Formulation and Evaluation of *Momordica dioica* Topical gel for Wound healing activity

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Abstract:
The aim of the present study is formulation and evaluation of Topical gel of *M. dioica* for its physio-chemical properties *in-vitro* permeation and excision wound healing activity in rats. Topical gels were prepared by different two polymers Carbopol and Hydroxypropyl Methyl Cellulose (HPMC) in a range (0.5-1.5%). The gels were evaluated for their viscosity, spreadability, content uniformity, *In-vitro* diffusion of gels using Franz diffusion cell. It was observed that with an increase in the polymer percentage, there was a proportional increase in the viscosity and reduced spreadability. Similarly, the rate of diffusion of the drug from the gels was also reduced with an increase in the polymer concentration. All the prepared formulations demonstrated content uniformity and moderate to good spreadability. Treatment of *M. dioica* Topical gel on Excision wound in rats significantly decreased wound contraction time and Percentage wound is compare to control group. It concluded that *M. dioica* loaded Topical gels is a viable option for the treatment of wounds.

Key words: *M. dioica* ,Zeta potential, SEM, Excision wound.

Introduction

Momordica dioica is a perennial, dioecious climber belonging to family (Cucurbitaceae) which is commonly known as spiny gourd, teasel gourd or small bitter gourd worldwide whereas in India, Fruits, leaves, and tuberous roots of *M. dioica* are used as a folk remedy for various medicinal properties such as antitumorogenic, analgesic, anti-diabetic, anti-inflammatory and anti-allergic activity [1-4]. The *M. dioica* fruit contains ashes, protein, lipid, fiber, carbohydrate, mineral are potassium , sodium , calcium , iron , and zinc [5].small quantities of essential vitamins like carotene, thiamin, riboflavin and niacin [6].

Cutaneous wounds are a serious health problem worldwide and are frequently associated with high costs and ineffective treatments [7]. They are characterized by a disruption of the cellular and structural integrity of skin tissue layers [8], caused by physical, chemical, thermal, microbial, or immunological damage to tissues [9-10]. Wound healing is a complex, dynamic, and integrated process involving three phases, inflammatory, proliferative, and maturation or remodeling [11-12]. Excessive production of reactive oxygen species, which is correlated with oxidative stress, in inflammations and infections prolong the wound healing process [13-14]. Therefore, compounds with antimicrobial, antioxidant, and anti-inflammatory properties will be helpful to wound healing [8,15-16]. The primary goals of wound treatment are rapid wound closure and the formation of a functional and esthetically satisfactory scar [17]. Dressings and topical products, such as creams and gels, are used in clinical practice. However, they are often costly or unsuccessful and have side effects [18]. The use of topical gel in excision wound models in rats should be the approach for the future studies on wound healing activities of *M. dioica* root extract.

MATERIALS AND METHODS

Plant Materials

Fresh fruit of *M. dioica* Roxb. (Family: Cucurbitaceae), were procured from local vendor Hyderabad, Telangana. Fruits are authenticated by authenticated by Dr.Vijaya Bhasker Reddy, Assistant Professor, Department of Botany , Osmania university, Hyderabad. A voucher specimen (No.OUAS-164).

PREPARATION OF EXTRACTION

The fresh fruits around 2kg shade dried for 15 days; fruit material was powdered using mixer grinder and passed through sieve no 85. Weight About 150gm of dried fruit powder was subjected to soxhlet's apparatus extraction using ethanol solvent for 72 hrs. The extract were concentrated in rotary flash evaporators and stored in refrigerator

Preliminary phytochemical analysis: the extracts were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents [19].

Description and Solubility

Ethanolic extract *M. dioica* as describe the organoleptic properties and solubility with polar solvents.

