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EXPLORING WOUND HEALING POTENTIAL OF *MALLOTUS PHILIPPENENSIS* INFUSED EMULGELIN DIABETIC RATS

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ABSTRACT: Traditionally *Mallotus philippinensis* Lam. Mull. Arg (Euphorbiaceae) has been reported to treat a variety of ailments, particularly skin conditions and wounds. The objective of the current work is to formulate and optimize the emulgel formulation containing *Mallotus philippinensis* (MP) Lam. Muell. Arg extract. Moreover, it seeks to validate the traditional claim by investigating the effects of topical emulgel on diabetic wound healing. Initially extract of *Mallotus philippinensis* Lam. Mull. Arg fruit was prepared using 70 % ethanol. The extract was evaluated for antimicrobial and antioxidant activity. The formulations (F1 to F6) incorporating varying concentrations of extract were prepared and Carbopol 934 used as a gelling agent. The formulations were assessed for rheological, physicochemical properties, drug release, stability and healing of diabetic wounds. Wound healing potential was assessed using an excision wound model in streptozotocin induced diabetic rats. The optimized formulation F6 demonstrated satisfactory drug release and prominent diabetic wound healing activity in comparison to other formulations. Hence, emulgel infused with ethanolic fruit extract of *Mallotus philippinensis* Lam. Mull. Arg would be a promising candidate for diabetic wound healing.

INTRODUCTION: Wound could be outlined as a loss of cellular and anatomic tissue as well as stability of living tissue [1]. Healing of Wounds is a Herculean task as several interdependent steps and factors come into play including hemostasis, inflammation, proliferation, and remodeling [2–5]. Delayed wound healing leads to chronic wounds such as diabetic ulcers, venous ulcers, pressure injuries, arterial ulcers, infections, sepsis and finally lead to amputation [6]. The impact of the altered healing is gigantic, considering the number of diabetic patients continues to rise [7].

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Around 80 % of the world population is using natural products as their healthcare needs. Almost 50 % of best-selling pharmaceutical products are natural products based. Globally Plants or natural products are becoming a choice for wound healing [8]. The current study was designed to target chronic problems of diabetic wound healing and to design formulations with potential herbal candidates.

Mallotus philippinensis Lam. Mull. Arg (Euphorbiaceae) (MP) commonly Termed Kamala, grows well between 300 and 1600 meters above sea level on limestone hills, stream valleys, and mountain slopes or valleys in Indian woods. It is widely dispersed in southern China, India, Sri Lanka, the western Himalayas, Malesia, and Australia. The plant leaves are alternate, simple, more or less leathery, ovate to lanceolate, have two glands at base. The male flowers are terminal and axillary, 2–10 cm long, with many stamens and

small, single or fascicled paniculate spikes. Female flowers have thin racemes or spikes, and the ovaries are stellate, hairy, three-celled, and have three papillose stigmas. The fruit has three lobed capsules, is stellate, puberulous, and has a lot of orange or reddish glandular granules. It has three seeds. The seeds are black and sub globose. The glandular hairs on the fruits are coated in scarlet powder [9].

Traditionally, its powder and fruit have been employed as a purgative, analgesic, anthelmintic, anti-inflammatory, detergent, and carminative [10]. These are also effective in the treatment of wounds, ulcers, bronchitis, stomach disorders, contraception, parasitic skin infections, and spleen enlargement. Several sources suggest that ancient Indian healers employed *Mallotus philippinensis* Lam. Mull. Arg [11].

