Research Article

Phytochemical Investigation and Pharmacological Evaluation of *Plumeria rubra* and *Murraya koenigii* L. Leaves Extracts for Wound Healing Activity

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ABSTRACT

The study focuses on the therapeutic potential of Plumeria Rubra and *Murraya koenigii* L. leaf extracts due to their rich phytochemical composition. These natural extracts serve as alternatives to synthetic medications, which may be costly and ineffective. The investigation entails the assessment of their wound healing and antimicrobial properties through various extracts: petroleum ether, acetone, and methanol. Preliminary phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, tannins, saponins, alkaloids, and phenolic compounds, all known for their antioxidant, anti-inflammatory, and antimicrobial effects. The methanol extract exhibited the highest total phenolic and flavonoid content when expressed as gallic acid and quercetin equivalents, respectively. In vitro antioxidant activity, assessed via DPPH assay, indicated superior antioxidant potential in the methanolic extract compared to the other two extracts, with ascorbic acid as a standard. Antimicrobial efficacy was tested using the agar and cup plate method against bacterial cultures, revealing that both acetone and methanol extracts significantly inhibited bacterial growth when compared to Amoxicillin. Additionally, wound healing activities of acetone and methanol extracts were evaluated in rats using an excision wound model, with Betadine as a control. Both extracts demonstrated notable wound healing activities, especially the acetone extract, which showed significant results compared to the control group. These findings suggest that Plumeria Rubra and *Murraya koenigii* L. may provide bioactive natural compounds for future pharmaceutical applications.

Keywords: Plumeria Rubra and *Murraya koenigii* L. Phytochemical screening, Wound healing activity, antimicrobial.

INTRODUCTION

The main aim of the study is to conduct a phytochemical investigation and pharmacological evaluation of *Plumeria rubra* and *Murraya koenigii l.* Leaf extracts for wound healing activity.

Wounds are defined as injuries that result in a loss or disruption of the cellular, anatomical, or functional continuity of living tissue, particularly involving the skin. They can be caused by various agents, including physical, chemical, or microbial factors, and lead to disturbances in normal skin anatomy and function. The primary objective of wound healing is to restore the anatomical structure and physiological functions of the affected area. This healing process involves tissue regeneration and consists of several stages: initial bleeding, coagulation, an acute inflammatory response, and the subsequent renewal and movement of connective tissue and parenchyma cells. During healing, extracellular matrix proteins are produced along with new parenchyma, connective tissue, and collagen, facilitating the repair of the injury to the skin and soft tissues. Healing is the body's response to tissue injury, aiming to restore normal structure and function. This process involves two distinct mechanisms: regeneration, in which parenchymal cells proliferate, leading to complete tissue restoration, and repair, in which connective tissue elements proliferate, resulting in fibrosis and scarring.[1,2]

Plumeria rubra L., or frangipani, is a tropical flowering plant known for its fragrant blooms and ornamental value. Leaves, flowers, stem bark, roots, and latex of Plumeria rubra exhibit various medicinal properties. Leaves are anti-inflammatory, analgesic, and antimicrobial, used for fever, wounds, skin infections, and rheumatic pain. Flowers have antioxidant and mild sedative effects, utilized for stress, fever, cough, and cosmetics. Stem bark shows antidiarrheal and anti-inflammatory properties, applied for diarrhea, dysentery, and toothache. Roots have antipyretic and purgative actions, used in fever and constipation. Latex is antimicrobial and caustic, used externally for warts and corns.[3,4,5] *Murraya koenigii* L., or curry leaf tree, is a small evergreen native to tropical India and Sri Lanka, valued for its aromatic culinary and medicinal leaves. Antioxidant, antidiabetic, antimicrobial, anti-inflammatory, hepatoprotective, neuroprotective, and hypolipidemic effects can be found in leaves; antimicrobial, analgesic, anti-inflammatory, and astringent properties can be found in bark; antioxidant, antidiarrheal, and antimicrobial activities can be found in fruits; and antimicrobial and insecticidal effects can be found in seeds.[6,7]

MATERIALS AND METHODS

The plants Plumeria rubra and *Murraya koenigii* are collected from Pune, Maharashtra, and identified at the Vidya Niketan College of Pharmacy, Lakhewadi. These plants were authenticated at the Shankarrao Mohite Mahavidyalaya, Akluj. From Dr. S.M. Satpute (HOD – Botany Department).