NAME OF THE INGREDIENTS	Quantities in w/w %(100 gm)					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
M. dioica extract	5	5	5	5	5	5
Tween 20 (V/V)	1	1	1	1	1	1
Carbapol 934(% W/V)	0.5	1	1.5	-	-	-
HPMC	-	-	-	0.5	1	1.5
Sodium benzoate (W/W)	0.5	0.5	0.5	0.5	0.5	0.5

Table1: Composition of *M. dioica* Topical gel formulations

Preparation of Ethanolic Extract of *M. dioica* Topical Gels

Topical Gels of ethanolic extract of *M. dioica* were prepared methods are describe below

1. Weigh 5gms of extract *M. dioica* in a beaker
2. Add 0.5ml of Tween stir continuesto form homogeuos suspension
3. Dissolved sodium benzoate, this mixture was incorporated to the above mixture and was subjected to magnetic stirred at 650-700rpm for 10min until homogeneous gels were obtained.
4. The specified amount of Carbopol 934 and HPMC polymers powder was slowly added to ultrapure water and kept for 12 hours for the polymer to swell.[20-21]
5. These formulations were then placed in wide-mouthed bottles for stability testing, with all samples equilibrated at room temperature. There were six different formulations as shown in (Table**1)(**F1, F2, F3,F4,F5 and F6)

Characterization of *M. dioica* loaded Topical Gel

Drug-excipient compatibility study

Extract and excipient compatibility study was carried out to investigate any possible interaction between *M. dioica* other excipients used in the formulation of the Topical gel, the samples were analyzed byFTIR spectroscopy.[22-23]

Zeta potential

Zeta potential of Topical gel formulations were measured by dynamic light scattering (DLS) technique (Malvern Zetasizer, Malvern Instrument, UK). Samples were dispersed in distilled water (3:25) before measurement.

Morphology

Topical gel morphology was observed using scanning electron microscopy (SEM) (Microscope Tecnai 200 kV D2360, USA). A drop of the Topical gel that had been dispersed by water was placed onto the carbon-coated copper grid and dried at room temperature, leaving a thin film. The film was colored using phosphotungstic acid solution and imaged.

Evaluation of *M. dioica* Topical Gel

Spreading diameter

By calculating the spreading diameter of 1 g of gel between two horizontal plates (20 cm 20 cm), the spreadability of the gel formulation was determined after one minute. On the upper plate, the normal weight was 125 g. [22-23]

Viscosity and pH measurement

Viscosity of Topical gelformulations was measured using Brookfield viscometer (Model No DV-III ULTRA) using spindle no 06 at 100 rpm, and pH measurements of the formulations were done using digital pH meter (RI-152-R).

***In vitro* diffusion studies**

In-vitro diffusion study was performed for topical gel dispersion (F1, F2, F3, F4, F5, and F6), Topical gel formulation using dialysis membrane (Hi media). Diffusion membrane was placed in Phosphate buffer solution (PBS) 7.4 for 6 h to attain saturation before starting permeation study and then mounted between the donor and receptor compartment of the Franz diffusion cell (fabricated with glass, the surface area available for diffusion was 2.54 cm2). The release rate of *M. dioica* was analyzed by placing the required sample in the donor cell compartment. To prevent contamination and evaporation, the donor compartment was covered with parafilm. The receptor chamber was filled with PBS 7.4 and was maintained at 37°C with continuous stirring. 1 ml aliquot of receptor phase solution was withdrawn at half an hour from the commencement of diffusion studies, followed by every hour till approximately 80% of the drug was released, the same volume of fresh medium was added back into the receptor compartment to maintain the sink conditions. The quantification was done using a UV spectrophotometer (Shimadzu Model No. 1800) at 208 nm. The cumulative amount of drug diffused versus the time graph was plotted. [22-23]

Stability study

Stability evaluation of Topical gel was performed by storing the gel at high (40°±2°C), room (25° ±2°C) and low (7°± 2°C) temperatures. During 12-weeks, organoleptic changes, pH in the *M. dioica* Topical gel were evaluated.