A plethora of literature is available on *Mallotus philippinensis* Lam. Mull. Arg but according to undocumented folkloric knowledge, it was reported that the fruit powder is used to treat the injuries along with oil. Therefore, to corroborate this claim, it was decided to investigate the effect of *Mallotus philippinensis* Lam. Mull. Arg fruit extract and effect of carrier oil in healing of diabetic wounds. The novelty of the current work is to check the effect of *Mallotus philippinensis* Lam. Muell. Arg emulgel formulation on diabetic wound healing which was not documented previously. Emulgel is a gelled emulsion prepared with a gelling agent. They are available in two forms: o/w and w/o *i.e* oil-in-water or water in oil type of emulsions [12]. Emulgel provides various advantages over traditional topical formulations, including good spreadability, grease lessness, thixotropy, a long shelf life, no odour, and a pleasant look. Emulgel contains gel and emulsion qualities and acts as a dual-control release system [13]. The emulgel was designed with the intention to promote the traditional claim that MP and oil together accelerate wound healing [10]. The pharmacological and physicochemical properties were assessed.

MATERIAL AND METHODS:

Materials: Streptozotocin (Sigma Aldrich), span 80, Sesame oil, and Carbopol 934 were acquired from Loba Chem. Pvt. Ltd. in Mumbai. Glycerol, triethanolamine, Methyl paraben, sodium and propyl paraben sodium were obtained from Hi Media laboratories. All the chemicals and reagents utilized were of analytical grade.

Plant Materials and Extract Preparation: The fruits of *Mallotus philippinensis* Lam. Mull. Arg were collected from Baneshwar village in the Pune area of Maharashtra in January, when the plant was in flower. The plant was authenticated with Jawaharlal Nehru Botanical Garden at Erandwane (Voucher No. 1442). The plant material was dried. The red powder that was found on the fruit's glandular hairs was gathered and cold macerated. The powder was extracted using 70% ethanol for 48hr followed by drying in a rotary vacuum evaporator at 50° C. After that, the extract was kept at room temperature in an airtight container.

Antibacterial Activity [10, 14]:

Microorganism: The total four strains of bacteria such as *E. coli* (MCC2246), *S. aureus* (MCC 2408), *Bacillus subtilis* (MCC 2511) were used. The National Centre for Microbial Resources in Pune, Maharashtra, supplied the microorganisms.

The dried fruit hair extract was dissolved in 70 % ethanol. The different concentrations of ethanolic extract like 20,30,50, 70 mg/ml were prepared. The disc diffusion method was used to perform the antimicrobial test, and 50µl of suspension containing each of the bacteria was spread out over nutritional agar. The plates were placed on agar plate. On the disc were 20µl of various extract dilutions. As a positive reference standard, chloramphenicol was used. The same solvent was used to produce negative controls. After being sealed, the inoculation plates were incubated for 24 hours at 30° C. The zone of inhibition against the test pathogens was measured to assess the antimicrobial activity.

Antioxidant Studies: Antioxidant study was performed using DPPH Radical Scavenging Activity. It was performed as per protocol described by Shinde *et al*., with some modifications [15]. The percentage of inhibition was determined in comparison to the blank.

Estimation of Total Flavonoid Content: The total flavonoid content of plant material was measured colorimetrically using quercetin as a reference,

following the procedure outlined by Suman Chandra *et al* [16].

Formulation Development Studies:

Preparation of Gel: The appropriate volume of *Mallotus philippinensis* Lam. Muell. Arg extract was dissolved in a 1:1 combination of ethanol and water. The required amount of carbopol 934 was weighed and added to the solvent mixture. The polymer was allowed to swell thoroughly without being stirred continuously. After that, the dispersion was constantly stirred for approximately two hours at 500 rpm. Later, the speed was decreased to avoid the air entrapment. Alkali triethanolamine was added to the hydrogel solution after two hours to neutralise it and get the highest viscosity possible.

