Research Article

Synergistic Effect of *Murraya koenigii, Carica papaya* and *Lawsonia inermis* in Wound Healing of Excision Wounds in Rats

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ABSTRACT

Historically, several illnesses have been treated with herbal plants. In the healthcare system, medicinal plants are frequently utilized either as a single medication or in combination. A polyherbal formulation provides numerous benefits that are not present in a single herbal formulation due to synergistic effects. A single multi-constituent formulation can clearly produce a better therapeutic impact. In this study, *Murraya koenigii, Carica papaya*, and *Lawsonia inermis* leaf extracts were used to form a polyherbal formulation that was intended to be a remedy. These three natural agents induce the healing and regeneration of lost tissue. By using an excision wound model in albino rats, the potency for wound healing was evaluated in polyherbal formulations. The potency of the polyherbal ointment, which was made in two formulations: F1(2%) and F2(4%) W/W was compared with the potency of standard povidone-iodine ointment. Because of the synergism of the phytochemicals present in these plant extracts, polyherbal formulations have shown impressive wound-healing characteristics, which may encourage the creation of future polyherbal formulations for the treatment of wounds.

Keywords: Wound healing, excision wound model, poly herbal formulation, epithelialization, wound closure.

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INTRODUCTION

Plants have served as an essential ingredient in medicines, flavors, beverages, cosmetics, and dyes. They have also made a significant contribution in enhancing human life quality and preserving human health. The development of herbal medicine was based on the idea that it would promote health and treat sickness. Recent years have seen a considerable increase in the amount of data gathered to show the immense potential of medicinal plants employed in traditional systems, mostly due to growing global interest in plant studies.[1]

Currently, we are witnessing a significant level of public interest in the utilization of herbal treatments. Moreover, plant extracts are the source of several western medications. They are capable of having a major therapeutic impact and can be used as drugs or dietary supplements to treat or manage a variety of disorders. Medicinal plants and herbal medicines have demonstrated a variety of biological activities in their extracts and isolated components. These have historically been used as traditional medicine or as food supplements to treat a wide range of diseases. Ethnopharmacological studies on such herbs, as well as medicinally important plants, continue to attract the interest of researchers from all over the world.[1]

Presently, there are numerous cutting-edge therapies for healing wounds, but the majority of them are expensive, and some even has adverse effects including allergic reactions. Herbal products are preferred widely because they are thought to be safer, and many herbs rich in nutrition value have been proven to heal sever wounds without much adverse effects. However, the lack of standardization procedures has made determining the true efficacy of these products more difficult, which necessitating more in-depth research.[1]

A wound can be described as a rupturing of skin's epithelial integrity or as a disruption of cellular, anatomical, or functional integrity of living tissue. The wound healing society describes that, a wound is any physical injury that breaks or opens the skin and alters the normal structure and function of the skin. They cause the epithelium to lose its continuity, either with or without underlying connective tissue loss. The complex process contains four stages, and they are as follows: hemostasis, inflammatory response, cell growth, tissue remodeling, and lastly, the healing of a wound.[2]

Murraya koenigii Linn, commonly known as a curry leaf, is a member of the *Rutaceae* family, which includes more than 150 genera and 1600 species.[3] It is a small tropical or subtropical tree or shrub that normally reaches heights of 6 to 15 feet. It is well known for its aromatic, spicy curry leaves, which are key ingredients in Indian and Asian

cuisine.[4] According the various reports, this plant exhibits, anti-oxidant, cytotoxic, antimicrobial, antibacterial, antibacterial, anti-ulcer, positive inotropic and cholesterol-lowering properties. In diabetic rats, leaves were also found to have significant wound healing properties.[5]

Carica papaya Linn, a member of the Caricaceae family, is grown all over the world such as in India and Sri Lanka.[6] *Carica papaya's* leaves have the ability to treat wounds and inhibit the growth of bacteria. Papaya leaves contain compounds that promote wound healing, including flavonoids, phenolics, papain enzymes, saponins, and chymopapain. In the process of healing a wound, flavonoids also have anti-inflammatory and antioxidant activities.[7]