Pharmacological study on wound healing activity

Experimental animals procured

Adult wistar rats of male 9 to 11 week age, weighing 160–180gm were procured from Mahaveera enterprises, Hyderabad. Animals were housed in standard laboratory conditions at 25°c with 12 hr light-dark cycle with free access to chow and water *ad libitum*. The research protocol was approved by (HKES/COP/MTRIPS/IAEC/105/2022).

Excision wound model

18 albino rats weighing between 160–180gm are divided in to 3 groups of 6 animal s each.

Group I -served as Excision wound

Group II - served as *M. dioica* Topical gel + Excision wound

Group III- served as AVOMEB ointment +Excision wound

Albino rats 160-180 gm were taken for studies, the rats were anesthetized prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using Anaesthetic Ether. Wound of 500 sq. mm on dorsal thoracic region was made. Animals were apply the gel daily and closely observed for any infection and those which showed signs of infection wereseparated and excluded from the study and replaced. The animals were observed for wound closure at 0, 4^{th}, 6^{th} ,8^{th} , 12^{th} and 15^{th}day and for period of epithelialisation. [24-25]

Measurement of wound area

The progressive changes in wound area were monitored by 2^{nd}, 4^{th}, 6^{th} ,8^{th} , 12^{th} and 15^{th}day. The size of the wound was also measured using a scale daily and the wound area was calculated. Wound contraction was calculated as percentage of the reduction in wound area.

$$\textbf{Percentage of wound contraction} = \frac{\textbf{(Initial wound area – Specific day wound area)}}{\textbf{Initial wound area}} \times \textbf{100}$$

STATISTICAL ANALYSIS

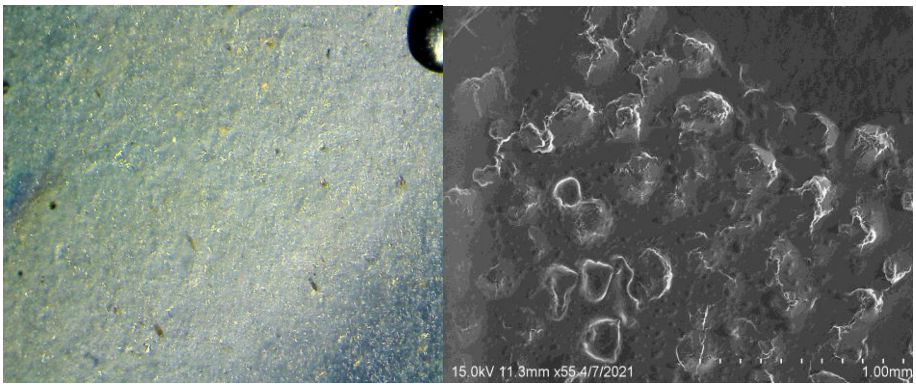
Data are expressed as mean ± standard error of mean. Differences in the *in vitro* release profile of prepared formulations were tested for significance using independent *t*-test using SPSS-17.0. Difference was considered significant when$P <$ 0.05. Graphs were prepared using GraphPad Prism 8 (Graph Pad Software, Inc). ***p<0.001, **p<0.01 and *p<0.05.

RESULTS AND DISCUSSION

Qualitative phytochemical studies shows the *M. dioica* contain primary and secondary metabolites such as Alkaloids, Glycoside, Flavonoids, triterponids and poly phenols etc.

The IR spectra for drug excipient compatibility study showed major peaks at 3342.13 cm^{-1},3231.58 cm^{-1}, 2981.15 cm^{-1},1735.98cm^{-}1,1643.22cm^{-1}, 1044.13 cm^{-1}, 2903.18 cm^{-1}, 2833.61 cm^{-1},1758.96 cm^{-1}and1621.87 cm^{-1}in pure extract the corresponding peaks were also obtained in the extract excipient mixture with slight shifting. It is evident from the data that the characteristics peaks of extract were not affected in the presence of carbapol implying that extract and excipient are compatible with each other.

The preliminary characterization of *M. dioica* topical gel (prior to sonication) was done by using an optical microscope.



