JCHR (2024) 14(4), 817-827

Journal of Chemical Health Risks

sanad.iau.ir/journal/jchr

ORIGINAL ARTICLE

Anti-inflammatory and Wound Healing Properties of *Curcuma amada* and Oregano Essential Oil Derived Hydrogel on Wistar Albino Rats

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Received: 2 July 2024 *Accepted: 17August 2024)*

KEYWORDS

Carbopol 934;
Essential oil;
Herbal hydrogel;
Inflammation;
Rhizome;
Sodium alginate

ABSTRACT: The development of herbal gels for the treating wounds and inflammation is increasing attention to researchers for finding alternatives to conventional drugs with minimal side effects. In the present study, the herbal gel was prepared using *Curcuma amada* rhizome extract, a combination of oregano oil extracted from *Origanum vulgare* leaves, and either of three polymers namely carbopol 934, sodium alginate, and sodium carboxymethyl cellulose. Rheological parameters for prepared herbal gel were tested for pH, viscosity, and spreadability. The *in-vivo* excision wound healing and anti-inflammatory studies were performed on three month-old-male Wistar rats. The results show the best wound healing potential found in herbal formulations containing carbopol 934. This formulation showed good viscosity (65 cPs × 10^3), spreadability (25 gm × cm sec^{-1}), *in-vitro* release (100%) and significant (350.97±0.27 mm^2) wound contraction compared to a positive control (neomycin sulfate). The results show the highest anti-inflammatory activity in rats group treated with 200 mg kg^{-1} of methanol extract of *C.amada* compared to control (indomethacin). The gel was prepared using methanol extract of *C.amada*, oregano oil, and carbopol 934 which are excellent drug candidates for treating wounds and inflammation.

INTRODUCTION

Wound healing is a lengthy and intricate process that involves interactions with various physiological systems. Most wounds are classified as acute or chronic in either case, if the wound is not treated it can lead to clinical complications [1]. This is common in developing countries with tropical climates: due to lack of adequate hygiene and health awareness. Inflammation is a secondary phenomenon accompanying the wound and has characteristics such as pain, redness, rubor, and swelling [2]. In addition to being costly, a large number of the pharmaceutical drugs recommended for managing wounds also lead to side effects like drug resistance and allergies. Therefore, drugs derived from plants have been the focus of recent pharmaceutical products for treating various illnesses. Many species of medicinal plants consist of active botanical compounds and elements useful in treating diabetes, hypertension, burns, wounds, and inflammatory diseases. Due to the negative health

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DOI: 10.60829/JCHR.2024.25724

effects and multidrug resistance of synthetic drugs, people around the world are now starting to choose herbal extracts over synthetic drugs since they are affordable and safe [3].

Earlier studies have shown that plant secondary metabolites can be used for wound healing as topical creams such as *Thespesia populnea* [2], *Raphanus sativus* [4], *Sansevieria trifasciata* [5], *Moringa oleifera* [6], *Curcuma longa*, *Centella asiatica*, *Rosmarinus officinalis*, *Calendula officinalis* [7]. To increase their efficacy, bioavailability, and spreadability, these plant-based metabolites are used as topical gels by combining them with other polymeric substances. Topical gel products are used to treat wounds because gel is a lipophilic substance that readily penetrates the skin's outer layer and has the potential to heal wounds. Gelling agents, such as carbomers (carbopol), are considered acceptable due to their biocompatibility [8]. They are also regarded as suitable alternatives, especially for controlled drug delivery systems and the delivery of drugs to a specific part of the body. Additionally, this hydrogel can be coupled with other active ingredients as a matrix substance to address different wound repairs [9]. Gels containing herbal extracts work best for healing wounds because they build a barrier of protection over the wound surface and function as humectants (hygroscopic substances).