Preparation of Emulgel [17]: The emulgel formulation was divided into three steps. Emulsion was prepared either o/w or w/o in Step 1. Gel-based was produced in Step 2. The emulsion was then continually mixed into the gel basis in step 3. Carbapol 934 was used as a gelling agent in the preparation of the various batches. Carbopol 934 was mixed with distilled water and continuously stirred at a moderate speed using a mechanical shaker to produce the gel base. Triethanolamine was added to all formulations to get their pH to a range between 6 and 6.5. The emulsion's oil phase was produced by combining sesame oil and span 80. *Mallotus philippinensis* Lam. Mull. Arg extract dissolved in water and methyl paraben dissolved in propylene glycol were mixed to formulate the aqueous phase. Next, the aqueous and oily phases were heated individually to 70°C to 80°C. With constant stirring, the aqueous phase was incorporated to the oil phase. It was allowed to cool to room temperature. After that, the produced emulsion was gently stirred and mixed with the gel in a 1:1 ratio to produce the emulgel. The formulation plan of MP gel and MP Emulgel is given in **Table 1.**

TABLE 1: FORMULATION BATCHES OF MP GEL AND EMULGEL

Ingredients (% w/w)	MP Gels			MP Emulgels		
	F_1	F_2	F_3	F_4	F_5	F_6
Extract	0.75	1	1.5	0.75	1	1.5
Carbapol 934	0.75	0.75	0.75	0.4	0.4	0.4
Propylene Glycol	4.5	4.5	4.5	4.5	4.5	4.5
Triethanolamine	0.12	0.12	0.12	0.15	0.15	0.15
Ethanol	9	9	9	1.5	1.5	1.5
Propyl paraben	0.15	0.15	0.15	0.15	0.15	0.15
Span 80	-	-	-	4	4	4
Sesame oil	-	-	-	1	1	1
Distilled water	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S

Evaluation of Gels and Emulgels [12, 18–20]: The formulated gel and emulgel of *Mallotus philippinensis* Lam. Mull. Arg extracts were evaluated for Physicochemical characteristics like pH measurement, viscosity, spreadability, homogeneity, grittiness, and stability as per protocol mentioned by Maria BR *et al.* The *ex-vivo* permeation study was carried out employing the method outlined by Panigrahi L *et al.*

***In-vivo* Study:**

Animals: Male albino Wistar rats weighing 180–200 g were purchased from the National Institute of Biosciences in Pune, Maharashtra. They were kept in groups of six rats each in polypropylene cages, with a 12-hour light-dark cycle and a room temperature of 22±3 °C, relative humidity of 30-70%. They were provided conventional laboratory animal feed and clean drinking water ad libitum. All animals were quarantined and acclimatized for about 7 days before the initiation of the study.

Experimental Design: The experimental animals were grouped into 5 groups of 6 rats each, as shown in **Table 2.**

TABLE 2: EXPERIMENTAL DESIGN FOR WOUND HEALING ACTIVITY OF EMULGEL

Groups	Treatment
Group 1	Normal control, (NC)
Group 2	Diabetic wound control (DC)
Group 3	Treatment group I MP Gel
Group 4	Treatment group II MP Emulgel
Group 5	Treatment group III 5% Povidone Iodine (Std)

Induction of Diabetes [21]: Streptozotocin induced diabetic rats were used to evaluate the wound

healing activities. A single intraperitoneal dosage of Streptozotocin (55 mg/kg) was administered to induce diabetes. The blood glucose levels were checked by Accu-Chek glucometer after three days of induction to confirm diabetes. Standard procedures were followed to select animals with diabetes (blood glucose level > 200 mg/dL) for the wound healing study.

Excision Wound and Treatment [1, 22–25]: The rats were anaesthetized with Isoflurane (2-3%) with O_2 on the days of wound formation (day 0). The Wistar rats dorsal skin were shaved first, then a circular wound was formed on the back of each rat using a flexible transparent plastic template, and a layer of full thickness skin with a 2.5 cm in width using toothed forceps surgical blades and pointed scissors were produced along the markings. After induction, the rats were given vehicle, formulation, and standard (Povidone-Iodine). Separate groups of rats were maintained with wound creation.

The formulated gel and emulgel were applied twice a day to wounded areas till the wound was completely healed. The wound areas were measured on 1st, 4th, 8th, 12th, 16th and 21st day by tracing the wound areas on graph paper. The results of reduction in wound areas were expressed as the percentage of original size of wound. The results were compared with the group of animals treated with standard.