A B

Figure: 1 [A] Optical microscope image of *M. dioica* [B] SEM image of *M. dioica* Topical gel

Overall, performance of transdermal drug delivery system is generally governed by morphology. [Figure1]. Phase contrast microscopy also showed the surface morphology of Topical gel (Figure 1 A and B). All the images depict smooth surface ZP is an important parameter that affects stability. Normal formulation was found to have negative ZP (−46.1 mV) due to the net charge of the lipid composition in the formulation. The negative ZP is responsible for enhanced percutaneous permeation of drug as shown in (figure2).

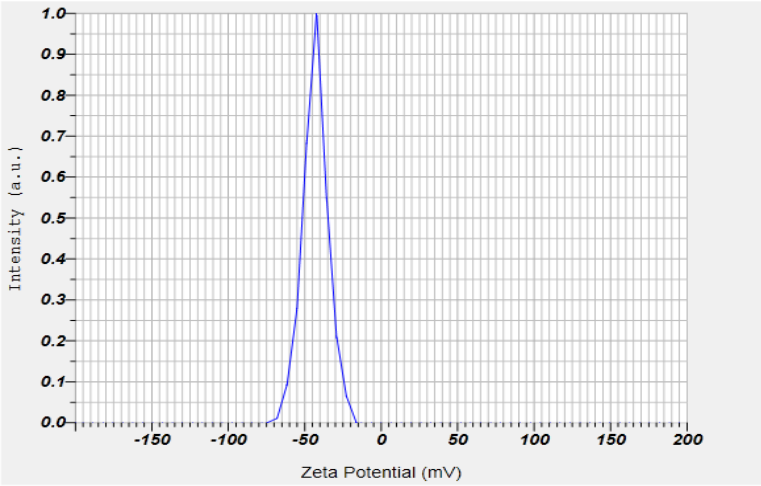


Figure 2: zeta potential of *M. dioica* Topical gel

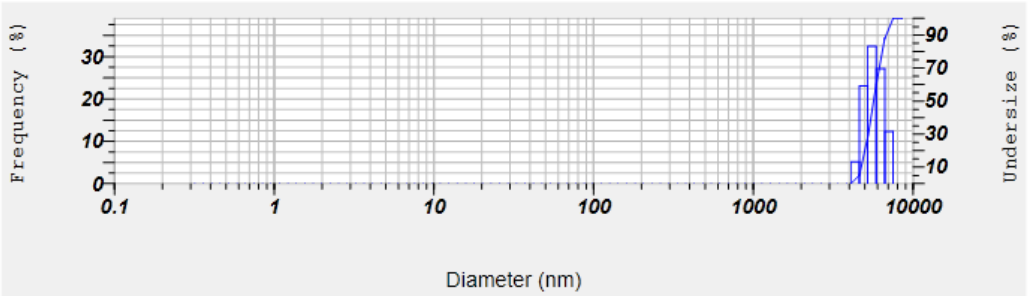


Figure 3: PDI of *M. dioica* Topical gel

Evaluation of *M. dioica* Topical Gel

The prepared gels were evaluated for physical appearance, pH, spreadability, viscosity, and drug content. Gels were found to be smooth, homogenous, yellowish white in color, pH lying in the normal skin pH range, easily spreadable, and viscosity ranging between 4500 and 4800 cps as shown in (table2)

The prepared topical gels of *M. dioica* demonstrated moderate to good spreadability and from the results it was evident that the gel spreadability was dependent on the polymer concentration and was reduced with an increment in the polymer concentration. The viscosity of the gels though increased with the increase in the polymer concentration, however, the gels remain easily spreadable. Polymer concentration affected the diffusion rate of the drug and the release was sustained with an increase in polymer concentration. Carbopol gels demonstrated higher viscosity compared to the corresponding HPMC concentrations.