Curcuma amada Roxb. (Zingiberaceae) is a rhizomatous, perennial, and aromatic herb generally called amada or mango ginger because of the raw mango-like scent of the rhizome. This plant is distributed in Southeast Asian countries of India, Myanmar, and Thailand. It has been used in traditional medicine and culinary preparations in these countries [10]. Studies have shown that, more than 100 bioactive compounds have been reported in this plant and it has anti-inflammatory, anti-oxidant, anti-bacterial, anti-cancer, anti-hyperglycemic, and anti-hypercholesterolemic activities [11].

Essential oils exhibit great potential as therapeutic agents due to their ability to promote wound healing and reduce inflammation and bacterial infection. Hydrogel is frequently used for loading essential oils as it helps maintain their stability and biological activity. Essential oils such as geraniol, cinnamaldehyde, eucalyptol, thymol, carvacrol, and menthol have antibacterial, antioxidant, and wound-healing properties [12].

Recent studies have shown that hydrogel and films infused with plant extract and essential oil are non-toxic and biocompatible, support fibroblast migration, and have antibacterial action in addition to enhancing the viability of skin cells and speeding up wound healing [13]. Therefore, we hypothesize combining oregano essential oil, *C.amada* rhizome extract, and polymers perform better than the existing topical creams.

MATERIALS AND METHODS

Collection and processing of plant material

Fresh rhizomes of *Curcuma amada* Roxb and *Origanum vulgare* leaves were collected from the SKM Herbal Research Centre in Erode, Tamil Nadu, India, and authenticated by the Department of Horticulture, Annamalai University, Tamil Nadu, India. The rhizomes were dried and powdered.

Preparation of plant extract and essential oil

The rhizomes of *C.amada* were extracted using the soxhlet apparatus with methanol as a solvent, while *O. vulgare* essential oil was obtained by hydro-distillation. Both extract and oil were stored at 4ºC for analysis [14].

Preparation of herbal gels

The topical gel was prepared by soaking the carbopol 934, sodium alginate, and sodium carboxymethyl cellulose with various concentrations (1.0 to 2.0%) in water for 24 hrs along with 5% of methanol extract of *C.amada* (Table 1). The herbal extracts were incorporated into the prepared gel base in nine different formulations. The pH was adjusted to 6.7 using triethanolamine and the formulated herbal gels were stored at room temperature for further analysis [15].

Table 1. Formulation of herbal gel using different gelling agents.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Carbopol 934 (%w/w)	1.0	1.5	2.0	-	-	-	-	-	-
Triethanolamine (ml)	0.1	0.1	0.1	-	-	-	-	-	-
Propylene glycol (ml)	5	5	5	5	5	5	5	5	5
Sodium alginate (%w/w)	-	-	-	1.0	1.5	2.0	-	-	-
Sodium CMC (%w/w)	-	-	-	-	-	-	1.0	1.5	2.0
Ethanol (ml)	5	5	5	5	5	5	5	5	5
Methanol extract of *C.amada* (%w/w)	5	5	5	5	5	5	5	5	5
Essential oil (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Distilled water (g)	84	83.5	83	84	83.5	83	84	83.5	83
Total volume (g)	100	100	100	100	100	100	100	100	100

Note: F1-F9 are formulations prepared by combining the ingredients listed in the table

Evaluation studies of herbal gel formulation

The characteristics of herbal gel formulations such as pH, viscosity, spreadability, and drug content were studied [15]. Each formulation was done in triplicate and average values were calculated. A precisely weighed amount of gel (approx 1 g) was dissolved in 100 ml of phosphate buffer (pH 6.8) and the drug release was studied after 24 hrs using Franz diffusion cell membrane. The obtained mixtures were then filtered *via* 0.45 mm membrane filters and absorbance was measured at 424 nm using a UV-Vis spectrophotometer (Labtronics/LT 2201).

Animal and treatments

Male Wistar albino rats weighing 150 to 200 g were procured from the Karpagam Academy of Higher Education's animal home in Coimbatore, Tamil Nadu, India. Institutional Animal Ethical Committee (IAEC) with reference number (KAHE/IAEC/2021/11-09/003) and the animals were housed in animal house with access to food, water, and care [16]. Animal research was conducted per the committee for the control and supervision of experiments with animals' ethical standards (CPCSEA).