Lawsonia inermis Linn is a widely branched, glabrous shrub with height of 2–6 meters, that belongs to the Lythraceae family. It's leaves, flowers, bark, roots, stem and seeds are used in traditional medicine.[8] This plant is popularly known as henna and is widely available in tropical and subtropical areas. The chemical components of this extract include naphthalene derivatives, quinoids, beta-sitosterol, flavonoids, and gallic acid. It has been used as an antibacterial, antidiarrheal, anti-inflammatory, and analgesic; it also has an antipyretic effect.[9] Burns, skin inflammations, wounds, and ulcers can all be treated with henna in the form of an ointment or decoction.[10]

Murraya koenigii, Carica papaya, and *Lawsonia inermis* leaf extracts are rich in alkaloids, carotenoids, essential oils, flavonoids, saponins, tannins and terpenes which give these plants good antioxidant potential. The goal of the current study was to investigate the potential effects of these ethanolic extracts. These plant materials were chosen for the creation of a newer polyherbal formulation in this investigation due to their various pharmacological properties and ease of availability.

Many of the wound-healing ointments on the market have negative side effects of one kind or another. The goal of this study is to create a novel polyherbal formulation with improved safety and efficacy that will heal wounds most effectively. The herbal remedy will lessen negative effects and effectively treat the wound. The active phytochemical components of particular plants have been thoroughly documented, although they are typically present in very little amounts and are never enough to produce the desired therapeutic effects. A polyherbal formulation gives several advantages not present in a single herbal formulation due to synergism. It is clear that a single multi-constituent formulation can achieve a superior therapeutic impact.

MATERIALS AND METHODS

Drugs and Chemicals

Povidone-Iodine (5%W/W) ointment, hard paraffin, cetostearyl alcohol, white soft paraffin, Ethanol, wool fat, surgical Spirit and Chloroform are the reagents which are used in the present study.

Collection of Plant Materials

All three plant leaves were cleaned with distilled water before being allowed to air dry for around two weeks in the shade. Each of the three plants' dried leaves was ground into a fine powder before being utilized in the extraction process. The Pilikula Development Authority examined the leaves and verified their authenticity.

Preparation of Leaf Extracts

Simple maceration procedure was selected for extraction. About 100 grams of each powdered leaves of *Murraya koenigii*, *Carica papaya* and *Lawsonia inermis* were kept for maceration by dissolving the samples with 500ml of absolute ethanol individually for about 48 hours with manual intermediate stirring. The macerate was filtered with Whatman filter paper, and the filtrate was kept for evaporation to obtain semi solid extracts.[5,7,11]

Preliminary qualitative phytochemical investigation

The ethanolic extracts of leaves of *Murraya koenigii, Carica papaya* and *Lawsonia inermis* was subjected to preliminary phytochemical investigations to check out the active components present in it. The tests were carried out based on standard methods.[11]

Formulation of Polyherbal Herbal Ointment

The 2% and 4% polyherbal ointment formulations were prepared by using specific ointment base formula proportion which is described in British Pharmacopeia. Hard paraffin, cetostearyl alcohol, white soft paraffin, and wool fat are the ingredients used in the preparation of ointment base. An electronic weighing balance was used to weigh all of the ingredients. Hard paraffin was first melted in the China dish over a water bath. The remaining ingredients were added in descending order of melting point until everything was melted (the order being cetostearyl alcohol, then wool fat and lastly white soft paraffin).

Table 1: Formula for poly herbal ointment

Sl. No.	Formulation Ingredients		F1(2%) (grams)	F2 (4%) (grams)
1.	Hard paraffin		5	5
2.	Cetostearyl alcohol		5	5
3.	White soft paraffin		79	73
4.	Wool fat		5	5
5.	Ethanolic extract	*Murraya koenigii*	2	4
		Carica papaya	2	4
		Lawsonia inermis	2	4

To ensure homogeneity, the mixture was constantly stirred. The polyherbal ointment formulations containing F1-2% and F2-4% strengths of ethanolic extracts and they were prepared by using 94 grams and 88 grams of simple

ointment base and then adding 6g (each of 2g *of Murraya koenigii, Carica papaya* and *Lawsonia inermis* ethanolic leaf extract) and 12g (each of 4g of *Murraya koenigii, Carica papaya* and *Lawsonia inermis* ethanolic leaf extract) of plant extract respectively. The formula for the preparation of the polyherbal ointment and the concentrations of various ingredients were given below in table 1.[12]

Physicochemical parameters evaluation for polyherbal ointments

Color and odor: Color and odor of all ointments was visually examined.

pH: 1gm of ointment was dissolved in 100ml water and pH was determined by using pH paper.