1. **Processing of crude drug**: The fresh leaves of the plants Plumeria Rubra and *Murraya koenigii* L. were subjected to shade drying and further crushed to coarse powder, and then the powder was passed through mesh no. 14 and stored in an airtight container for further use.

   The dried leaves of the plant were used to determine physicochemical parameters such as total ash, acid-insoluble ash, water-soluble ash, and moisture content (LOD).

2. **Preparation of Extracts:** Three extracts of powder of Plumeria Rubra and *Murraya koenigii* L. leaves were prepared. Petroleum ether, Acetone extract, Methanol extract

   a) **Preparation of Petroleum Ether Extract**: The coarse powder material was extracted with petroleum ether by the continuous hot extraction method in a Soxhlet apparatus for defatting. Petroleum ether extract of powdered leaves of Plumeria Rubra and *Murraya koenigii* L. was prepared in a Soxhlet extractor according to the standard method till colorless solution was observed in the siphon tube. 240 gm of the powder and 1000 ml petroleum ether were used for extraction. After completion of the extraction, the extract was cooled and dried. The extract is stored in an airtight container till use. The percentage yield of the extract was calculated.

   b) **Preparation of Acetone extract**: Acetone extract of powdered Plumeria Rubra and *Murraya koenigii* L leaves was prepared in a Soxhlet extractor according to the standard method till colorless solution was observed in the siphon tube. 236 gm of the powdered husk and 1000 ml acetone were used for extraction. After completion of the extraction, the solvent was cooled and dried. The extract was stored in an airtight container till use. The percentage yield of the extract was calculated.

   c) **Preparation of Methanol extract**: Methanolic extract of powdered pods was prepared in a Soxhlet extractor according to the standard method till colorless solution was observed in the siphon tube. 234 g of the powder and 1000ml Methanol was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in an airtight container till use. Percentage yield of extract was calculated.

$$\%\ \text{yield} = \frac{\text{Wt. of extract}}{\text{Wt. of powdered drug}} \times 100$$

3. **Preparation of Ointment by fusion method**:[8,9]

   a) **Preparation of simple ointment (100 g)**: Wool fat- 5 g, hard paraffin- 5 g, cetostearyl alcohol- 5 g, and white soft paraffin-85g. Each ingredient was mixed and heated gently with stirring, then cooled. The base was packed in a wide-mouth container.

   b) **Preparation of 1% Acetone extract ointment (100 g):** 1 g of acetone leaves extract of Plumeria Rubra and *Murraya koenigii* L was added slowly to the 99 g of ointment base and stirred thoroughly until the mass cooled down and a homogeneous product was formed & the ointment was packed in a wide-mouth container.

   c) **Preparation of 2% Acetone extract ointment (100 g):** 2 g of acetone leaves extract of Plumeria Rubra and *Murraya koenigii* L. was added slowly to the 98 g of ointment base and stirred thoroughly until the mass cooled down and a homogeneous product was formed & the ointment was packed in a wide-mouth container.

   d) **Preparation of 1% Methanolic extract ointment (100 g**): 1 g of Methanolic leaves extract of Plumeria Rubra and *Murraya koenigii* L. was added slowly to the 99 g of ointment base and stirred thoroughly until the mass cooled down and a homogeneous product was formed & the ointment was packed in a wide-mouth container.

   e) **Preparation of 2% Methanolic extract ointment (100 g):** 2 g of Methanolic leaves extract of Plumeria Rubra and *Murraya koenigii* L. was added slowly to the 98 g of ointment base and stirred thoroughly until the mass cooled down and a homogeneous product was formed, & the ointment was packed in a wide-mouth container**.**

4. **Animal used**:

For the study Wistar rats of either sex, of weight 150-200gm were selected.