Skin irritation test

A Skin irritation test was done as per the standard protocol [17]. Skin irritation test was conducted on the dorsolateral part of the rats using methanol extract of *C.amada* and the reactions were monitored up to 72 hrs post-application.

Excision wound model

For the wound healing experimental study, all rats separated into five groups with six animals (n=6), were acclimated to a temperature of 22°C. Animals in group I served as a control. Carbopol 934 hydrogel was administered to group II animals. Commercial neomycin sulfate ointment, which serves as positive control, was given to Group III. At the same time, Groups IV and V were treated with herbal gel formulations containing 5% and 7.5% methanol extract of *C.amada* in 1.5% carbopol respectively, and marked as 5% herbal gel and 7.5% herbal gel. A 500 mm^2 surgical wound was inflicted on the back of each rat. In the treatment groups and positive control groups, 0.5g of each topical application of the hydrogel and neomycin sulfate ointment was applied every 24 hrs for 14 days. Wound contraction rates were monitored at the interval of 3,7,10 and 14 days after the start of the experiment. The percentage of wound contraction was calculated based on initial and subsequent measurements [18].

Acute oral toxicity of the extract

Acute oral toxicity was assessed using OECD 425 standards, administering a maximum oral dose of 2000 mg kg^{-1} body weight to three rats. Animals were

observed for 14 days for mortality or illness, followed by organ examination for pathological alterations [17].

Acute anti-inflammatory activity

Acute anti-inflammatory activity was assessed by carrageenan-induced rat paw edema [19]. Rats were divided into four groups of six each after a 12-hour fasting. Group I only received carrageenan which served as a control group. Group II received the standard drug indomethacin (3 mg kg^{-1} body weight). Group III and IV animals received methanol extract of *C.amada* at oral dose of 100 and 200 mg kg^{-1} respectively. 1% carrageenan in normal saline was administered to right paw of the experimental and control rats, resulting in acute inflammation. After injecting carrageenan into each group, the paw volume was evaluated at 15 minutes, 30 minutes, 1 hr, 2 hrs, and 4 hrs. The edema was calculated using the difference between the readings, and the percentage of anti-inflammatory action was estimated [20].

$$\% \text{ of inhibition rate} = [(V_e - V_t) / (V_e)] \times 100$$

Where V_e is the edema value of the control group and V is the edema value of treated groups.

Statistical analysis

Experimental data are presented as mean ±standard error of the mean (SEM), and statistical analysis was conducted using one-way ANOVA and student's t-test in GraphPad PRISM version 9.3.1. A p-value <0.05, <0.01 and <0.001 was considered statistically significant when compared to the control group (*p<0.05, **p<0.01 and ***p<0.001).

RESULTS

Formulation and evaluation of herbal gel

A herbal gel containing methanol extract of *C.amada* with *O.vulgare* essential oil was formulated using varying concentrations of carbopol 934, sodium alginate, and sodium CMC polymers (Table 1). Nine formulations were prepared and the rheological characteristics like pH, viscosity, spreadability, and drug content were measured (Table 2).

Table 2. Rheological studies of herbal gel using different gelling agents

Formulations	pH	Viscosity ($cPs \times 10^3$)	Spreadability ($gm \times cm\ sec^{-1}$)	Drug content (%)
F1	6.35	55	30	99.47
F2	6.14	63	25	100.78
F3	6.31	78	16.6	99.98
F4	6.64	58	21.4	102.64
F5	6.90	72	18.75	101.72
F6	6.72	89	15	99.56
F7	6.65	32	50	100.67
F8	6.54	49	37.5	99.06
F9	6.47	55	30	98.14

Upon handling the compositions exhibited the appearance of the gel was smooth with a soft and silky texture. The drug content of the methanol extract of *C.amada* in each formulation was determined at a peak absorption wavelength (λmax) at 424nm. The pH range of all formulations was calculated to be neutral (6.1 to 6.9), which is considered suitable for reducing the risk of skin irritation upon application. The viscosities of the formulated were within acceptable limits (Table 2). The formulation F7 demonstrated maximum spreadability (50 $gm \times cmsec^{-1}$) and maximum drug content was achieved in the F4 formulation (102.64%) followed by the F5 (101.72%).