RESULTS AND DISCUSSION:

Antibacterial Studies: The Antimicrobial activity of *Mallotus philippinensis* Lam. Mull. Arg ethanolic extracts were assessed by presence or absence of inhibition zone. The results have been displayed in **Table 3.** It revealed enhanced antimicrobial activity with increase in the concentration of extract. The maximum inhibition zone was obtained against microorganisms at a concentration of 75mg/ml.

TABLE 3: ANTIMICROBIAL ACTIVITY OF *MALLOTUS PHILIPPINENSIS* EXTRACT

Concentration (mg/ml)	Inhibition Zone (Cm)		
	E. coli	***S. aureus***	***B. subtilis***
Standard	3	3.3	3.5
25	-	-	-
35	1	0.9	1
50	2	1.2	1.5
75	2	1.3	1.5

Antioxidant Studies: *In-vitro* antioxidant activity of MP ethanol extract was determined by DPPH radical scavenging method. The findings of this assay revealed that the formulations scavenged the free radicals in a concentration-dependent manner. At 250 µg/ml concentrations, the *Mallotus philippinensis* Lam. Mull. Arg extract's percentage inhibition of DPPH scavenging activities was 62.12%. The resulting IC_{50} value was 167.7. Antioxidant activity is dependent on the amount of total polyphenolic components. *Mallotus philippinensis* Lam. Mull. Arg formulations antioxidant activity was determined by an *in-vitro* antioxidant study. Since *Mallotus philippinensis* Lam. Mull. Arg extract contains polyphenolic components, it could possess antioxidant properties. The antioxidant activity values are displayed in **Fig. 1.**

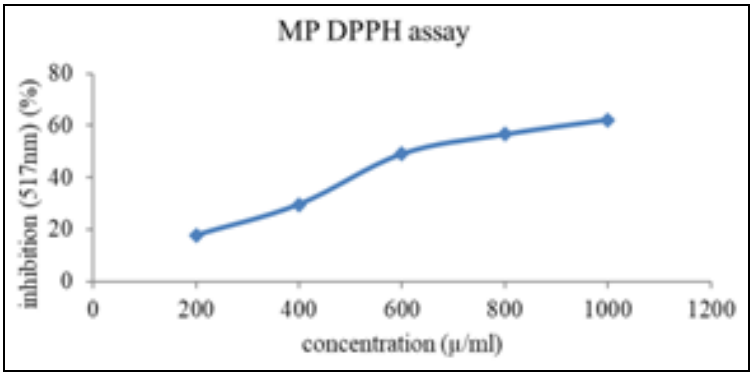

FIG. 1: DPPH RADICALS SCAVENGING ACTIVITY OF ETHANOLIC EXTRACT OF FRUIT OF *MALLOTUS PHILIPPINENSIS*

Total Flavonoid Content: The MP extract's total flavonoid concentration was 2.66 mg/g of quercetin equivalent per milligram of plant extract. The results are displayed in **Fig. 2.** As *Mallotus philippinensis* Lam. Mull. Arg extract contains an abundance of flavonoids, it may have potent antioxidant and free radical scavenging properties that aid in wound healing.

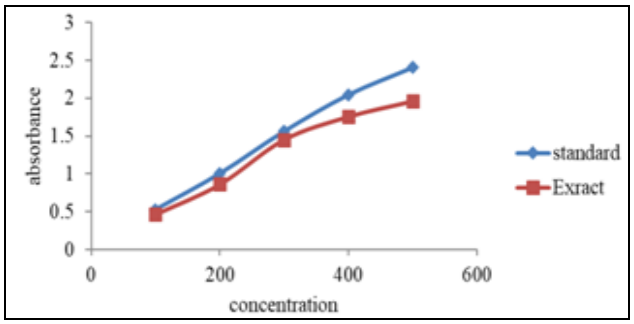

FIG. 2: THE TOTAL FLAVONOID CONTENT OF *MALLOTUS PHILIPPINENSIS* EXTRACT

TABLE 4: PHYSICOCHEMICAL CHARACTERISTICS OF FORMULATIONS

Formulation	Homogeneity	Grittiness	Colour	Phase Separation
F1	+++	-	Orange transparent	-
F2	+++	-	Orange transparent	-
F3	++	-	Orange transparent	-
F4	++	-	Translucent gel with reddish orange colour	Slight separation
F5	+++	-	reddish orange slightly translucent	-
F6	+++	-	reddish orange slightly translucent	-

