with total ash

P_w = Pre-weight of crucible

W = Total weight of powdered plant material

2.5. Extraction

The dried Plant leaves (1kg) was grinded coarse powder and pass through sieve no 10. The powder was packed in impenetrable container until further use. Extraction has been performed employing hot continuous extraction process with the help of Soxhlet apparatus. Distilled water and 95% ethanol used as solvent for the extraction[14],[15].

$$\% \text{ Yield} = \left(\frac{\text{Weight of dried extract (g)}}{\text{Weight of Dry Plant Material (g)}} \right) * 100$$

2.6. Preliminary Phytochemical screening

Preliminary phytochemical screening of both aqueous and ethanolic extracts were performed to check the presence of various active chemical constituents such as Flavonoids, Tannins, Triterpenoids, Glycoside, Cardiac Glycoside, Steroids, Alkaloids, Carbohydrate, Saponins, Phenols, Starch, Protein[16],[17].

2.7. Total Phenolic content

Total Phenolic content has been calculated by Folin-Ceocalteu (F-C) using gallic acid as the marker. Absorbance has been recorded at 750nm. Each extract sample was then added with 0.1ml Folin-ceocalteu reagent followed by addition of 0.1ml Na₂CO₃. The mixture were kept in a dark room for 30min. at room temperature. The absorbance were measured on the Spectrophotometer (Simadzu, PharmaSpec, UV-1700). The calibration curve has been constructed using the absorbance of various concentrations of gallic acid. Total Phenolic content of extract was calculated with the help of line equation that has been obtained from the calibration curve and expressed as mg gallic acid equivalent (GAE) per gm of sample in dry weight (mg/g)[18],[19].

The graph has been plotted using the absorbance and the concentration used for various solutions. From the equation in the graph of Gallic acid standardization curve.

$$y = mx + c$$

2.8.Total Flavonoid Content

Total Flavonoid content of the extract were calculated by the Aluminium Chloride colorimetric method. Quercetin was used as marker to obtain the standard calibration curve. 0.5ml Aluminium Chloride (10%) and 0.5ml of 1M Potassium Acetate has been added to the extracts and marker compound. The solutions were kept in a no light room for 30min. at room temprature absorbance was taken at 450nm using a UV-Visible Spectrophotometer(Simadzu, PharmaSpec,UV-1700) [18],[20].

The graph has been plotted using the absorbance and the concentration used for various solutions. From the equation in the graph of gallic acid standardization curve.

$$y = mx + c$$

2.9.Anti-Oxidant Activity

The antioxidant activity of each extract was calculated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. 1ml DPPH was mixed to each of the different extract and ascorbic acid concentration. Methanol acts as a blank sample solution, and Ascorbic acid is used as a reference to determine the value, and the antioxidant activity is stated in terms of IC₅₀ and % inhibition. The solution is allowed to stand for about half an hour in fully dark room at room temprature and absorbance has been taken at 517nm on UV Visible Spectrophotometer (Simadzu, PharmaSpec,UV-1700) [18],[21].

2.10.Herbal Ointment

The herbal ointment has been formulated by Fusion method in which oleaginous base and aqueous base has been melted separately and triturated in unidirectional movement in order of decreasing order of melting point. Two of the extract has been dissolved in cetosteryl alcohol and all the other ingredients were triturated and cooled to form a ointment[22].

Table 1. Ingredients of Ointment Formulation

INGREDIENT	MF	RF
Hard paraffin	50g	5g
Wool fat	50g	5g
White soft paraffin	850g	85g
Cetostearyl alcohol	50g	5g
Extract (aq. & eth.)	20g	2g
Total	1000g	100g
MF- Master Formula,RF- Reduced Formula		

2.11. Evaluation of Ointment

2.11.1. PH

2.5gm ointment was taken in dry beaker and 50ml of water was added. Beaker containing ointment was heated on water bath at 60-70°C. The pH of herbal ointment was measured employing digital pH meter. The determination has been carried out in triplicate and average of three readings were noted and expressed in Mean±SEM.

2.11.2. Spreadability

Spreadability of the formulation was determined by using two TLC plate. 2gm of ointment is placed in between the plate at the centre of the TLC Plate sandwich it, apply a weight of 250gm and the length of the ointment spreading has been noted in triplicate manner and the spreadability is expressed in terms of Mean $\pm$ SEM.

2.12. Thin Layer chromatography

The Thin Layer Chromatography of both the extract of *Michelia champaca* were subjected to TLC with various solvent systems to confirm the presence of the various active constituent through separation on the TLC Plate made with Silica gel G[23],[24].

2.13. High Performance Thin Layer Chromatography

Both aqueous and ethanolic extract of *Michelia champaca* were subjected to HPTLC with solvent systems (Toluene: Ethyl Acetate: Formic acid: Methanol; 3:6:1:0.4) to confirm the presence of the various active constituent through separation[25].

2.14. Procurment of Animals

Male Albino wistar rat weighing 250-280gm were used in the study. Animals procured from laboratory Animal house facility of Central Drug Research Institute (CDRI) lab of CSIR, Lucknow with IAEC approval No- IAEC/UIP/Aug.2023/031.

2.15. Preparation of Animals

The Institutional Animal Ethical Committee's guidelines were rigorously adhered to in all animal experiments. The animals were housed in polyacrylic cages with a conventional temperature range of 24-27°C, a humidity range of 60-65%, and a light-to-dark cycle of 12:12. They spent seven days acclimatization period. Food came in the shape of dry pellet and water [26],[27].

2.16. Study design

Excision wound model was used to determine the wound healing potency of the extract of *Michelia champaca* which was formulated in ointment base. The no. of animal is 5 for each group.

Table 2. Study design for Excision wound model

Group	Treatment	Description
Group A	Control	No treatment
Group B	Standard	Received Standard Povidone Iodine (10% w/w)
Group C	AEOMC	Received Aqueous Extract ointment of <i>Michelia champaca</i> (2% w/w)
Group D	EEOMC	Received Ethanolic Extract Ointment of <i>Michelia champaca</i> (2% w/w)

2.17. Excision Wound Model

Rats were anesthetized using ketamine (50 mg/kg) and Diazepam (2mg/kg) I.P an area of about $\approx 31.4 \text{ mm}^2$ was then cut carefully with the help of biopsy punch of 1cm diameter. Wounds were traced on 1mm graph paper on the day of wounding and subsequently at a gap period of 1 day till 14th day, until healing was complete. After wound is induced successfully various herbal ointments were applied. To check the effect of test drug determination of period of epithelialization and wound contraction rate has been assessed[9],[28].

$$\% \text{ contraction} = \frac{(\text{Area at 0th day} - \text{Area at nth day})}{\text{Area at 0th day}} \times 100$$

2.18. Statistical Analysis

All estimates are expressed as mean $\pm$ SEM of three replicates. Statistical analysis was performed with the help of GraphPad Prism 8.0.2 employing two-way Analysis of Variance (ANOVA) with replication followed by Tuckey's test. Mean, Standard Error in Mean (SEM) and Differences were considered significant at $P < 0.0001$ and $P < 0.0025$.

3. RESULTS

Results for all the activities are expressed in Mean $\pm$ SEM.

3.1.% Physicochemical Standardization of Leaf Powder

The Physicochemical Parameters of *M. champaca* leaf powder were determined following WHO Pharmacopoeial guidelines. The results are summarized in table 3 and Graph plotted available in the figure 1.