Formulation	pH	Viscosity (CPS)	Spreadability (g.cm./sec.)	Grittiness
F1	6.8	36092	34.09	No
F2	6.7	37271	30.16	
F3	6.7	32134	31.83	
F4	6.8	32983	33.98	
F5	6.8	35175	32.81	
F6	6.9	31092	31.18	

Table 2: showing the evaluation of *M. dioica* topical gel paramater

***In vitro* drugs release studies**

In vitro release profile of topical gels is shown in (figure4) The drug release from Topical gel was observed, Maximum 76% and %79 drug release was achieved in F3 and F6 formulation. The topical gel were incorporated into carbopol gel and three gel formulations (F1,F2 and F3) were evaluated for drug release. The drug release from F3 topical gels was significantly higher than that of corresponding formulations. The drug release at higher ratios of carbopol and HPMC was decreased and it could be due to increased thickness of Topical matrices which leads to increased diffusional distance and therefore reduced drug release rates.

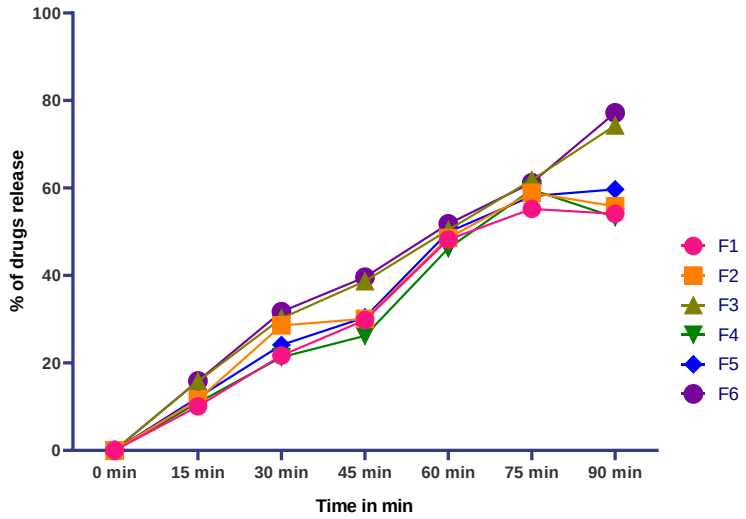


Figure 4: *In vitro* release profile of topical gels

Wound Healing Activity

The obtained results of Herbal Extract of *M. dioica* loaded Topical Gel on excision wound activity as shown in (figure 5 and 6). The wound healing control group are observed the wound contraction rate and % wound contraction healing are observed in days 19-20(0.473±0.073) and % wound contraction 85.03 0n 20th day. On treatment with Topical gel decreased wound contraction observed to monitor the fall of eschar leaving no raw wound behind (0.214±0.071) and % wound contraction (95.34) in 14-15 days, the results are comparable with that of showing *M. dioica* healing compared to control. The treatment *M. dioica* was day 14-15 are observed to monitor the fall of eschar leaving no raw wound behind, the results obtained indicate enhancement of wound contraction rate and increased epithelization followed by fall of escha,The treatment Standard Avomeb was day 14-15 are observed to monitor the fall of eschar leaving no raw wound behind, the results obtained indicate enhancement of wound contraction rate (0.189±0.010) and %98.20 wound contraction and increased epithelization followed by fall of escha, With the incision wound model.