+++ Excellent, ++ Good

Evaluation of Gels and Emulgels: The results of the physicochemical analysis of the prepared gel and emulgel are shown in **Table 4.** It was found that all the formulations were homogeneous, devoid of phase separation, and free of grittiness. They had a white, creamy, viscous preparation, a smooth, uniform texture, and a glossy appearance. The results obtained for the evaluation of pH, viscosity, spreadability and drug content studies are presented in **Table 5**. pH values of the formulations were found to be between 6.2 and 7.1, which is very close to neutral and indicates that they could not irritate skin. Additionally, it showed that the ingredients utilised for the emulgel formulation were not affecting the formulation's pH.

TABLE 5: RESULTS OF EVALUATION OF FORMULATION

Formulation code	pH	Viscosity (centipoise)	Drug content (%)	Spreading area (g.cm/sec)
F1	6.4	47450	89.37	19.50
F2	6.2	49863	95.77	29.02
F3	7.1	51729	96.34	35.95
F4	6.5	11192	89.44	38.22
F5	6.9	12212	97.50	63.18
F6	6.8	12502	98.40	62.18

The viscosity of gels and emulgels increased as the polymer concentration increased. Emulgels were prepared through mixing emulsion with carbopol gel in a 1:1 ratio, resulting in a higher viscosity than comparable gels. In comparison, the emulgels exhibited a higher percentage of drug content than the similar gel formulations. This indicated that the drug was distributed uniformly throughout the emulgels, which might be because of the high drug entrapment in the internal phase of emulsion. Additionally, it was found that the emulgels exhibited excellent spreadability due to their reduced viscosity and the oil phase's ability to lessen shearing stress.

Stability Studies: Stability studies were carried out on the prepared gels. All formulations exhibited no signs of colour fading. All formulations had pH values between 6.2 and 7.2, which were unaffected. Especially at room temperature and 8 °C, the viscosity and spreadability of all gels remained constant; however, a slight decrease in viscosity

was observed at 45°C. For all gel formulation, the drug concentration was determined to be within the range of 90% to 103% at all temperature environments. All the above-mentioned criteria indicated that the F3 and F6 formulations were appropriate, and they were further evaluated for *in-vivo* wound healing efficacy and *ex-vivo* penetration studies.

***Ex-vivo* Permeation Study:** Franz diffusion cells were used to carry out an *ex-vivo* release evaluation through the rat skin of the optimised formulations (F3 and F6) which were selected. The *ex-vivo* release showed a more precise estimation of the drug penetration characteristics through animal skin. *Mallotus philippinensis* Lam. Mull. Arg emulgel exhibited more skin release than MP extract dissolved in the hydrophilic carbopol gel matrix. The oil phase enhances drug penetration through the stratum corneum, which is the main barrier limiting drug penetration through the skin. After 8 hours, the percentage of drug that penetrated the skin from F3 and F6 was 60.59 and 71.93%. The results of the study on *ex vivo* permeation is shown in **Fig. 3.**