In vitro diffusion profile and release kinetics

Table 3 presents the kinetic studies and *in vitro* release characteristics of the herbal gel formulations.

Table 3. *In vitro* drug release percentage of herbal gel formulations at various time intervals.

Time (h)	Carbopol 934			Sodium Alginate			Sodium CMC		
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	12.5±0.34	10.9±0.55	16.4±0.21	13.6±0.19	12.1±0.21	14.2±0.20	16.3±0.55	11.2±0.32	9.9±0.21
2	27.2±0.39	21.3±0.72	22.6±0.25	29.4±0.23	19.7±0.28	21.5±0.26	29.5±0.72	23.6±0.37	17.2±0.26
3	43.9±0.48	34.9±0.89	32.7±0.32	38.7±0.28	27.1±0.32	31.4±0.31	41.7±0.89	36.5±0.44	24.2±0.31
4	64.8±0.53	46.2±0.96	41.6±0.36	50.3±0.32	46.7±0.39	37.9±0.35	59.2±0.92	41.4±0.53	33.6±0.40
5	76.5±1.02	52.6±1.25	48.4±0.41	65.6±0.36	59.2±0.45	47.6±0.40	68.4±1.25	50.2±0.69	35.2±0.35
6	85.2±1.24	66.2±1.38	53.6±0.48	77.8±0.45	74.6±0.50	48.7±0.49	86.7±1.38	57.9±1.02	46.9±0.49
7	97.3±1.35	73.8±1.44	60.1±0.54	93.2±0.50	88.3±0.57	59.2±0.55	94.2±1.44	69.4±1.23	54.1±0.57
8	99.9±1.44	89.3±1.52	67.9±0.62	100±0.56	96.5±0.64	66.7±0.61	100±1.52	83.7±1.35	61.7±0.62
9	100.0±1.56	99.6±1.68	73.8±0.67	100±0.70	100±0.72	72.1±0.68	100±1.68	97.2±1.46	65.3±0.68
10	100.0±1.69	100.0±1.92	80.5±0.82	100±0.79	100±0.85	78.2±0.83	100±1.92	100±1.67	71.2±0.82

Note: Values are expressed as mean ±SEM (n=6). SEM: Standard Error of Mean; h- hrs

Except for the formulations F3 (80.5±0.82%), F6 (78.2±0.83%), and F9 (71.2±0.82), all other formulations had nearly reached 100% of peak levels after 10 hrs of their *in vitro* release profiles (Table 2). Sodium alginate and sodium CMC gel formulations released their drug content within 8 to 9 hrs, In contrast, carbopol 934 delayed the release of active components for up to 10 hrs (100.0 %), making it appropriate for sustained release and improved clinical compliance. So, formulation F2 was chosen for kinetic studies due to its controlled release activity of up to 10 hrs to other formulations. Results of J-flux and permeability studies show we discovered that F2 formulation obeys zero order kinetics. Zero order kinetics is the gold standard for sustained drug release, so for further *in vivo* study, a gel formulation (F2) containing 1.5% carbopol 934 was chosen.

Skin irritation test

The skin irritation study results indicated that the formulations were safe and devoid of allergic reactions. Numerous investigations have shown that hydrogel enriched with plant extracts and essential oils is safer and non-cytotoxic than commercial ointments due to active metabolites in these components [13].