Texture and traces of gritty particles: small quantity of ointment was rubbed between 2 fingers.

Skin irritancy test: small quantity of ointment was rubbed onto the skin.[12]

Selection of animals

Rats of either sex, about 4-6 weeks of age, weighing (180-200gms) were collected from the central animal house. The rats were randomly grouped and then sheltered in distinct cages. The cages were kept under standard lab conditions of temperature 25 ± 2c° with appropriate dark and light cycle of 12 hours animals were freely accessed for standard food and water.[13]

Experimental Design for Excision Wound Model

Twenty Wister albino rats were divided into four groups. The rats were anaesthetized with chloroform before having excision wounds made on them. The fur on each animal's dorsal side (below the rib cage) was removed with a hair removal cream, and the surface was disinfected by wiping with a sterile disinfectant. The wound was then inflicted on the hair-free surface by incising the skin flap aseptically with a sterile surgical knife, scissors, and forceps. Each injured rat was housed in its own sterile polypropylene cage. Throughout the experiment, proper aseptic conditions were maintained. On post-wounding days 0, 3, 5, 7, 9, 12, 14, and 16, the wound was traced with mm2 graph paper to determine the epithelialization time and percentage of wound closure. The polyherbal ointments (F1 & F2) are applied twice daily to the wound surface. The animals in the control group are only given ointment base, whereas the standard group is treated with Povidone-Iodine (5%W/W) ointment.[13]

Percentage wound closure was determined by using the formula:

$$\% \text{ Wound closure} = \frac{\text{wound area on day 0} - \text{wound area on treatment day}}{\text{Wound area on day 0}} \times 100$$

Group I (control group): The wound of the animals was treated with only ointment base till 16th days of post wounding.

Group ll (standard group): The wound of the rats was treated with povidone- iodine ointment (5% w/w) till 16th days.

Group III (Test-1): The wound of the rat was treated with 2% poly herbal ointment formulation by applying twice a day for 16 days.

Group lV (Test-2): The wounds of the rat were treated with 4% poly herbal ointment formulation by applying twice a day for 16 days.

Statistical analysis

Results obtained were analyzed by using one-way ANOVA method followed by Dunnett's test. All the results were expressed as the mean ± standard error of the mean (SEM). All the treatment groups were compared with control. P values are less than 0.005 was considered as statistically significant.

RESULTS AND DISCUSSION

Percentage yield of ethanolic extracts

30 grams of ethanolic extract was obtained from the maceration of 300 grams of fine powder of the leaves of *Murraya koenigii, Carica papaya* and *Lawsonia* inermis by using ethanol as a solvent. Accordingly, the percentage yield of the ethanolic extract was 9.09%. The extract was in dark green color with semi solid consistency.

Preliminary phytochemical analysis of ethanolic extracts of *Murraya koenigii, Carica papaya* and *Lawsonia inermis*

The preliminary phytochemical analysis of combination of ethanolic leaf extracts of *Murraya koenigii, Carica papaya* and *Lawsonia inermis* indicated the presence of alkaloids, carbohydrates, flavonoids, tannins, proteins and saponins.

Physicochemical evaluation of the polyherbal ointment preparations

The physicochemical evaluation of the polyherbal ointment preparation was evaluated for different parameters like color, odour, nature, texture, traces of gritty particles, skin irritancy. The polyherbal ointment formulation was observed with yellowish cream, semisolid, greasy compound with characteristic odour. No traces of gritty particles were observed, and did not cause any skin irritation when applied locally and the pH of the poly herbal ointments were found to be 6.5.

Results analysis in Excision wound model

The above polyherbal formulations resulted in dose dependent significant increase in rate of wound healing when compared with standard group. The groups of animals treated with the 4% polyherbal formulation have shown almost comparable results as that of standard (5% Povidone- iodine ointment) treated group. However, both doses of polyherbal formulations and standard treated groups are statistically significant (p) when compared to the control. On day 0 the wound size was around 3.05 cm in all the groups of animals. Effect of polyherbal ointments

have been evolved by measuring wound size on 0,3,5,7,9,12,14 and 16 days respectively. The observations of wound area size during post wound days were given in table 2. Wound area size of different treatment groups from day 0 to day 16 was depicted in figure 1.

In the excision wound model, the wound area size values are analyzed by one way ANOVA method followed by Dunnett's test and results were expressed as Mean ± SD, where n=5 all the treatment groups results were significant **** p<0.0001 when compared with the control.