Table 3. Physicochemical Standardization parameters of *M. champaca* Leaf powder

Parameter	Observed Value (Mean $\pm$ SEM)
% Loss on Drying (Moisture Content)	12.5 $\pm$ 1.668% (w/w)
Total Ash Value	6.66 $\pm$ 0.001% (w/w)
Acid Insoluble Ash	5 $\pm$ 0.008% (w/w)
% Yield (Aqueous Extract)	16%
% Yield (Ethanolic Extract)	19%

Note. SEM= Standard Error of the Mean, WHO= World Health Organization, AEMC= Aqueous Extract of *M. champaca*

3.2. Physical Characteristics of Ethanolic and Aqueous Extract

Table 4. Physical characteristics of aqueous and Ethanolic extract

Parameters	AEMC	EEMC
Colour	Light brown	Dark green
Odour	Earthy	Characteristic
Taste	Tasteless	Slightly bitter
Consistency	Solid	Semi-solid
Sense of touch	Rough	Smooth but slightly sticky
% Yield (w/w)	16	19

3.3. Preliminary-Phytochemical Screening test

Table 5. Preliminary Phytochemical Screening test table

S.No.	Phytoconstituent	Test Applied	EEMC	AEMC
1	Alkaloids	Wagner's Reagent / Hager's Test	+	+
2	Flavonoids	Lead Acetate / Shinoda Test	+/-	+/-
3	Phenolics	Ferric Chloride Test	+	+
4	Tannins	Brayer's Test / 10% NaOH	+/+	+/-
5	Proteins	Biuret / Xanthoproteic Test	-/+	-/+
6	Carbohydrates	Barfoed's Test	-	-
7	Starch	Resorcinol Test	-	+
8	Cardiac Glycosides	Killer-Killani Test	-	-
9	Saponins	Foam Test	-	-
10	Triterpenoids	Salkowski's Test	+	-

Note. + = Present; - = Absent; EEMC = Ethanolic Extract of *M. champaca*; AEMC = Aqueous Extract of *M. champaca*.

3.4. Physicochemical Evaluation of Herbal Ointment

Table 6. Physical Evaluation, pH, and Spreadability of Herbal Ointment Formulations (AEOMC and EEOMC

S.No.	Specification	AEOMC	EEOMC
1	State	Semi-solid	Semi-solid
2	Colour	Creamy Brown	Green
3	Odour	Characteristic	Characteristic
4	Texture	Smooth	Smooth
5	pH	6.2	6.5
6	Spreadability (cm)	16.0	19.16

Note. AEOMC = Aqueous Extract Ointment of *M. champaca*; EEOMC = Ethanolic Extract Ointment of *M. champaca*; pH = potential of Hydrogen.

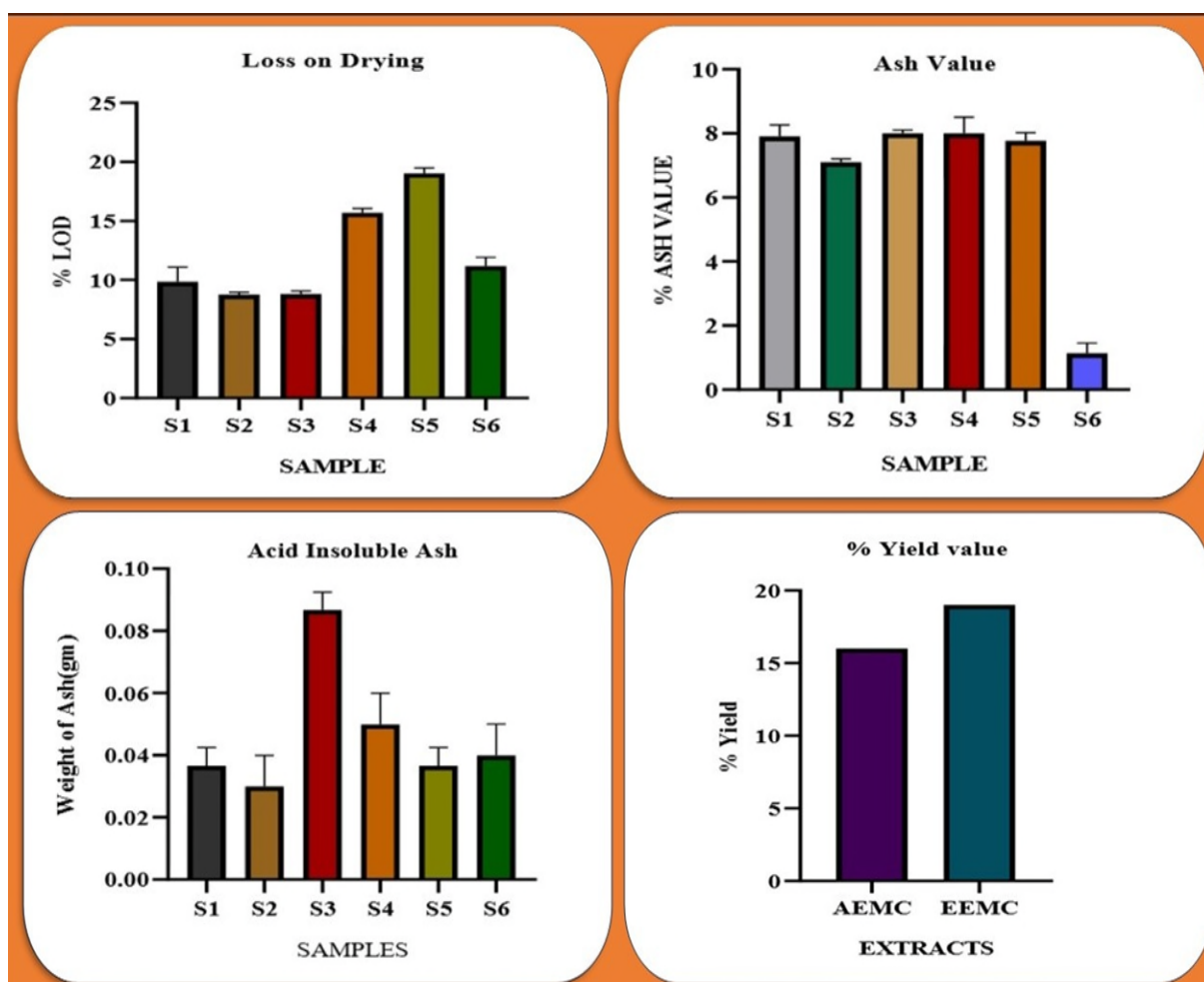


Fig 1. Graphs plotted for the % LOD, Ash value, Acid Insoluble ash, % Yield value, Values expressed as Mean±SEM

3.5. Total Phenolic Content:

The Total Phenolic content and Total Flavonoid content of both aqueous and ethanolic extract has been Tabulated in the Table No.7

Table 7. Total Phenolic and Flavonoid content and DPPH Antioxidant Activity of *M. champaca* leaf extracts

Parameter	AEOMC(Mean±SEM)	EEOMC(Mean±SEM)
TPC(mg GAE/g dry weight)	86.39±3.43	494.85±18.40*
TFC(mg QE/g dry weight)	272.10±10.35*	107.14±4.40
DPPH % Inhibition	51.14±8.22	72.36±6.41*
IC ₅₀ (µg/ml)	35	25

Note: TPC = Total Phenolic Content; TFC = Total Flavonoid Content; GAE = Gallic Acid Equivalents; QE = Quercetin Equivalents; IC₅₀ = Half-Maximal Concentration; *denotes statistically superior values within row