Wound Contraction studies

The wound healing progress was tracked for 14 days, starting from the initiation of the first day, followed by post-wound treatment assessments on days 3, 7, and 10 culminating in a final wound healing analysis on day 14 (Figure 1 and 2). The rate of wound healing was significant (*p<0.05) in all treatment groups (groups II, III, and IV) compared to control group I. The initial wound size was 20.58±1.79 mm on the first day (day 1), which confirms no wound healing (0 mm^2) in all groups. From day 3 to day 14, group V exhibited notable wound healing compared to the control and other groups (Figure 1). By the 14th day, the wound contraction rates in the herbal gel-treated groups were comparable to the standard (neomycin) and were significantly higher ((56.17±0.79 for Group I, 274.10±0.68 for Group II, 330.67±0.40 for Group III, 328.33±0.83 (***p<0.001) for Group IV (**p<0.01) and 350.97±0.27 for Group V (***p<0.001)) as compared to the negative control (Figures 1 and 2).

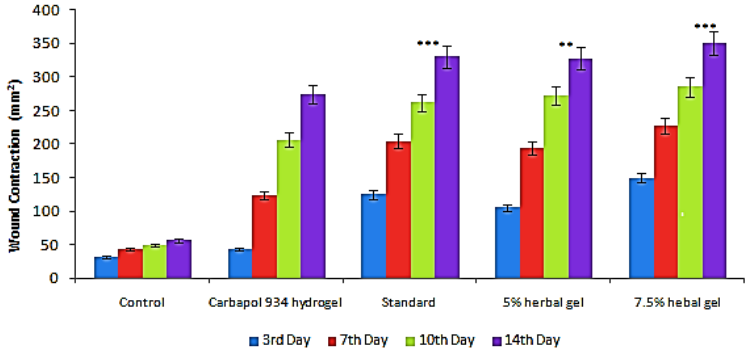


Figure 1. Wound healing activity of various formulations on percentage contraction of excision model in rats (n=6) Control (No treatment), Carbapol 934 hydrogel (Gel only), Standard (neomycin sulfate ointment), Low dose - 5% herbal gel (1.5% carbapol 934 with 5% methanol extract of *C.amada*), High dose- 7.5% herbal gel (1.5% carbapol 934 with 7.5% methanol extract of *C.amada*). Data: Mean ± SEM; *p<0.05 **p<0.01 ***p<0.001, compared to the normal control group (ANOVA test)

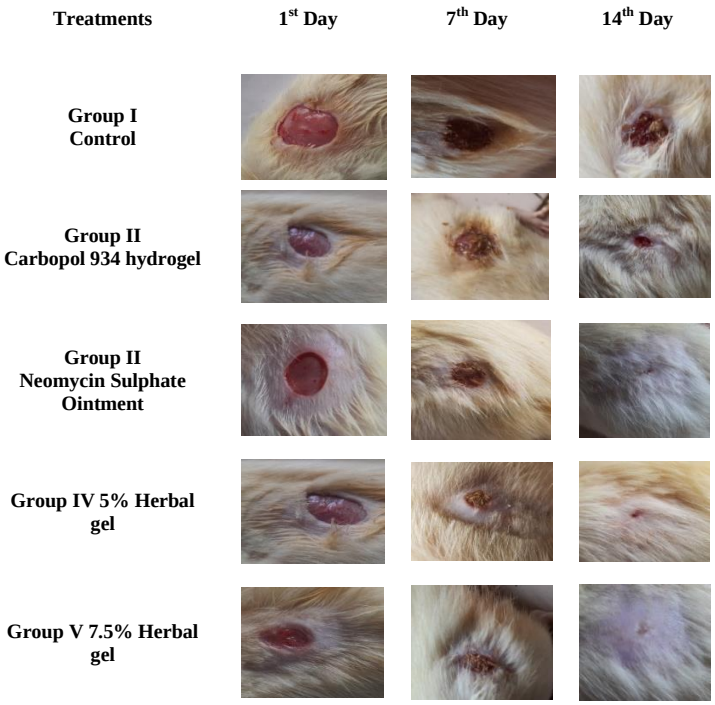
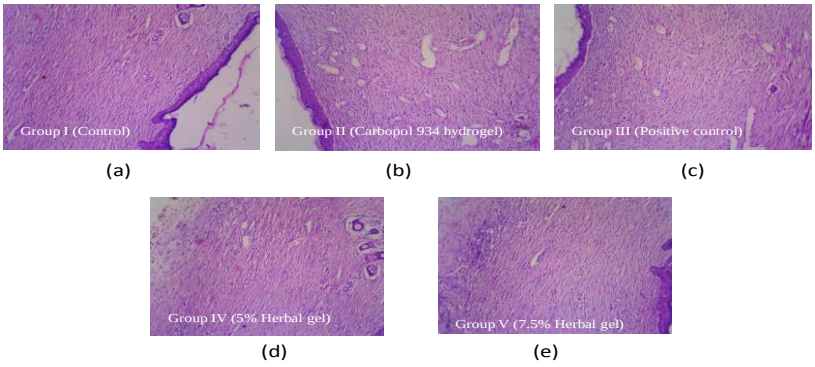