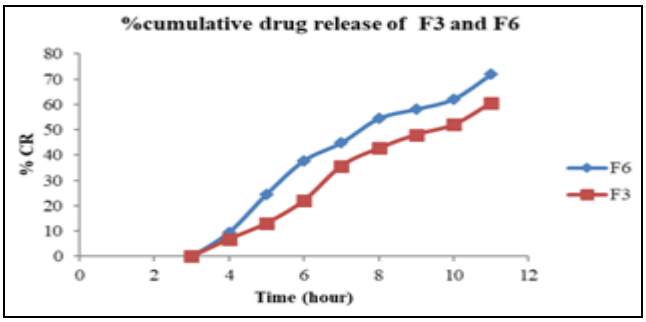

FIG. 3: *EX-VIVO* RELEASE STUDY OF OPTIMIZED FORMULATIONS F3 AND F6

***In-vivo* Study:** In streptozotocin induced diabetic rats, the effects of *Mallotus philippinensis* Lam. Mull. Arg gel and emulgel formulations were studied using an excision wound model. The percentage of wound healing for the MP emulgel and standard group was significantly greater than control and gel treated groups. The *Mallotus philippinensis* emulgel-treated group showed 65% wound closure on the eighth day as compared to 50% closure in the standard-treated group.

The optimised formulation (F6) treated exhibited almost complete wound closure at the end of the study while the control groups showed 90%. The results are shown in **Fig. 4**. The macroscopical changes of wound have been displayed in **Fig. 5**.

This wound healing effect could be due to the interaction between phytoconstituents of *Mallotus philippensis* (Lam.) Mull. Arg and the multiple targets of chronic wound healing process as reported in our previous *in-silico* study.

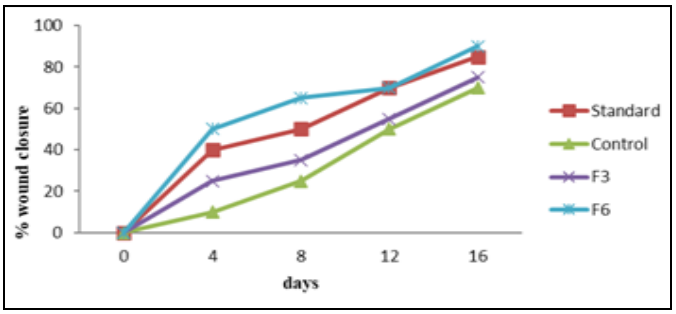

FIG. 4: COMPARISON OF *IN-VIVO* WOUND HEALING ACTIVITY OF OPTIMIZED FORMULATIONS F3 AND F6 WITH THAT OF CONTROL AND STANDARD GROUPS

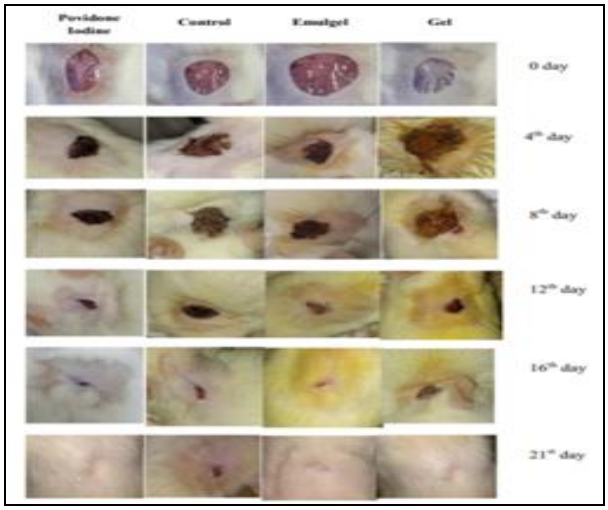

FIG. 5: MACROSCOPICAL CHANGES OF WOUND HEALING ACTIVITY

CONCLUSION: The findings of the present study revealed that ethanolic fruit extract of *Mallotus philippinensis* Lam. Mull. Arg has antimicrobial and antioxidant properties that may be due to presence of polyphenolic compounds in plant. The emulgel formulation (F6) showed satisfactory physicochemical, rheological properties, drug content, stability, spreadability and *Ex-vivo* release profile.

Also, the emulgel formulation containing ethanolic *Mallotus philippinensis* Lam. Mull. Arg extract demonstrated significant diabetic wound healing activity. It was finally concluded that *Mallotus philippinensis* Lam. Mull. Arg emulgel formulation was found to be a potential candidate for diabetic wounds management.

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CONFLICT OF INTEREST: Nil

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