The phytochemicals present in the leaf extracts of plants like alkaloids, glycosides, flavonoids and tannins may have various effect on wound healing physiology. The polyherbal ointments (F1and F2) have exhibited significant wound healing activity on day 3, 5, 7, 9,12, 14 and 16. There is substantial decrease in wound length in rats treated with F1 and F2 polyherbal ointment when compare to rats treated with Povidone – Iodine (5%w/w) which was considered as standard. 0n the treatment day of 16th the percentage wound closure in F1 polyherbal ointment treated group was 93.80%, F2 polyherbal ointment treated group was 100% and, in the standard drug (Povidone-Iodine 5%) treated group was 100%. The % wound closure in the various treatment groups on the post wounding day 16 was given in table 3. Graphical representation of percentage wound closure in various treatment groups on the day of 16th were depicted in figure 2.

Table 2: Epithelialization and size of wound area in animal of different treatment groups during post wounding days.

Wound area size (in cm) during post wounding days				
Day	**Treatment groups**			
	Group-I Control	**Group-II Standard**	**Group-III F1 (2%)**	**Group-IV F2(4%)**
0th	3.16±0.015	3.09±0.035****	3.22±0.011****	3.22±0.022****
3rd	2.78±0.023	2.26±0.016****	2.56±0.038****	2.48±0.054****
5th	2.66±0.024	2.15±0.02****	2.43±0.023****	2.19±0.028****
7th	2.50±0.019	1.97±0.017****	2.20±0.031****	1.78±0.023****
9th	2.39±0.014	1.53±0.056****	1.8±0.0312****	1.37±0.022****
12th	2.24±0.016	1.24±0.047****	1.60±0.038****	1.07±0.047****
14th	2.11±0.027	0.68±0.091****	0.7±0.031****	0.39±0.030****
16th	1.35±0.063	0.17±0.049****	0.37±0.043****	0.17±0.049****