Figure 2. Photographs show the effect of hydrogel on wound contraction at different day intervals (1st, 7th, and 14th days). Group I- Control (No treatment), Group II-Carbapol 934 hydrogel (Gel only), Group III- Standard (neomycin sulfate ointment), Group IV- 5% herbal gel (1.5% carbapol 934 with 5% methanol extract of *C.amada*), Group V- 7.5% herbal gel (1.5% carbapol 934 with 7.5% methanol extract of *C.amada*).

Histopathological study

To confirm the wound healing, a histopathology study was done on the wound tissue. Tissue sectioning was done using a microtome with a section thickness of 5 microns followed by Hematoxylin eosin used for tissue staining. Group I sections showed inflammatory cells, decreased collagen fibers, fibroblast cells, and blood vessels, along with visible scar tissue because of the untreated wound skin. Group II (1.5% carbopol 934 hydrogel) exhibited necrotic cells and fewer collagen fibers and blood vessels. The results indicated that the hydrogel acts as a better vehicle to promote the healing process than untreated groups. Group III demonstrated complete tissue regeneration, evidenced by increased fibroblast cells, collagen fibers, and blood vessels, along with reduced inflammatory cells due to the neomycin sulfate treatment. Sections from group IV treated with

5% herbal gel treated tissue showed reduced cellular necrosis alongside increased collagen fibers and blood vessels. Group V (7.5% herbal gel) exhibited significantly increased fibroblast cells, blood vessels, and well-organized collagen fibers compared to the control group. As a result, the wound treated with 5% and 7.5% herbal gel manifested epithelial tissue proliferation along with keratinization (Figure 3).



Histopathological section of 14th day healed tissue stained with hematoxylin eosin at 40X magnification : (a) Group I - (control) untreated wound tissue (b) Group II - 1.5% of carbapol 934 hydrogel treated tissue (c) Group III- neomycin sulphate commercial drug treated wound tissue (d) Group IV - 5% methanol extract of *C.amada* in 1.5% carbapol 934 herbal gel treated tissue (e) Group V - 7.5% methanol extract of *C.amada* in 1.5% carbapol 934 herbal gel treated tissue.

Figure 3. Photomicrograph of histopathological section of wound tissue of rats.

Acute oral toxicity study

No deaths were observed in any of the experimental groups while administration at a dose rate of 2000 mg kg^{-1}. This finding indicates that the herbal medicine is safe to consume even at the maximum test dose recommended by OECD 425. Therefore, the methanol extract of *C. amada* can be considered entirely non-toxic.

Acute anti-inflammatory activity

The methanol extract of *C. amada* was assessed for its anti-inflammatory activity and compared to a commercial drug (indomethacin) in a carrageenan-induced paw edema model in rats (Figure 4). Anti-inflammatory activity was dose-dependent and found to be statistically significant (***p<0.001) at 200 mg kg^{-1} and equally effective as a standard drug (indomethacin).