Test group: For the study, six groups of animals were made. Each group having six rats.

Route of administration: Topical

Housing condition: Animals were housed in six groups in separate cages under controlled temperature conditions (22±2 Celsius). All animals were given standard diet (golden feed, New Delhi) and water regularly. Animals were randomly divided into six treatment groups, each consisting of three rats.

IAEC Approval: Wistar rats of either sex weighing 150 to 200 g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house of Nanded Pharmacy College, which is approved by the committee for the purpose of control and supervision of experiments on animals (CPCSEA) Protocol. Animals were kept under 12 h light/dark cycles and controlled temperature (24 ± 2°C) and fed with a commercial pellet diet and water ad libitum. All animals were acclimatized to the laboratory environment for at least one week before the commencement of the experiment. The experimental protocol for the study was followed according to the norms of the Institutional Animal Ethics Committee.

5. ***In-vivo* studies Wound Healing Activity**:

A wound is described as the disruption of the cellular and anatomic discontinuities of a tissue wound. A tissue wound

may be generated by a chemical, physical, thermal, microbiological, or immunological insult. Some illnesses, including diabetes, ischemia, malnutrition, local infections, and local tissue damage from burns, cause a delay in the healing of wounds.

Excision wound model: Excision wound model was the 21 days study and the animals were anaesthetized by using ketamine. With the help of Veet (hair-removing cream) hairs on the dorsal thoracic region of rats were removed. The excision wound was created 5cm away from ear, a circular wound of approximately 2cm2 area and 2 mm in depth was excised on the depilated dorsal thoracic region of the excised rats. The entire wound was left open. The treatment was done topically in all the cases. The wounds were measured by scale, and wounds Area measured by day-to-day progress for all groups and observed for complete healing of the wound.[10]

Grouping of animals:

Animals were divided into six groups, each consisting of six rats.

Group-I: Treated with plain ointment base (Positive control)

Group-II: Treated with the standard drug, i.e., Betadine

Group-III: Treated with 1% Acetone extract ointment (CSAE 1%)

Group-IV: Treated with 2% Acetone extract ointment (CSAE 2%)

Group-V: Treated with 1% Methanolic extract ointment (CSME 1%)

Group-VI: Treated with 2% Methanolic extract ointment (CSME2%)

Wistar rats (150- 200 g) of around two months old were used in this experiment, divided into six groups: control, standard drug-treated, and four extract-treated groups. The rats were housed under standard conditions with a 12-hour light/dark cycle and provided with a standard pellet diet and water ad libitum. After anesthesia and depilation of back hair, a 5 cm circular excision wound (2 cm^2 area, 2 mm depth) was created. The wounds were left open, and topical applications of extract and vehicle ointments were administered. Healing was monitored via camera every other day, concluding with the calculation of the wound area at treatment's end.

Statistical Analysis: Presentation of results was done in tabular form. All results are expressed as Mean ± Standard Error. The results were expressed as mean ± S.E.M. Data was analyzed by one-way ANOVA test.

RESULTS AND DISCUSSION

Determination of Total Ash Content

Determination of Total Ash Content of *Plumeria Rubra* and *Murraya koenigii L.* Leaves Powder

Weight of empty crucible (g)	- 14.82
Weight of crucible + air-dried material (g)	- 16.82
Weight of crucible + ash (g)	- 15.00
Weight of total ash (g)	- 0.18
Total ash value (%w/w)	- **9%**