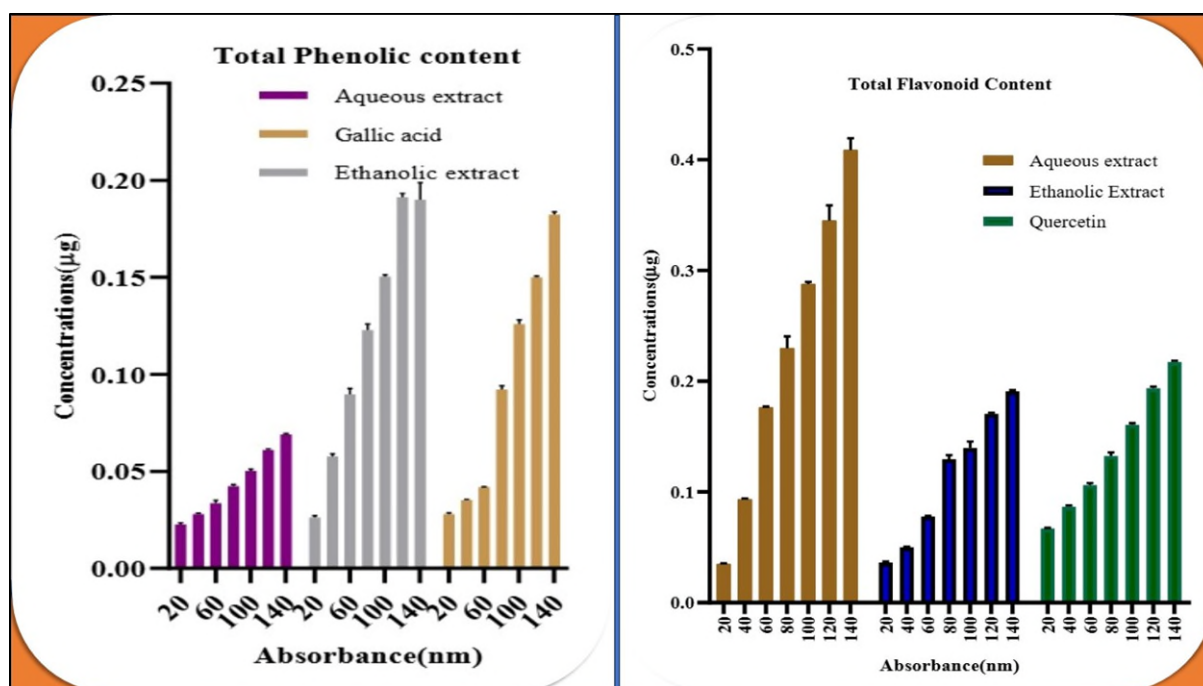


Fig 2. Comparative Total Phenolic Content and Total Flavonoid content of Aqueous (AEMC) and Ethanolic (EEMC) leaf extract of *M. champaca* Values expressed as mean $\pm$ SEM

3.6. Anti-Oxidant Activity:

DPPH Assay

The % inhibition value for Ethanolic extract was found to be 72.36 ± 6.411 and 51.14 ± 8.22 for Aqueous extract. The IC₅₀ Value has been found out to be $0.51 \mu\text{g/ml}$, $2.90 \mu\text{g/ml}$, $3.02 \mu\text{g/ml}$ for Ethanolic extract, Aqueous extract, Ascorbic acid respectively.

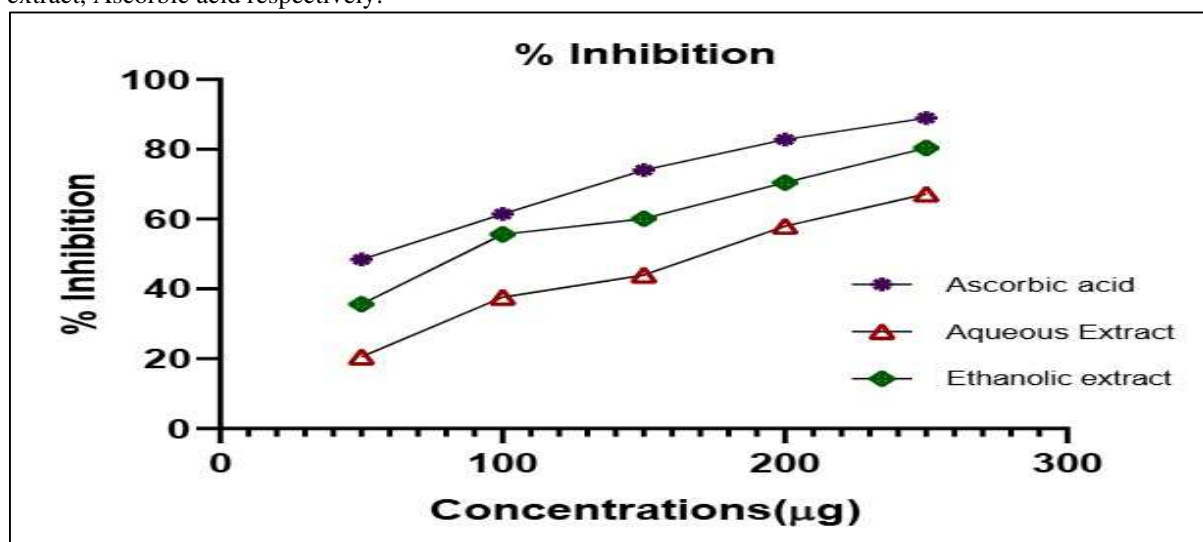


Fig 3. % inhibition by Aqueous extract, Ethanolic extract of *M. champaca* and Standard curve of Ascorbic acid

3.7. Evaluation of Ointment

Table 8. Physical evaluation, PH, spreadability of herbal Ointment

S.no	Specification	AEOMC	EEOMC
1	State	Semi-solid	Semi-solid
2	Colour	Creamy Brown	Green
3	Odour	Characterstics	Characterstics
4	Texture	Smooth	Smooth
5	PH	6.2	6.5
6	Spreadability	16	19.16

Note: AEOMC = Aqueous Extract Ointment of *M. champaca* EEOMC = Ethnolic Extract Ointment of *M. champaca* pH = Potential of Hydrogen

3.8. Thin Layer Chromatography (TLC)

Table9. TLC of EEMC with various Solvent System

S.no.	Solvent System (Ratio)	Separation Quality	Rf Value	Compound Detected
1	Chloroform : Methane : Acetonitrile (8:1:1)	Very good	0.51, 0.21	Quercetin, Rutin
2	Chloroform : Ethanol (9:1)	Very good	0.84	Flavonoid (Apeogenin)
3	Chloroform : Methanol : Water (7:2:1)	Poor	0.91	Terpene (Limonene)
4	Chloroform : Ethanol : Acetonitrile (8.5:1:0.5)	Very good	0.92, 0.56	β -Sitosterol, Kaempferol
5	Chloroform : Methanol : Water (8:1:1)	Good	0.88	Flavonoid (Luteolin)

Note: R_f = Retardation Factor. Plates prepared with Silica Gel G

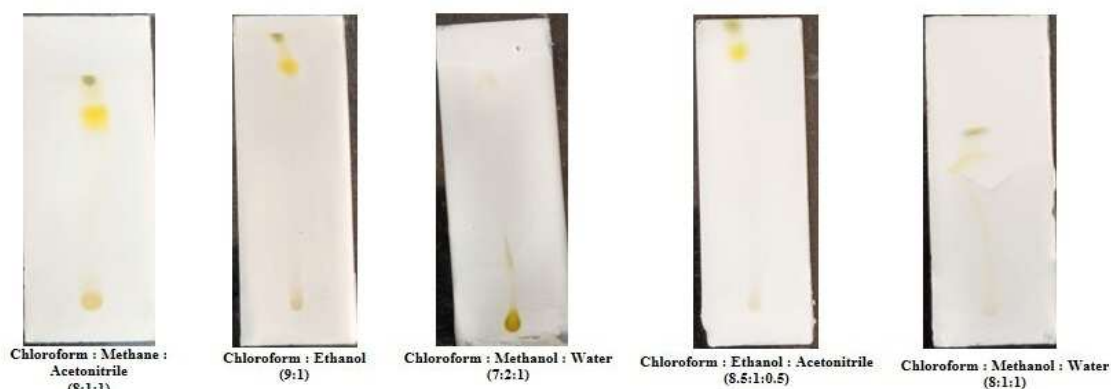


Fig 4. Images of TLC Plate showing separation

3.9. High Performance Thin Layer Chromatography(HPTLC):

HPTLC Fingerprint Profile of EEMC & AEMC

Application- Linomat-5 Applicator (CAMAG)

Sample Volume Applied- 10, 20 μ l

Solvent System- Toluene : Ethyl Acetate : Formic Acid : Methanol (3:6:1.6:0.4)