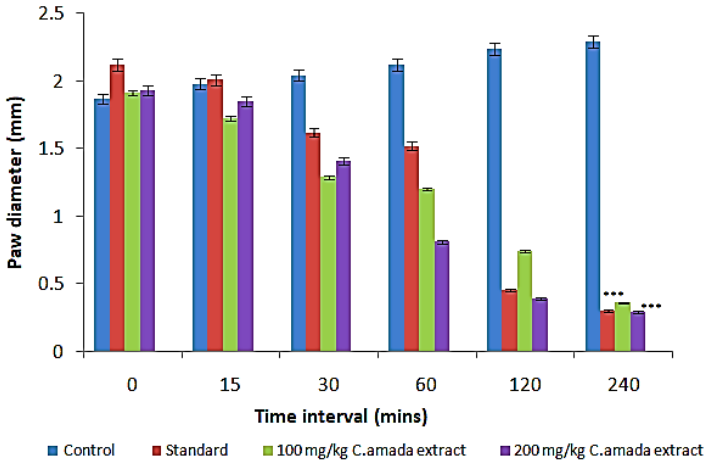


Figure 4. Measurement of hind paws volume at different time intervals after administering of methanol extract of *C. amada* on Wistar albino rat model (n=6). Control (No treatment), Standard (indomethacin), 100 mg kg^{-1} methanol extract of *C.amada* (Low dose), 200 mg kg^{-1} methanol extract of *C.amada* (High dose). Data: Mean ± SEM; *p<0.05, **p<0.01, ***p<0.001, compared to a normal control group (ANOVA test)

DISCUSSION

Historically, herbal medicines have played a significant role in the global public health system. Herbal medicines have gained popularity in the past ten years due to their low cost and lower adverse effect rate than standard dose forms [7]. Ideally, a gel formulation with an herbal base is preferred over other topical semisolid formulations because it has better release characteristics, a longer duration of remaining on the skin, a high viscosity, and a moisturizing effect on dry skin because of its occlusive properties. It also has more bio-adhesiveness and less irritation.

The pH of the formulation was adjusted by the addition of triethanolamine. To maximize medication penetration while lowering the risk of skin harm from DMSO, propylene glycol was selected as a permeation enhancer [21]. There is an inverse relationship between drug release and gel viscosity. Increasing polymer concentrations may decrease drug release because of higher microviscosity [22]. Gel spreadability is vital to their bioavailability, and carbopol 934 has shown promise as a polymer for controlled release. The diffusion studies supported Lauffer's molecular diffusion theory, indicating that higher polymer concentrations resulted in lesser drug release due to decreased diffusion coefficients [23]. Synthetic membranes were utilized for their affordability and data fidelity in the diffusion studies.

According to Emre et al [24], of the two polymers studied, carbopol 934 was shown to have stronger gelling properties than carbopol 940, which is consistent with our study. In the gel formulation, carbopol 934 was a better carrier for releasing active phyto-constituents. After making gels at several concentrations ranging from 0.5 to 2.5%, the percentage of the polymer was adjusted. The overall quality of an herbal extract is determined by the presence or absence of various structurally different chemical components found in plant extracts [25]. Nine different gel formulations (F1 to F9) were prepared using different concentrations (1, 1.5, and 2% w/w) of carbopol 934, sodium alginate, and sodium CMC polymer with 5% methanol extract of *C.amada*.

In pharmaceutical formulations, polymers are essential because they enable rapid drug release, which is necessary to attain and sustain therapeutic concentrations within the intended range. There was no change in viscosity in any of the gel compositions because the polymer concentration was kept constant at 1.5%. It is known that the optimal viscosity for topical gel formulation made with carbopol polymers is between 0.38 and 0.39 poise. Gels containing 1.5% of carbopol (934 or 940) were compatible with the specifications of gel formulations [26]. All the formulations tested in this study were found to have good viscosity and spreadability. The observed drug content was varied from 98.14% to 102.64% in nine formulations (F1 to F9). Satisfactory drug content was achieved among different formulations where it ranged from 98.11% to 101.7% [27].

In vitro studies showed formulation F2 (1.5% of carbopol 934 containing 5% methanol extract of *C.amada*) was superior in terms of having better rheological characteristics. Over the years, *in-vitro* release testing has been commonly utilized to ensure the consistency of a product. As such, the test might be applied to assess the *in vivo* efficacy of a topical treatment. The correlation between THP's percutaneous penetration, rheological properties, and *in vitro* release was the subject of a study by Li et al [28] that included similar observations for emulgel formulations. This understanding could yield important insights and facilitate the development of customized hydrogels.

