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Research Article

Phytochemical and Anti-Inflammatory Evaluation of Herbal Gel Prepared from Bark Extract of *Mesua Ferrea* Linn

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ABSTRACT

The present research work involves formulation of herbal gel containing stem bark extracts of *Mesua ferrea* Linn and its evaluation for *in vitro* anti-inflammatory activity. The gel formulations were prepared using ethanolic extracts along with different polymer. The physicochemical parameters of formulations like; pH, viscosity and spreadability etc. also evaluated. Phytochemical analysis revealed presence of phenols, flavonoids and alkaloids, etc. The ethanolic extracts showed appreciable anti-inflammatory activity compared to the standard drug. Study confirmed that potential anti-inflammatory formulation can be developed from bark extract of *Mesua ferrea* Linn.

Keywords: *Mesua ferrea* Linn., Anti-inflammatory, Herbal Gel, Phytochemical, Albumin denaturation.

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INTRODUCTION

Inflammation is a body response that acts as a protective mechanism and induces as a resultant of injury. The symptoms of inflammation are redness, swelling and pain, while chronic conditions of inflammation may induces pathogenesis of disease like; arthritis. Vasodilatation, increased permeability of capillary and leukocytes migration these are some biological responses that are associated with inflammation [1, 2].

Generally non-steroidal anti-inflammatory drugs (NSAIDs) are used to control inflammation; however these drugs suffer some disadvantages like; gastric irritation and renal dysfunction. Therefore it is required to develop some naturally originated drugs as anti-inflammatory agents which can overcome adverse effects of classical NSAIDs.

Mesua ferrea Linn. is a plant of medicinal importance belongs from family *Clusiaceae* distributed around countries like Burma, India, Indochina and Thailand. *Mesua ferrea* used for various medicinal purposes [3-5] like; treatment of inflammation, ulcers, hemorrhoids, wounds and piles. Bark of plant used as astringent, also used for bronchitis and

gastritis. Plant traditionally recommended for diseases associated with pain and inflammation. Considering above mentioned aspects present study was planned for the phytochemical and pharmacological evaluation of gel prepared from *Mesua ferrea*.

MATERIALS AND METHODS

Ethanol, Folin-ciocalteu reagents and albumin were purchased from Loba Chem. All other chemicals and reagents used were of analytical grade. The stem bark of *Mesua ferrea* Linn. was collected from the local forest area of Karnataka state of India. Stem bark was first washed with running water to remove dirt and other waste materials, after that it was rinsed with distilled water and dried under shade for extraction purpose.

Extraction of the plant material

The bark of *Mesua ferrea* was dried under shade and pulverized in a mechanical grinder into coarse powder. The dried powder first passed through sieve no. 40 to optimize uniformity then 500 gm of dried powder was subjected to extraction using soxhlet apparatus. Extracts were

concentrated to dryness using rotary evaporator and stored in desiccator to avoid any moisture content [6].

Phytochemical studies

The preliminary phytochemical analysis was performed using methods described earlier with slight modification [7, 8]. The phytochemical analysis was performed to confirm presence of phytoconstituents such as flavonoids, alkaloids, phenols, saponins, glycosides and tannins, etc.

Quantitative analysis of extracts

Total phenol content:

Total phenol content in extract was determined using Folin-Ciocalteu method, about 1 ml of 100µg of extracts was mixed with 2 ml of Folin-Ciocalteu reagent (previously diluted up to 1:10, v/v). The mixture was allowed to stand for 90 min. at room temperature after addition of 2 ml of sodium carbonate solution (7.5% w/v). Absorbance was measured after incubation period against the blank at 750 nm using UV-visible spectrophotometer (Shimadzu) [9].

Determination of total flavonoids content

Total flavonoid content of extract was determined as per the method described by Zhishen *et al.*, [10] with slight modification. About 2.5 ml of 100µg of extract was added in a mixture of 300µl of 5% sodium nitrite and 300µl of 10% aluminum chloride. Sodium hydroxide (2 ml of 1 M) was added after the incubation of mixture for 6 min. at room temperature. Absorbance was measured at 510 nm using UV-visible spectrophotometer (Shimadzu).

Preparation of Herbal Gel

The gel formulations of herbal extract was prepared using the compositions depicted in **Table 1**. First purified water containing sodium benzoate soaked in carbapol. HPMC/Sodium Alginate solution was prepared and homogenized separately. The drug solution was transferred to HPMC/Sodium Alginate solution followed by homogenization; that after this solution containing drug & polymer added to the previously prepared carbapol solution and finally neutralized using triethanolamine followed by homogenization to obtain gel formulation of uniform consistency [11, 12].

Table 1: Composition of Herbal Gel Formulations (%w/w):

Ingredients	Gel prepared from ethanolic extracts
HPMC	1
Carbapol	1
Sodium Alginate	1
Drug extract	4
Propylene Glycol	6
Sodium benzoate	0.3
Triethanolamine	As required (up to neutralization)
Distilled Water	q.s.