Scan Wavelength- 254nm, 366nm

TLC Plate Development- Pre-saturated Twin Trough Chamber

Reference Standards : Gallic Acid, Quercetin, Caffeic Acid & Rutin [24]

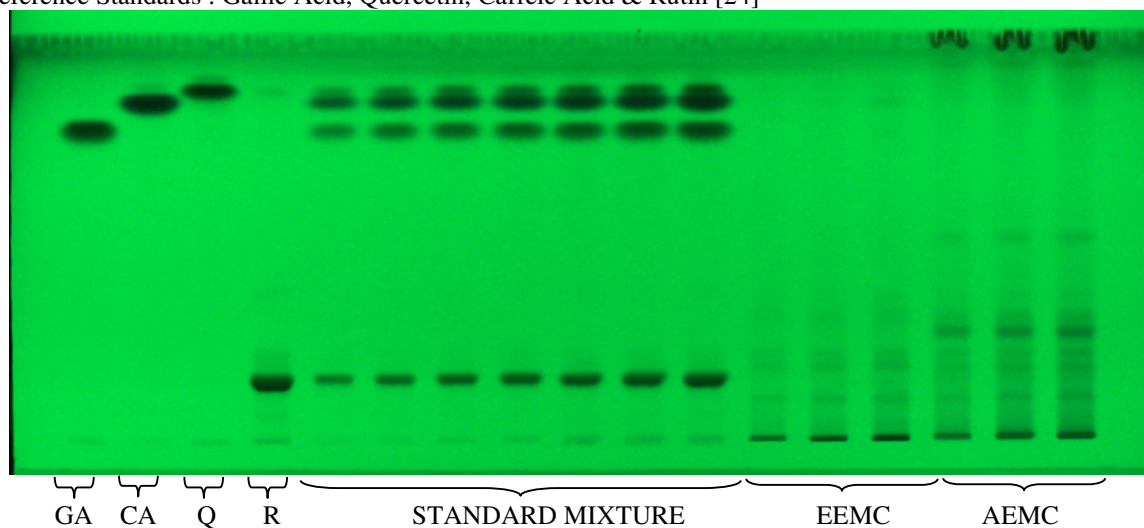


Fig 5. Image of HPTLC Plate at 254nm

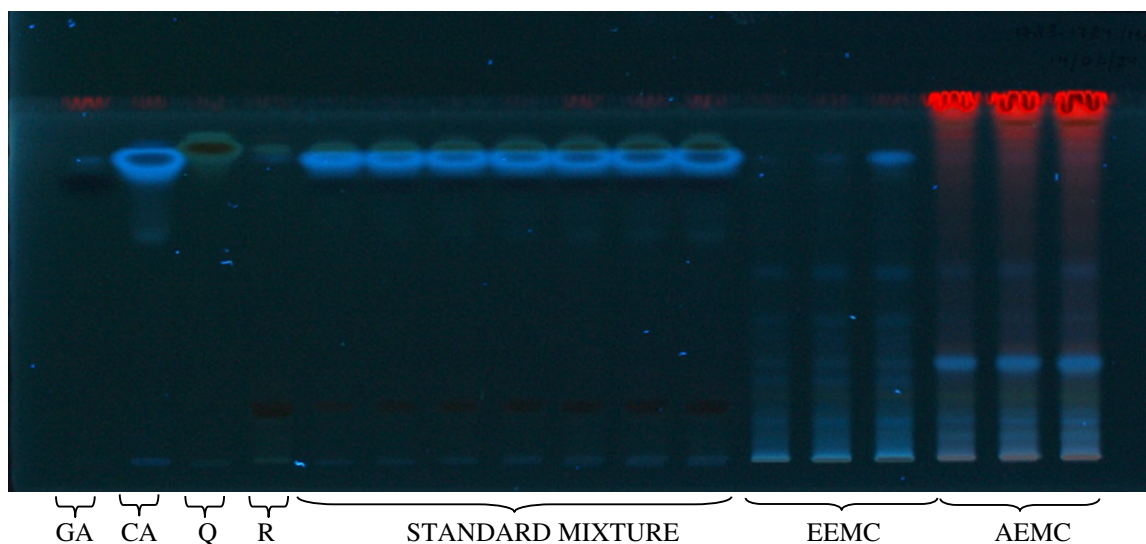


Fig 6. Image of HPTLC Plate at 366 nm

GA- Gallic Acid
 CA- Caffeic Acid
 Q- Quercetin
 R- Rutin
 Standard Curve:

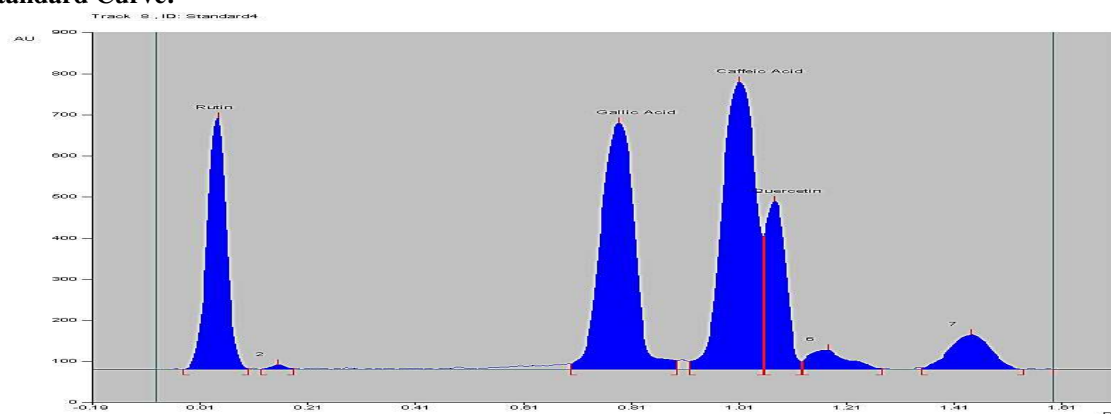


Fig 7. Graph of Standard Curve

Table10. Data of Reference Standard compounds

Peak	Start Position	Start Height	Max position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02Rf	0.0AU	0.04Rf	611.9AU	24.84%	0.10Rf	2.5AU	10377.1AU	16.93%	Rutin
2	0.12Rf	0.3AU	0.15Rf	11.6AU	0.47%	0.18Rf	2.3AU	148.2AU	0.24%	Unknown
3	0.70Rf	13.4AU	0.79Rf	599.6AU	24.35%	0.90Rf	21.8AU	17906.5AU	29.21%	Gallic acid
4	0.92Rf	19.7AU	1.01Rf	698.8AU	28.37%	1.05Rf	22.6AU	20736.6AU	33.82%	Caffeic acid
5	1.06Rf	326.4AU	1.08Rf	408.8AU	16.60%	1.13Rf	19.7AU	7365.2AU	12.01%	Quercetin
6	1.13Rf	20.6AU	1.18Rf	47.3AU	1.92%	1.28Rf	2.3AU	1635.5AU	2.67%	Unknown
7	1.35Rf	3.7AU	1.44Rf	85.0AU	3.45%	1.54Rf	0.1AU	3137.2AU	5.12%	Unknown