For *in vivo* studies, low and high doses of the plant extract were used to assess its ability to heal. For topical application, a low dose of *Bauhinia purpurea* (BP) of 2.5% (w/w) and a high dose of BP 5% (w/w) were utilized [29]. Since the formulation containing 1.5% carbopol with 5% methanol extract of *C.amada* was found to be a potential vehicle for herbal gel formulation, we used a slightly higher dose of 7.5% methanol extract of *C.amada* was added to 1.5% of carbopol 934 and act as a high dose formulation for *in vivo* studies. The pH of this formulation was 6.21, viscosity was 65 $cPs \times 10^3$, spreadability was 25 $gm \times cmsec^{-1}$, drug content was 100.58%, and 100% drug release was achieved on 10^{th} hrs.

All the experimental groups showed notable wound contraction on the 15th day ($p<0.05$). Particularly the animals treated with hydromethanolic *Dioscorea bulbifera* extract (group III) revealed significant wound contraction (87.21%) compared to the negative control group [30]. The efficiency of the herbal gel in accelerating wound healing was proven *in vivo* tests on rats, especially in the 7.5% *C.amada* extract formulation. Re-epithelialization, granulation tissue regeneration, and modifications to cell membranes are all part of the wound healing process. Neomycin with green synthesized AgNP/ZnONP formulated with carbopol 934, has optimal consistency, viscosity, and spreadability along with desirable thixotropic behavior and accelerates wound healing effects in rats. In addition to the above features, this formulation also promotes growth factors, fibroblast proliferation, angiogenesis, and the synthesis of collagen, hyaluronic acid, and dermatan sulfate components of the extracellular matrix-the plant plays a therapeutic role by increasing the number of cross-links between the collagen molecules in the skin, bone fractures, and gastric lesions [31].

In oral acute toxicity tests, 2000 mg kg^{-1} doses of *C.amada* methanol extract did not exhibit any harmful effects in rats. The ethanol extract of *C.amada* is not toxic to albino rats up to a dose of 1 g kg^{-1}, according to research reports published by Mujumdar et al [32]. Rajasekaran et al [33] conducted a thorough investigation on the acute and sub-chronic toxicity and found that the extracts of *Cardiospermum halicacabum L* and *Vitex negundo* were safe up to 2000 mg kg^{-1}. In an acute carrageenan-induced rat paw edema model, a recent study [26] showed that the inflammation was gradually reduced at 200 mg kg^{-1} concentration, and ethanol extract of *C.amada's* demonstrated significant anti-inflammatory at 200 mg kg^{-1}. According to these findings, mango ginger therapy is also useful in the treating rheumatoid arthritis by inhibiting the osteoclastic and inflammatory processes [34]. These data highlight the potential health advantages of extract from *C.amada*, demonstrating encouraging outcomes in wound healing and anti-inflammatory properties.

CONCLUSIONS

The study concludes that the herbal gel formulation containing 1.5% carbopol 934 and 7.5% *C.amada* extract with oregano essential oil, demonstrates significant wound healing and anti-inflammatory properties, outperforming the standard drug used for comparison. This efficacy is attributed to the gel's sustained drug release, high drug content, optimal viscosity, and excellent spreadability. The formulation proved highly effective in promoting wound contraction and epithelialization in rat models, suggesting its potential for similar benefits in humans. Additionally, the *C.amada* extract showed dose-dependent anti-inflammatory activity, positioning it as a promising natural remedy with minimal adverse effects.

CONFLICT OF INTEREST

We declare that we do not have any associative interest that represents a conflict of interest in this work.

Funding source

This work was supported by the Department of Biotechnology (DBT) -Competitive Research Grant Project under Sanction order Number BT/PR32521/TRM/120/246/2019 Letter dated 30.10.2019.

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