Evaluation of gel formulations [11, 12]

Physical evaluation

Color, appearance and consistency were observed as physical parameters. The typical gel like appearance was considered as standard formulations.

pH Measurement

pH of the formulations were measured using digital pH meter which was calibrated previously using standard buffer solutions meant for pH calibration.

Spreadability

Spreadability was measured using an apparatus that involve a wooden block along with a pulley at one end. The slip and drag characteristics of gels were utilizes to measure spreadability. There are two slides in apparatus one is ground slide while another one is fixed slide that is attached with a hook. Excess amount of gel formulation was applied between two slides and weight around 1 kg was applied for 5 minutes to obtain a uniform film of gel between the slides. Excess amount of gel was scrapped out from the edges of slides and top slide was pulled off with the help of hook and time in seconds was noted down required by the slide to cover a fix distance. Spreadability was calculated using the following formula:

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

Where, S = Spreadability, M = Weight in the pan, L = Length moved by the glass slide, T = Time (sec.) required for separation of slides.

Homogeneity

The herbal gel formulation was tested for homogeneity by visual inspection; it is done by inspecting gel formulation for their consistent appearance and presence of aggregates.

Viscosity

Viscosity of gel formulation was measured using Brookfield viscometer fitted with spindle. The instrument measures shear stress that applied on a spindle rotating at a constant speed when placed in the sample solution.

Analytical Standardization

Thin Layer Chromatography (TLC)

Silica gel G60F254 was used as stationary phase; the plates were developed in twin trough glass chamber using ascending method. The sample solution was prepared by dissolving dry extract in ethanol. The sample solution was applied as bands using 100 µl syringe maintaining 2 cm distance from the bottom of the plate.

Infra-red spectroscopy (IR)

The IR spectrum of ethanolic extract was scanned on FT-IR spectrophotometer over the range of 4000-400 cm⁻¹.

Inhibition of albumin denaturation

The preliminary *in vitro* anti-inflammatory activity of *Mesua ferrea* extract was performed using "inhibition of albumin denaturation" method [13]. The test solution consisted of extracts with 1% aqueous solution of albumin, 1N HCl was used to adjust the pH of reaction mixture. The solutions were incubated at 37 °C for 20 min. followed by heating at 51 °C. Turbidity was measured at 660 nm after cooling the samples solution. Inhibition of albumin denaturation was calculated as anti-inflammatory response of extracts using the following formula:

$$\text{Percent inhibition} = [(Ac - At)/Ac] \times 100$$

Where "Ac" is absorbance of control (solvent) and "At" is the absorbance of test solution (extract).

RESULTS AND DISCUSSION

The qualitative screening of extract confirms presence of phenols, alkaloids, tannins and glycosides (**Table 2**). The total phenolic and flavonoids contents were found to be 59.7µg/ml and 9.0µg/ml respectively in plant extract.

Table 1: Qualitative and quantitative chemical analysis:

Qualitative test		Quantitative chemical analysis	
Test performed for	Present (+) / Absent(-)	Test performed for	Amount found ($\mu\text{g/ml}$)
Flavonoids	+	Total Phenols	59.7
Alkaloids	+	Total Flavonoids	9.0
Phenols	+		
Saponins	+		
Glycosides	+		
Tannins	+		

The gel formulation was prepared by trial & error method. Initially concentrations of polymer varied many times to developed optimized formula and finally herbal gel was prepared using composition mentioned in **table 1**.

The physical test revealed that gel formulation is light brown in color and having translucent appearance. Gel was found good in homogeneity without any lumps, the spreadability observed within acceptable range which indicated that gel formulation is spreadable even by small amount of shear. The pH of formulation was found to be slightly neutral side which is prerequisite for topical gel like preparation. Viscosities of formulation along with other parameter reported in **Table 3**.

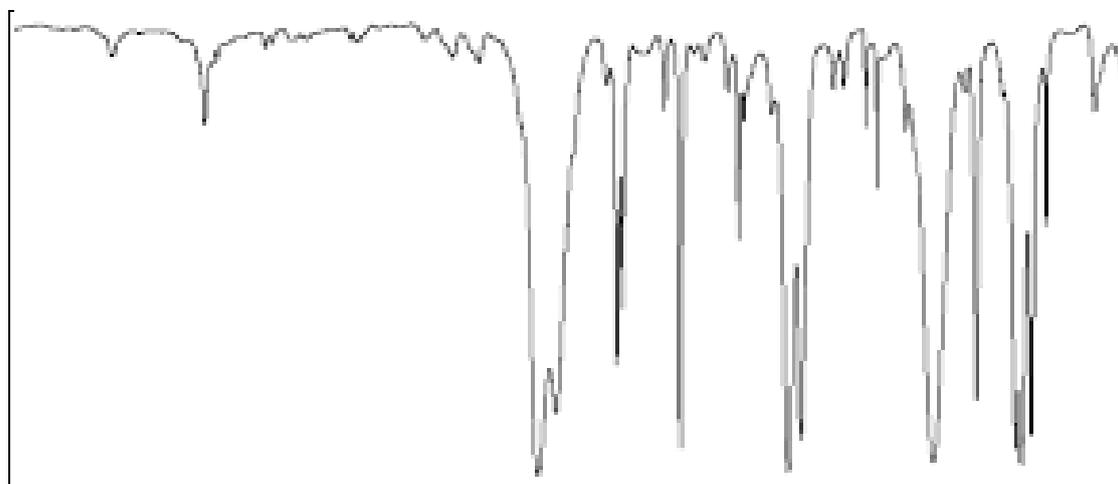
Table 4: Evaluation of herbal gel prepared from ethanolic extracts of *Mesua ferrea*.