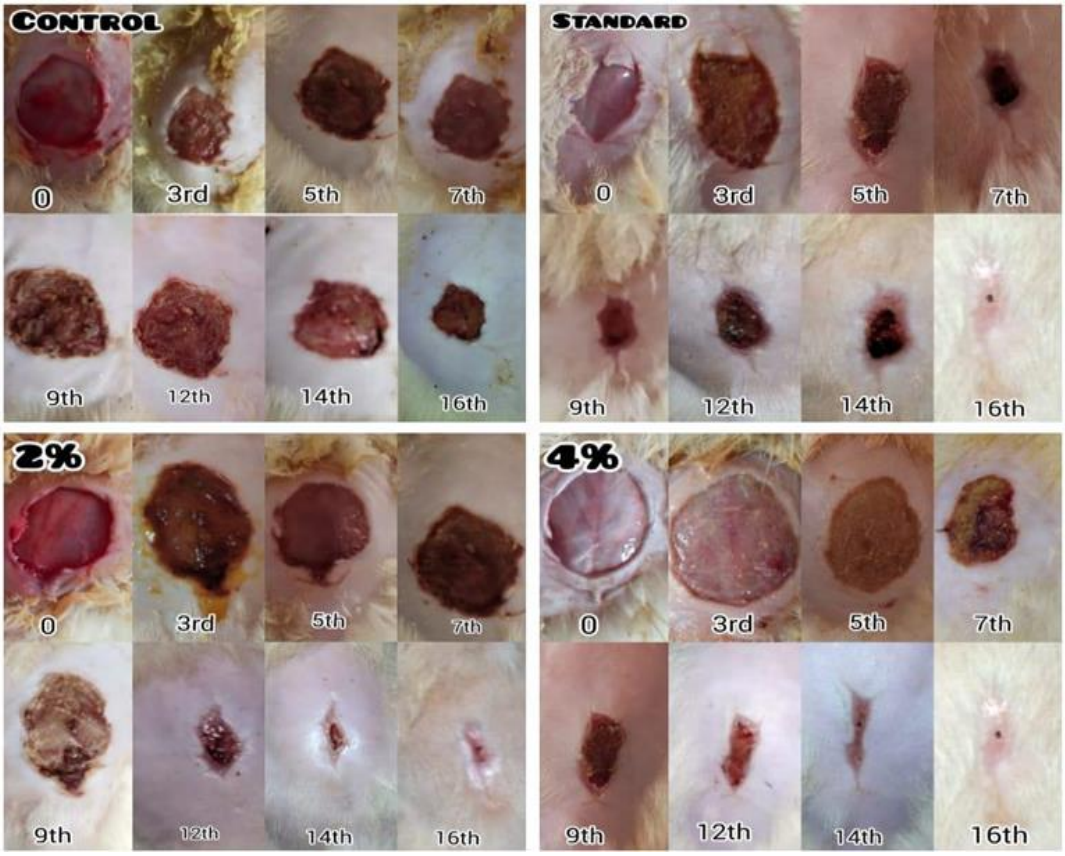

Figure 1: Observation of wound healing activity in various treatment groups during post wound days

Table 3: % wound closure in various treatment groups on post wounding day 16

Animals	Control	Standard	F1(2%)	F2(4%)
Animal I	49.68%	100%****	93.80%****	100%****
Animal II	55.55%	93.54%****	87.5%****	90.15%****
Animal III	62.5%	89.33%****	86.15%****	96.96%****
Animal IV	58.06%	92.69%****	89.32%****	92.81%****
Animal V	60.56%	96.66%****	85.44%****	93.65%****

In the excision wound model, the wound size values are expressed as %wound closure, where n=5, all the treatment groups have shown significant (**** p<0.0001) results when compared to control by using one way ANOVA method.

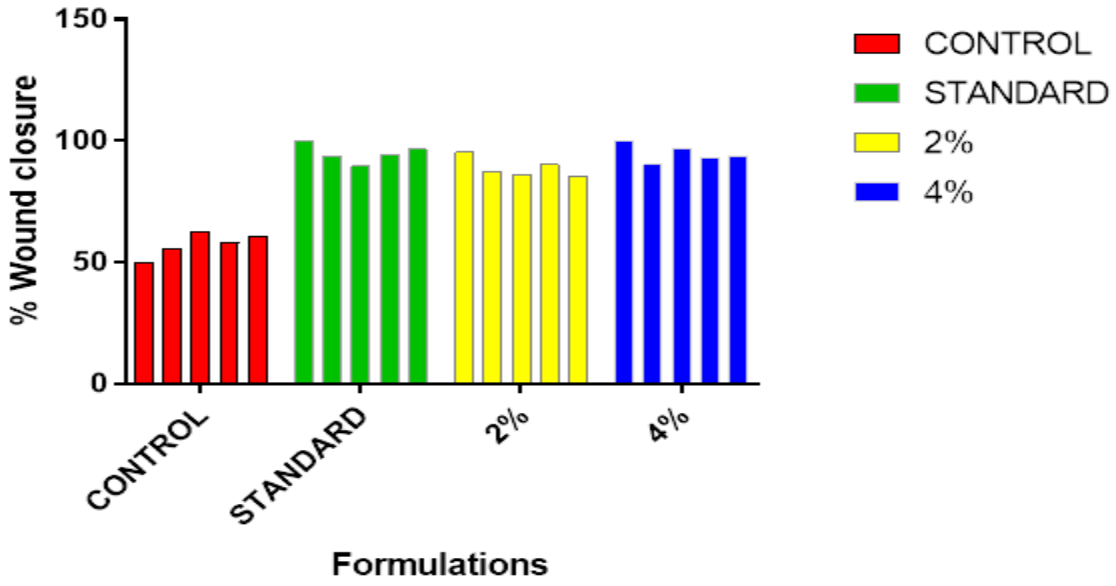

Figure 2: Graphical representation of % wound closure in various treatment groups on post wounding day 16

CONCLUSIONS

Polyherbal formulation is a type of formulation that combines multiple plant extracts in a single dosage form and is beneficial because it has additive or synergistic effects on the healing process. Based on the foregoing, we conclude that 4% polyherbal formulation (F2) has demonstrated superior wound healing activity when compared to 2% polyherbal formulation (F1), which is comparable to standard (Povidone-Iodine 5% w/w). Polyherbal formulation's wound healing efficacy can be attributed primarily to its anti-inflammatory, antioxidant, antibacterial, and free radical scavenging properties. Based on the findings, we can conclude that our polyherbal formulation is safe, has no side effects, and has a high rate of healing in the case of severe deep wounds, and it can be used in traditional medicine for the management of various kinds of severe life-threatening wound conditions.

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