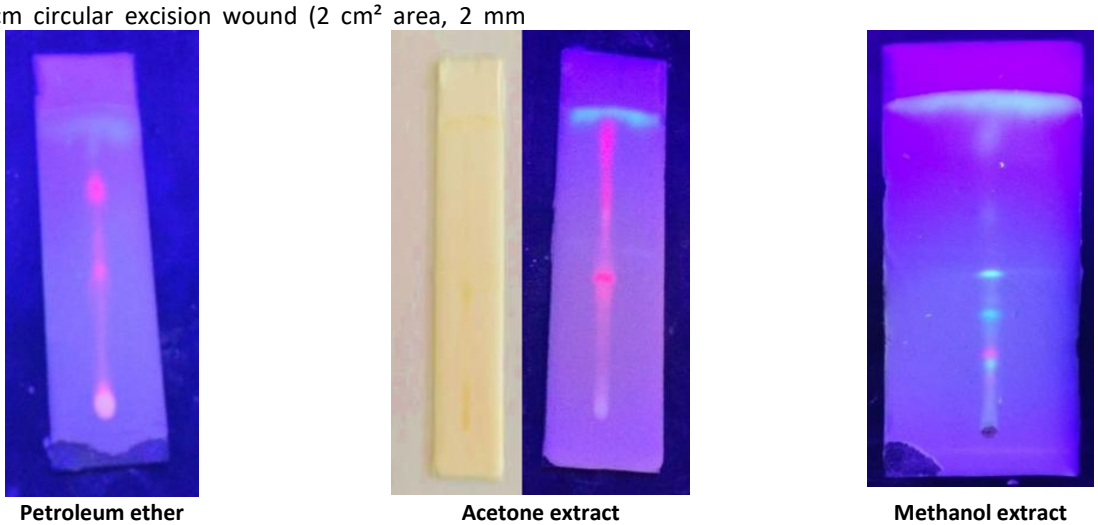
The total ash content of *Plumeria Rubra* and *Murraya koenigii L.* Leaves powder was found to be 9%.

1. Phytochemical evaluation of Plumeria Rubra and *Murraya koenigii* L. Leaves extract

Above observations table 1 reveals that Petroleum ether, acetone and methanolic extracts have percentage yield respectively. Highest yield was obtained for Methanolic extract.

2. Phytochemical tests of Plumeria Rubra and *Murraya koenigii* L. Leaves extract

The above observation in table 2 shows the presence of phytoconstituents in the extracts. It reveals all three (i.e., Pet ether, Acetone and Methanolic) extracts contains Carbohydrates, Glycosides, Tannins, Alkaloids, Amino acid, Protein, Steroid and Flavonoids were presence or absence.



Petroleum ether **Acetone extract** **Methanol extract**

Figure 1: TLC of Petroleum ether, Acetone, and Methanol extract

Table 1: Physical parameters and yield of extracts

Sr. No.	Drug taken (gm)	Solvent used (ml)	Consistency	Color of extract	Yield (gm)	% Yield (w/w)
1	240	Petroleum ether	Sticky	Dark green	**6**	**2.5**
2	236	Acetone	Sticky	Dark green	**4**	**1.69**
3	234	Methanol	Sticky	Green	**2.5**	**1.06**

Table 2: Observations for Phytochemical qualitative analysis

Sr. No.	Chemical test	Petroleum ether	Acetone	Methanol
1.	Test for Alkaloids			
	a) Dragendroff's test	-	+	+
	b) Mayer's test	-	-	+
	c) Wagner's test	+	+	+
	d) Hager's test	+	-	-
2.	Test for Carbohydrates			
	a) Molish's test	+	-	+
	b) Benedict's test	+	+	+
	c) Barfoed's test	-	-	-
	d) Fehling's test	-	+	+
3.	Test for Protein			
	a) Millions test	-	+	+
	b) Biuret test	-	-	-
4.	Test for Amino acid			
	a) Ninhydrin test	-	-	+
	b) Tyrosine test	-	+	-
5.	Test for Glycoside			
	a) Keller-killani test	-	+	-
	b) Borntrager's test	+	-	-
	c) Legal test	+	+	+
6.	Test for Tannins and Phenols			
	a) Lead acetate test	+	-	+
	b) Pot. dichromate	-	+	+
7.	Test for Flavonoids			
	a) Lead acetate test	+	+	+
	b) Shinoda test	-	+	+
	c) Zinc dust & HCL test	-	+	-
8.	Test for Steroid			
	a) Salkowski's test	-	+	-