S. No.	Parameters	Results
1	Color	Light brown
2	Appearance	Translucent
3	pH	6.7
4	Spreadability (gm. cm./ sec)	29.35
5	Homogeneity	Homogenous
6	Viscosity (cps)	1538

The analytical standardization was performed using chromatographic and spectral techniques. Thin layer chromatography was performed as chromatographic method to separate out important phytoconstituents from herbal extract. The combination of solvents; Ethyl acetate: methanol: formic acid (7: 2.5: 0.5 v/v/v) was used as mobile phase and separated chromatogram were observed at R_f value above 0.25 as mentioned in **Figure 1**.

**Figure 1:** TLC profile of extract of *Mesua ferrea*

IR spectroscopy was performed as spectral technique of analysis and IR spectra of plant extract recorded at range $4000-400\text{ cm}^{-1}$, spectra confirmed the presence of characteristic peaks of important phytoconstituents like; alkaloid, phenols and flavonoids, etc. The IR spectra also revealed characteristic overtone of aromatic compound which indicates presence of aromatic ring in herbal extract (**Figure 2**).

**Figure 2:** IR spectra of extract of *Mesua ferrea*.

The *in vitro* anti-inflammatory activity was performed to ascertain pharmacological profile of extract of *Mesua ferrea*. The inhibition of albumin denaturation was performed as a measure of anti-inflammatory potential of test compound. The inhibition of protein denaturation was observed in dose

dependent manner and maximum activity was observed with higher concentration of extract (500µg/ml). The extract at concentration level 500µg/ml exerted 79% inhibition of protein denaturation which was found to be comparable to the standard drug Diclofenac sodium (Figure 3).

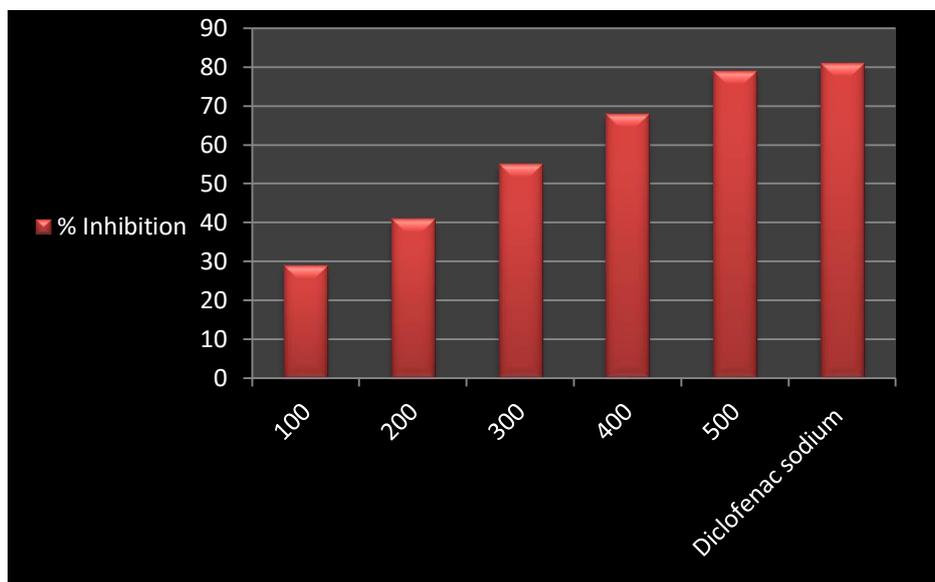


Figure 3: *In vitro* anti-inflammatory activity of extract of *Mesua ferrea* (%inhibition of albumin denaturation).

CONCLUSION

Study revealed that developed herbal formulation consisting extract of *Mesua ferrea* possesses most of the desired properties of topical formulation. Study concluded that *M. ferrea* Linn. offers appreciable anti-inflammatory activity and this response can be attributed to the presence of phtoconstituents like; alkaloids, flavonoids and tannins, etc. The study support folkloric use of *M. ferrea* as anti-inflammatory agent. However further study on animal population is required to confirm mechanisms of anti-inflammatory potential of plant extract, additionally it is also required to identify active constituents responsible for anti-inflammatory activity of *M. ferrea* bark extract. Study suggests that gel formulation prepared from bark extract of *Mesua ferrea* can be employed as topical anti-inflammatory agent for various therapeutic purposes.

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