EEMC peak at 10 μ l

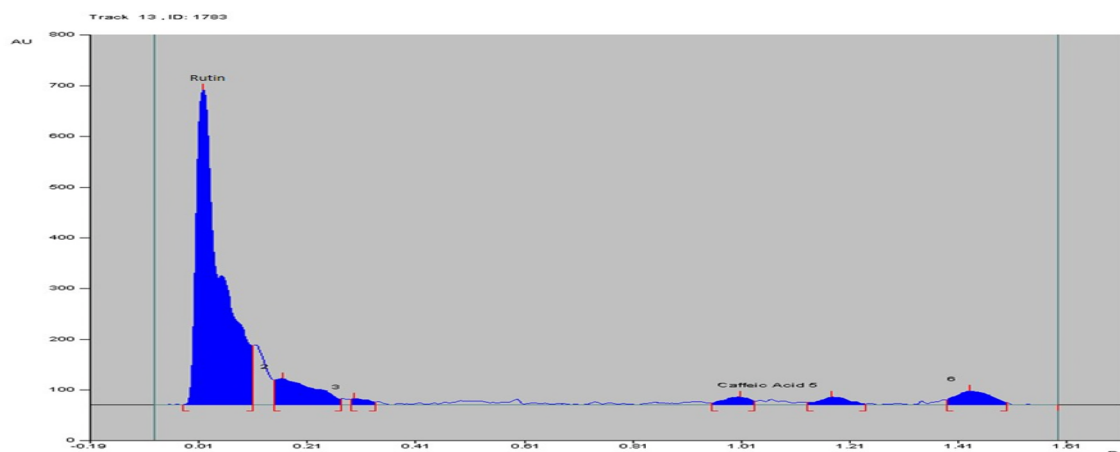


Fig 8. HPTLC Curve of EEMC at 10µl

Table 11. Data of EEMC at 10µl

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned Substance
1	-0.02Rf	0.1AU	0.02Rf	622.1AU	83.56%	0.11Rf	16.9AU	13452.2AU	78.46%	Rutin
2	0.15Rf	49.1AU	0.16Rf	51.9AU	6.97%	0.27Rf	11.3AU	1845.5AU	10.76%	Unknown
3	0.29Rf	11.4AU	0.30Rf	11.8AU	1.58%	0.34Rf	5.4AU	193.1AU	1.13%	Unknown
4	0.95Rf	3.1AU	1.01Rf	16.1AU	2.16%	1.04Rf	7.1AU	3764.3AU	2.20%	Caffeic acid
5	1.13Rf	4.3AU	1.18Rf	15.3AU	2.05%	1.24Rf	1.7AU	403.1AU	2.35%	Unknown
6	1.39Rf	10.0AU	1.43Rf	27.4AU	3.68%	1.50Rf	4.3AU	876.2AU	5.11%	Unknown

EEMC peak at 20µl

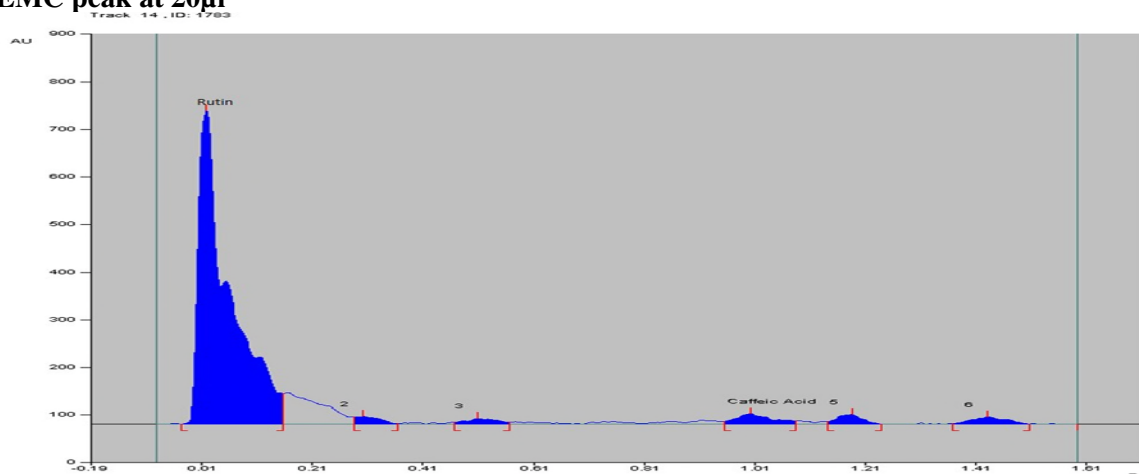


Fig 9. HPTLC curve of EEMC at 20µl

Table 12. Data of EEMC at 20µl

Peak	Start Position	Start Height	Max position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned Substance
1	-0.03Rf	0.0AU	0.02Rf	658.6AU	88.67%	0.16Rf	64.8AU	17397.1AU	88.47%	Rutin
2	0.29Rf	15.4AU	0.30Rf	15.9AU	2.14%	0.36Rf	0.9AU	353.2AU	1.80%	Unknown
3	0.47Rf	3.5AU	0.51Rf	11.6AU	1.57%	0.57Rf	3.7AU	325.4AU	1.65%	Unknown
4	0.95Rf	5.8AU	1.00Rf	21.6AU	2.91%	1.09Rf	6.5AU	680.8AU	3.46%	Caffeic acid
5	1.14Rf	5.4AU	1.19Rf	20.1AU	2.71%	1.24Rf	0.1AU	450.3AU	2.29%	Unknown
6	1.37Rf	1.6AU	1.43Rf	14.8AU	2.00%	1.51Rf	0.5AU	456.7AU	2.32%	Unknown

AEMC peak at 10µl

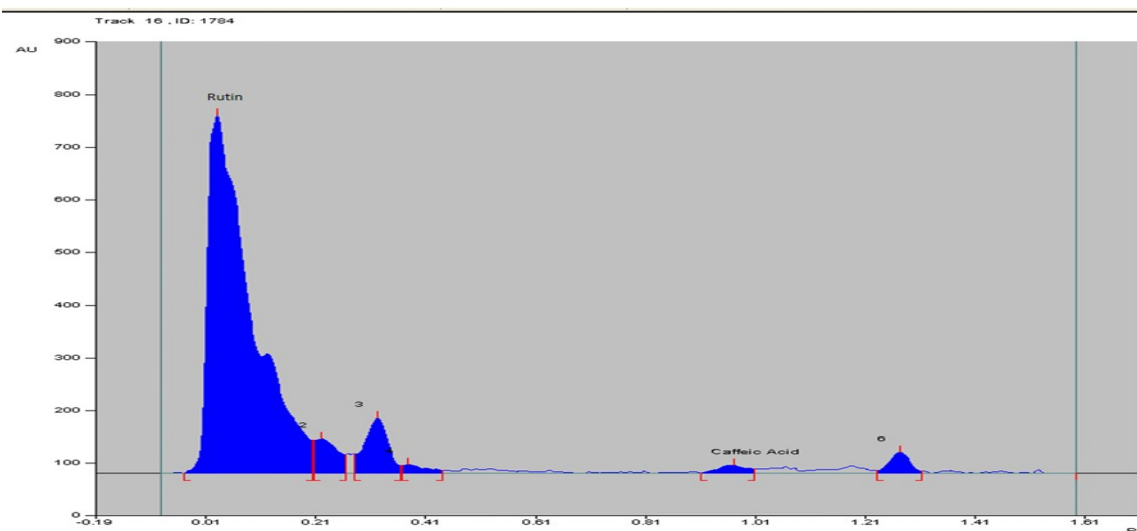


Fig 10. HPTLC curve of AEMC at 10µl

Table 13. Data of AEMC at 10µl

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned Substance
1	-0.03Rf	0.1AU	0.03Rf	679.8AU	73.74%	0.20Rf	32.3AU	26063.1AU	84.4%	Rutin
2	0.21Rf	62.4AU	0.22Rf	65.4AU	7.10%	0.26Rf	36.0AU	1343.5AU	4.33%	Unknown
3	0.28Rf	36.7AU	0.32Rf	105.4AU	11.43%	0.36Rf	14.4AU	2147.0AU	6.92%	Unknown
4	0.37Rf	14.4AU	0.38Rf	16.2AU	1.76%	0.44Rf	5.3 AU	346.0AU	1.12%	Unknown
5	0.91Rf	0.4AU	0.97Rf	15.2AU	1.65%	1.01Rf	6.6 AU	397.3AU	1.26%	Caffeic Acid
6	1.23Rf	4.9AU	1.27Rf	39.9AU	4.33%	1.31Rf	4.5 AU	714.8AU	2.30%	Unknown