Table 3: Results of TLC Profile of extracts

Sr. No.	Extracts	Solvent system	Proportion	Spraying agent	Rf value	Colour	Chemical constituent
1	Petroleum ether	Chloroform: Methanol	8 : 2	10% H_2SO_4	0.46	Red	Flavonoid
					0.53	Pink	Glycoside
					0.12	Grey	Phenols
2	Acetone	Toluene: Methanol	7 : 3		0.45	Yellow	Flavonoid
					0.67	Yellow	Flavonoid
					0.90	Brownish red	Alkaloid
					0.31	Pink	Glycosides
3	Methanol	Chloroform: Methanol	5 : 5		0.36	Green	Phenol
					0.47	Blue	Flavonoid
					0.56	Green	Phenol
					0.78	Bluish red	Tannin

Thin Layer Chromatography:

Petroleum ether extract of *Plumeria Rubra* and *Murraya koenigii L.* Leaves extract when subjected to TLC showed Rf value at 0.46, 0.53 in the solvent system of: Chloroform: Methanol (5 : 5). Acetone extract of *Plumeria Rubra* and *Murraya koenigii L.* Leaves when subjected to TLC showed Rf value at 0.12, 0.45, 0.67, 0.90 in the solvent system of Toluene: Methanol (7: 3). Methanolic extract of *Plumeria Rubra* and *Murraya koenigii L.* Leaves when subjected to TLC showed Rf value at 0.31, 0.36, 0.47, 0.56, 0.78 in the solvent system of Chloroform: Methanol (5: 5)

Total Phenolic content of Plumeria Rubra and *Murraya koenigii* L. Leaves extracts

Acetone and Methanol extract of *Plumeria Rubra and Murraya koenigii L. Leaves* have found phenolic content as 67±0.57 mg GAE/gDW and 53±0.02 mg GAE/gDW respectively. Acetone extract shows more phenolic content than Methanol as per comparative evaluation of phenolic content of extracts.

Total Flavonoid Content of *Plumeria Rubra and Murraya koenigii L.Leaves* extract

The Observation table 4 reveals that Acetone and Methanol extract of *Plumeria Rubra and Murraya koenigii L. Leaves* have flavonoid content as 54.4(mg/gDW) and 68.05(mg/gDW) respectively. Acetone extract shows more flavonoid content than Methanol as per comparative evaluation of flavonoid content of extracts.