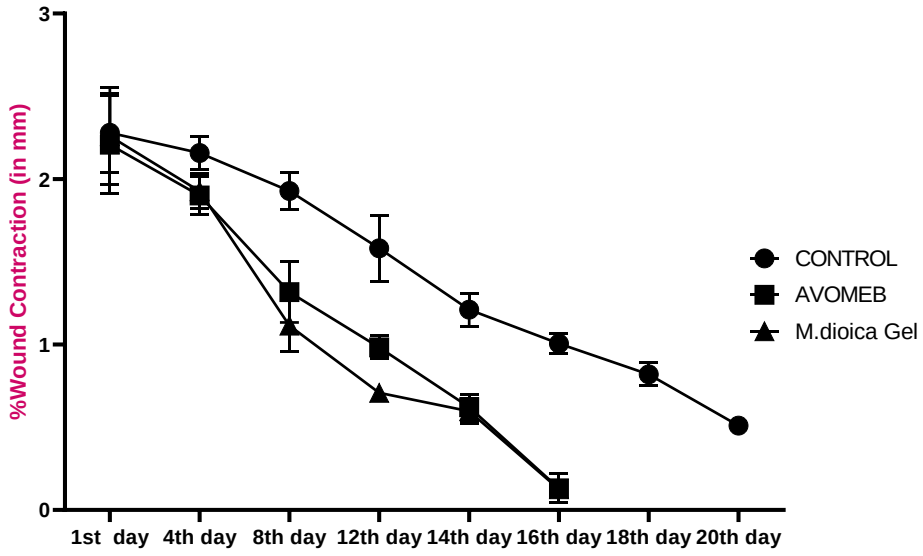


Figure5: Effect of *M. dioica* Topical gel on %wound contraction in excision wound

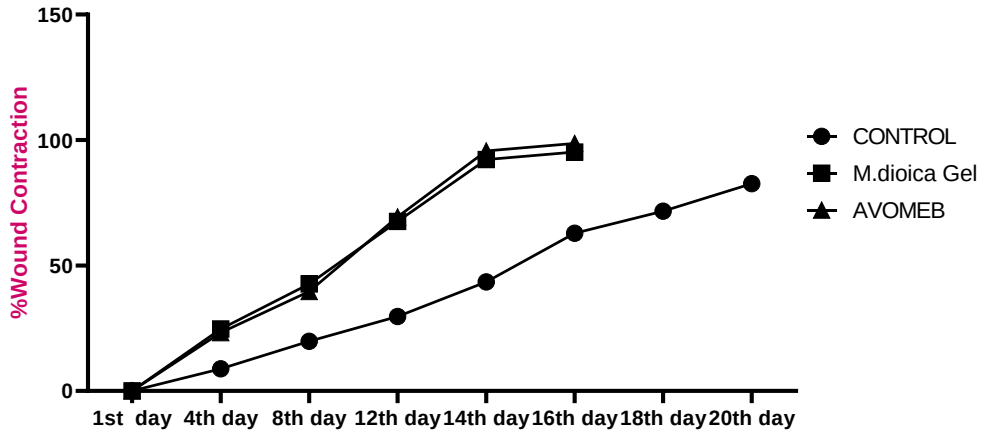


Figure 6 : Effect of *M. dioica* Topical gel on %wound contraction in excision wound

The use of traditional medicinal plants for wound healing is based on their antiseptic, astringent, anti-inflammatory, and antimicrobial properties [26]. Arekar *et al*. reported antibacterial activities more active against *E. coli* compared to *S. aureus, S. paratyphi*, and *P. mirabilis* bacteria. [27] This antimicrobial activity is related to facilitating the wound healing process, since open wounds are particularly prone to infections, especially by bacteria, and they also provide an entry point for systemic infections [28]. this effect is related to the presence of tetracyclic triterpenoids and their glycosides, most of which are known as cucurbitanes and are known for their bitterness and diverse biological effects [29].
The antioxidant activities of methanol and aqueous extract of fruits were analyzed and the presence of phenolic compounds, flavonoids, sterol, alkaloids, amino acids were found , Among those compounds, due to the presence of flavonoids, its fruit was reported as a potent antioxidant [30].is also related to the process of accelerating wound healing, since in the normal physiology of wound healing, it depends on low levels of reactive oxygen species and oxidative stress [31]; therefore, overexposure to oxidative stress leads to poor wound healing [32].

CONCLUSION

Topical formulations prepared based on the ethanolic extract of *M. dioica* can accelerate the healing of the induced wounds in mice. The 1.5% gel formulation was the most effective treatment. The healing mechanism of *M. dioica* may be related to phytoconstituents, such as anthocyanins and phenolic compounds that exert antioxidant, antimicrobial, and anti-inflammatory effects.

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

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