AEMC peak at 20µl

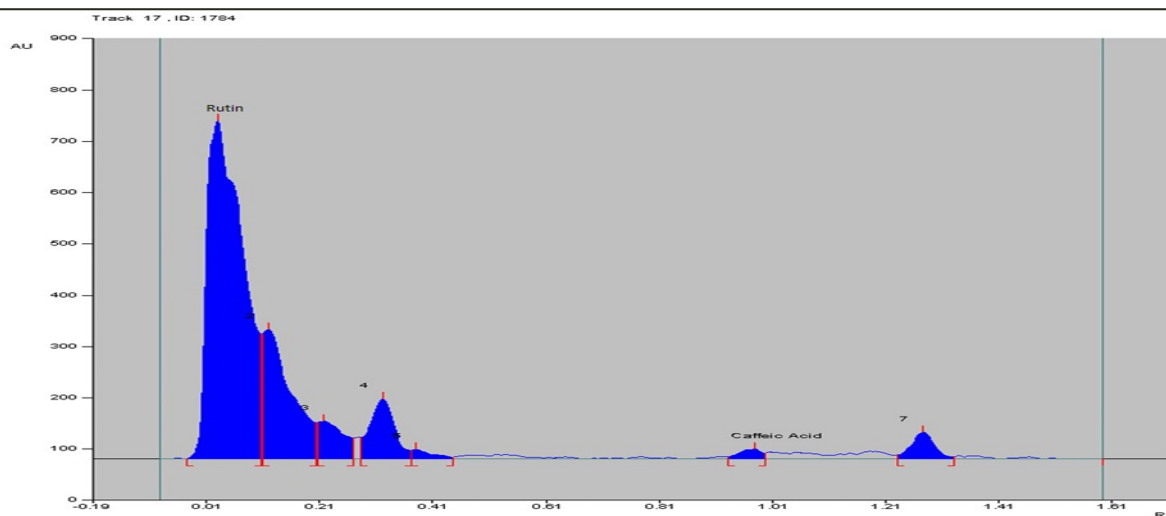


Fig 11. HPTLC curve of AEMC at 20µl

Table 14. Data of AEMC at 20µl

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned Substance
1	-0.02Rf	0.3AU	0.03Rf	659.5AU	55.33%	0.11Rf	43.5AU	20423.3AU	62.95%	Rutin
2	0.11Rf	243.7AU	0.12Rf	252.4AU	21.17%	0.20Rf	71.0AU	6255.9AU	19.28%	Unknown
3	0.21Rf	71.1AU	0.22Rf	73.3AU	6.15%	0.27Rf	40.8AU	1603.9AU	4.94%	Unknown
4	0.28Rf	42.0AU	0.32Rf	117.2AU	9.84%	0.37Rf	16.3AU	2441.0AU	7.52%	Unknown
5	0.37Rf	165.5AU	0.38Rf	18.5AU	1.55%	0.45Rf	3.0AU	317.6AU	0.98%	Unknown
6	0.93Rf	7.6AU	0.98Rf	19.0AU	1.59%	1.00Rf	11.5AU	373.5AU	1.15%	Caffeic acid
7	1.23Rf	7.6AU	1.28Rf	52.1AU	4.37%	1.33Rf	4.1AU	1025.9AU	3.16%	Unknown

Table 15. Quantitative HPTLC concentration of Rutinand caffeic acid in EEMC and AEMC

Sample	Rutin Run-1 (%)	Rutin Run-2 (%)	Average Rutin(%)
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EEMC	0.15	0.20	0.175
AEMC	0.18	0.27	0.225
Sample	Caffeic acid Run-1 (%)	Caffeic acid Run-2 (%)	Average Caffeic acid(%)
EEMC	0.091	0.082	0.0864
AEMC	0.096	0.090	0.0929

Note. EEMC = Ethanolic Extract of *M. champaca*; AEMC = Aqueous Extract of *M. champaca* % = w/w in dry extract

3.10. PHARMACOLOGICAL ACTIVITY:

Excision Wound Model

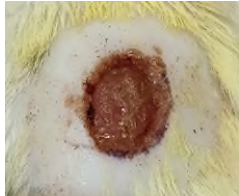
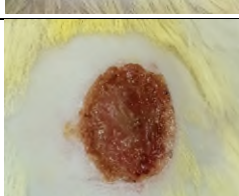
After inducing wound the area of the wound has been measured at a gap of 1 day. Dropping of the Ischar recognised as the endpoint of successful re-epithelialization while the day necessary for this was taken as a period of re-epithelialization. The time of re-epithelialization has been calculated by monitoring the falling of eschar. The falling of eschar shows the complete re-epithelialization of tissue.

Table 16. % wound contraction from day 0 to day 14

Grouping	0 th day	2 nd day	4 th day	6 th day	8 th day	10 th day	12 th day	14 th day
Control	0	2±1.22	9±2.91	25±2.23	49±1.87	59±1.87	70±1.58	82±1.22
Povidone-Iodine 10%	0	14±2.91	35±2.23	55±2.23	66±1.87	86±1.87	100±0	100±0
AEMC 2% (Aqueous)	0	5±0	23±2	39±2.44	56±2.91	76±1.87	95±1.58	100±0
EEMC 2% (Ethanolic)	0	8±1.22	28±2	50±1.58	62±1.22	84±1.87	100±0	100±0

Note. Values expressed as % wound contraction (Mean ± SEM). *p < 0.0001 vs. Control; p < 0.0025 EEMC vs. Povidone-Iodine; AEOMC = Aqueous Extract Ointment; EEOMC = Ethanolic Extract Ointment.

Table 17. Images showing the healing of wound over the period of 14 days

GROUPING	CONTROL	STANDARD	AEOMC 4%	EEOMC 4%
0 th day				
2 nd day				
4 th day				

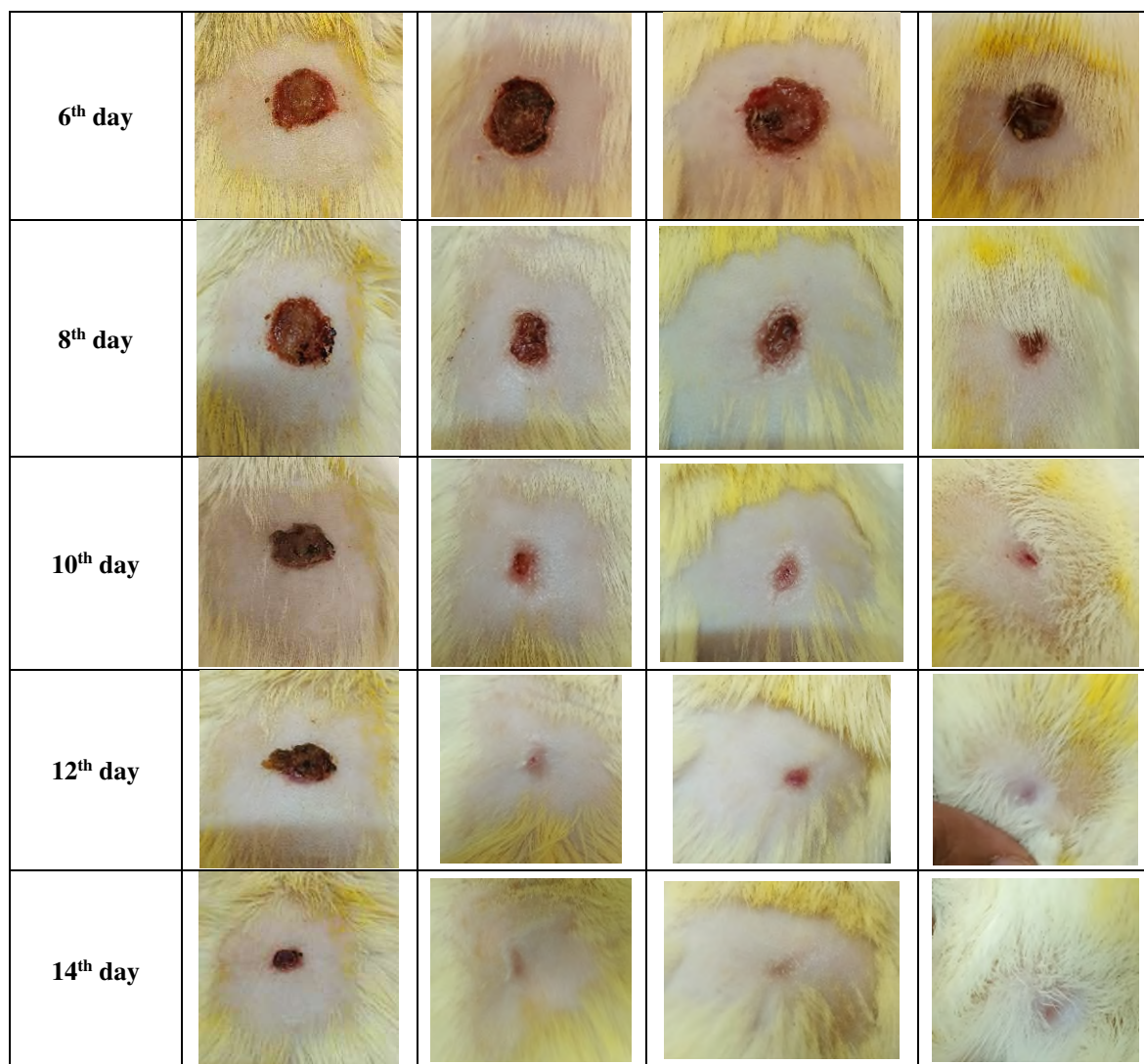


Table 18. Period of Re-epithelialization Across Treatment Group (Mean±SEM,n=5)

Animal group	n	Treatment	Re-epithelialization(Days)
Group 1	5	Blank	19.8±0.3
Group 2	5	Povidone iodine 10%	10.8±0.4*
Group 3	5	2% AEOMC	12.2±0.2*
Group 4	5	2% EEOMC	11±0.31*

Note. * $p < 0.0001$ vs. Blank Control group; two-way ANOVA with Tukey's test. AEOMC = Aqueous Extract Ointment of *M. champaca*; EEOMC = Ethanolic Extract Ointment of *M. champaca*.

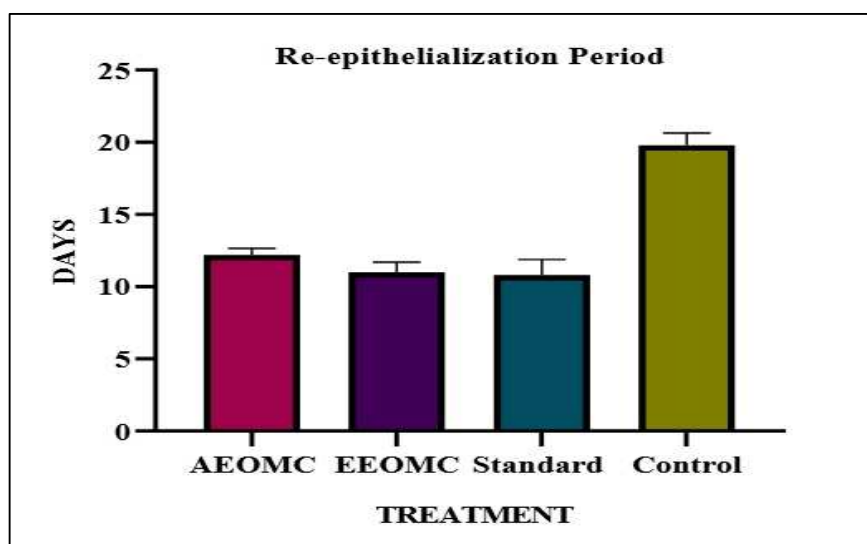


Fig 12. Re-epithelialization period (days) across all treatment groups. Shorter duration indicates faster wound healing. Values expressed as mean $\pm$ SEM (n = 5). *p < 0.0001 vs. Control.

The results depicted that EEOMC ointment was superior among the formulations in comparison to AEOMC ointment and showed complete wound healing in 12 days while the AEOMC represent entire wound healing in 13 days. Results were compared with standard drug (Povidone iodine) which represent complete healing in 11 days. The result depicted that selected plant (*Michelia champaca*) has marked wound healing property, which is nearby to standard drug formulation.

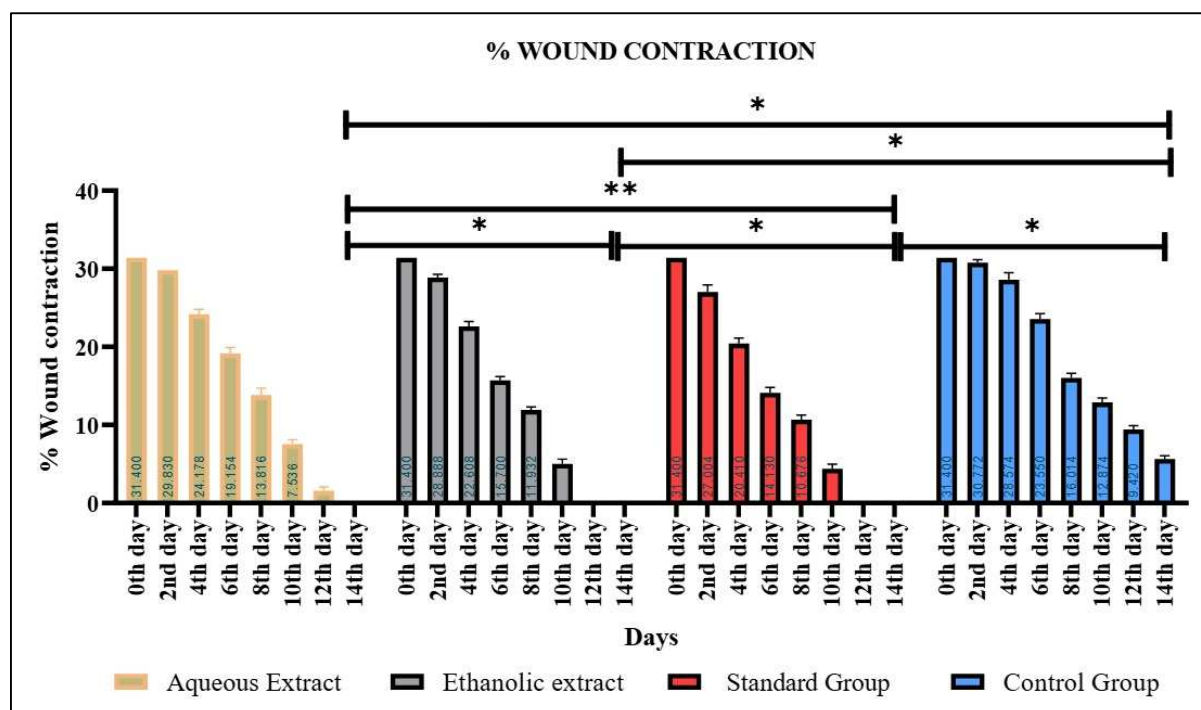


Figure 13. Graph showing % wound contraction from day 0 to day 14, [At the 14th day of the study the (*)AEOMC found out to be statistically significant with P<0.0001 with respect to Control, (*)EEOMC found out to be statistically significant with P<0.0001 with respect to Control, (*) Povidone iodine(STD) found to be statistically significant with the P<0.01 with respect to control, but (*)AEOMC found out to be significant with P<0.0001 with respect to Povidone iodine, (b*)EEOMC found out to be significant with P<0.0025 with respect to Povidone iodine, (b*)AEOMC does not found out to be significant with P<0.0001 with respect to EEOMC.]

DISCUSSION

The present investigation evaluated the phytochemical composition and wound healing potential of aqueous (AEMC) and ethanolic (EEMC) leaf extracts of *Michelia champaca* Linn. by integrating quantitative phytochemical profiling with in vivo pharmacological evaluation. The ethanolic formulation (EEOMC) demonstrated wound healing efficacy comparable to the standard antiseptic Povidone-Iodine (10% w/w), achieving complete wound closure by Day 12 and a re-epithelialization period of 11.0 ± 0.31 days. The Folin–Ciocalteu assay revealed a markedly higher TPC in EEMC (494.85 ± 18.40 mg GAE/g) compared with AEMC (86.39 ± 3.43 mg GAE/g), indicating that ethanol more effectively solubilises polyphenolic constituents from the leaf matrix. This finding is consistent with polarity considerations, wherein medium-polarity solvents such as ethanol facilitate diffusion through intact cellular membranes and extraction of a broader spectrum of phenolic compounds [15]. In contrast, AEMC exhibited superior TFC (272.10 ± 10.35 mg QE/g vs. 107.14 ± 4.40 mg QE/g), suggesting differential solubility profiles of glycosylated flavonoids in aqueous media, a phenomenon previously documented in *Caesalpinia bonduc* and other Magnoliaceae species [18]. The DPPH radical scavenging assay corroborated the polyphenol data: EEMC demonstrated $72.36 \pm 6.41\%$ inhibition ($IC_{50} = 0.51$ μ g/mL) versus $51.14 \pm 8.22\%$ for AEMC ($IC_{50} = 2.90$ μ g/mL). The lower IC_{50} of EEMC relative to ascorbic acid ($IC_{50} = 3.02$ μ g/mL) is noteworthy and aligns with the well-established antioxidant synergism of mixed polyphenolic matrices [29]. Antioxidant capacity is a pharmacologically relevant determinant of wound healing, as reactive oxygen species (ROS) generated at the wound site impair fibroblast proliferation and delay re-epithelialization [30]. The superior free radical scavenging activity of EEMC thus provides a mechanistic basis for its enhanced in vivo wound healing efficacy. HPTLC fingerprinting using a Toluene:EthylAcetate:FormicAcid:Methanol (3:6:1.6:0.4) mobile phase unambiguously confirmed the presence of rutin and caffeic acid in both extracts, with average concentrations of 0.175% and 0.225% rutin in EEMC and AEMC respectively, and 0.086% and 0.093% caffeic acid. Rutin, a bioflavonoid glycoside, exerts pronounced anti-inflammatory activity via inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes and promotes angiogenesis - a critical determinant of wound bed vascularization and granulation tissue formation [24]. Caffeic acid attenuates oxidative stress in wound microenvironments by scavenging ROS and modulating the Nrf2/HO-1 antioxidant signalling axis [25]. The co-presence of these bioactive markers in both leaf extract formulations provides a phytochemically rationalised mechanistic basis for the observed wound healing activity. The excision wound model is a widely validated paradigm for evaluating topical wound healing agents and provides simultaneous assessment of wound contraction rate and period of re-epithelialization [26], [28]. In the present study, all treatment groups exhibited significantly accelerated wound contraction compared with untreated controls ($p < 0.0001$). On Day 10, EEOMC achieved 84% wound closure versus 59% in the control group. Complete closure (100%) was observed in both the EEOMC and Povidone-Iodine groups by Day 12. The AEOMC group achieved 95% contraction by Day 12 and complete closure by Day 14. The statistically comparable performance of EEOMC versus the standard antiseptic ($p > 0.0001$ at Day 12) represents a clinically significant outcome, supporting its candidacy as a botanical wound care agent. Re-epithelialization, marked by eschar detachment, is a definitive endpoint of wound closure that reflects the restoration of epidermal barrier integrity. Untreated animals required 19.8 ± 0.3 days for complete epithelialization, whereas EEOMC and AEOMC reduced this to 11.0 ± 0.31 and 12.2 ± 0.2 days, respectively ($p < 0.0001$ vs. control). The EEOMC re-epithelialization period was not statistically different from that of the Povidone-Iodine standard (10.8 ± 0.4 days; $p > 0.0001$), demonstrating equivalence of healing velocity. The acceleration of re-epithelialization likely reflects enhanced keratinocyte migration and

fibroblast proliferation, processes known to be promoted by flavonoids and phenolic acids through stimulation of transforming growth factor- β (TGF- β) and fibroblast growth factor (FGF) pathways[31].The multi-mechanistic wound healing activity of *M. champaca* leaf extracts may be attributed to the synergistic interaction of their phytoconstituents. Flavonoids (quercetin, rutin, luteolin, kaempferol, apigenin) and phenolic acids (caffeic acid, gallic acid) confirmed by HPTLC and TLC analyses collectively exerts: (a) antioxidant effects neutralising ROS at the wound site; (b) anti-inflammatory activity via COX/LOX pathway inhibition reducing TNF- α and IL-6 cytokine release; (c) pro-angiogenic effects facilitating granulation tissue vascularization; and (d) collagen-stimulating properties via Nrf2/HO-1 and TGF- β signalling[31], [32]. Tannins detected in EEMC further contribute via astringent-mediated reduction of exudation and formation of a protective protein–tannin complex over the wound bed [33]. Although histopathological validation and collagen quantification were beyond the scope of the present study, the wound contraction data strongly imply enhanced dermal matrix remodelling in extract-treated animals. The present study extends the prior work of Shanbhag et al. (2011), who demonstrated wound healing activity of *M. champaca* floral extracts in Wistar rats. The current investigation focused on leaf extracts the most accessible and pharmacologically under-characterized organ of the plant and, for the first time, correlated quantitative HPTLC fingerprinting with in vivo wound contraction kinetics. The additional identification of rutin and caffeic acid as principal biomarkers advances the translational profile of *M. champaca* leaf preparations and establishes a Pharmacopoeial reference point for quality standardization of future formulations [32]. Notwithstanding the favourable outcomes, several limitations merit acknowledgement. First, histopathological evaluation of wound tissue and quantitative collagen analysis by hydroxyproline assay were not performed, which would have provided direct evidence of connective tissue regeneration. Second, the antimicrobial contribution to wound healing was not individually assessed, and future studies should include an infected wound model. Third, nano-formulation of the ethanolic extract may enhance bioavailability and sustained-release characteristics of the identified biomarkers. A mechanistic investigation incorporating cytokine profiling (TNF- α , IL-1 β , VEGF) and Nrf2/HO-1 pathway analysis is warranted to delineate the precise molecular targets.

CONCLUSION

Our findings from this study shows that Ethanolic extract of *M. champaca* shows marked presence of Total Flavonoid Content, Total Phenolic Content and better antioxidant activity as compared to aqueous extract. TLC of both extract shows presence of many active compounds Compound Detected; Quercetin, Rutin, Apeogenin, Limonnene, β -Sitosterol, Kaempferol, Luteolin. HPTLC confirms the presence of two very important active constituents Rutin and Caffeic acid which may be responsible for wound healing activity. The wound healing activity of ethanolic and aqueous extract found out to be significant as compared to control group. The wound healing effects are likely mediated through combined actions of Phenolics, Flavonoids, Tannins, other bioactive constituents that enhance tissue regeneration, wound contraction, and epidermal restoration. These findings provide scientific support for the traditional use of *M. champaca* and suggest its potential as a promising natural wound healing agent.

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AUTHORS CONTRIBUTION

Nikhil Kumar - Data curation, phytochemical and animal activity, writing
 Dr. Jyoti Nanda Sharma - Conceptualization, supervision, Revision, finalizing manuscript

DISCLOSURE OF INTEREST

The authors report there are no competing interest to declare.

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ETHICAL APPROVAL

This study involves the experiment on Rat (Albino Wistar) so all the research work has been performed according to the IAEC guidelines after the approval (Approval No. - IAEC/UIP/Aug.2023/031).

DATA AVAILABILITY

The authors claims that the data supporting the investigations of this research work are available within the paper. On reasonable request, the appropriate author will provide any raw data files that are required in a different format.

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