Excision wound model

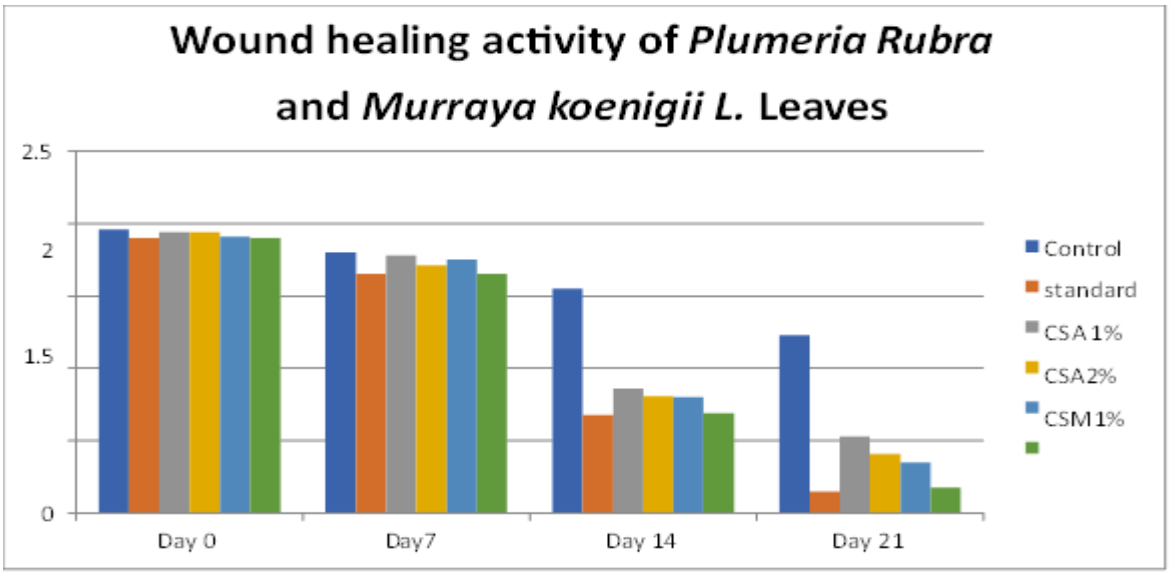
Wound healing activity: Excision model

The study assessed the wound healing effects of extracts from Plumeria Rubra and *Murraya koenigii* L. through a series of tests involving six animals in each group (n=6). The data were presented as means ± standard error of mean (S.E.M) and analyzed using one-way analysis of variance (ANOVA). A P-value of < 0.05 indicated a significant difference when comparing the standard and test treatments with the positive control. The treatments included a standard ointment (Betadine) and test groups (CSA 1%, CSA 2%, CSM 1%, CSM 2%). On the 7th day, the Betadine group showed no significant reduction in wound area compared to the positive control, while the test groups exhibited a significant decrease in wound area ($P < 0.05$). By the 14th day, Betadine showed a significant decrease compared to the positive control, and the test groups displayed a highly significant decrease ($P < 0.001$). However, there was no notable change between the test and standard groups. On the 21st day, both Betadine and test groups continued to show highly significant decreases in wound area ($P < 0.001$) compared to the positive control, but again there were no significant differences between the test and standard treatments. The findings indicate that extracts from Plumeria Rubra and *Murraya koenigii* L. exhibit significant wound healing activity, particularly CSA 1% and CSA 2%, which demonstrated highly significant reductions in wound area compared to the positive control and significant differences relative to the standard.

Table 4: Total Flavonoid Content of *Plumeria Rubra and Murraya koenigii L. Leaves* extract

Sr. No.	Extracts	Concentration (µg//ml)	Absorbance	TFC (mg/gDW)
1	Acetone	100	0.030±0.0011	54.4
2	Methanol	100	0.026±0.008	68.05

Value represents mean ± SEM (n=3)



Graph 1: Comparative in vivo wound healing activity of Plumeria Rubra and *Murraya koenigii* L. Leaves

Table 5: Day-to-day progress of wound healing effect

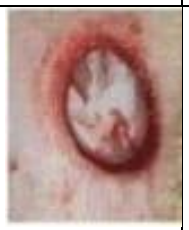
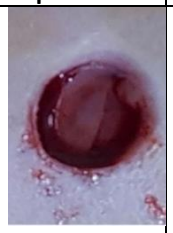
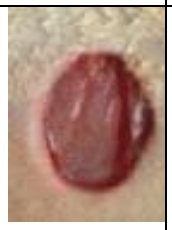
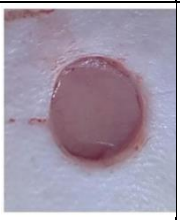
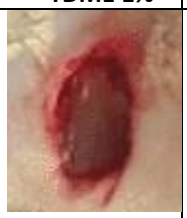
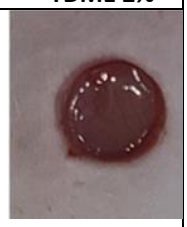
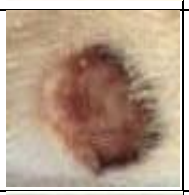
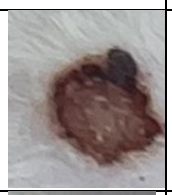
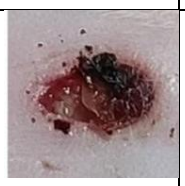
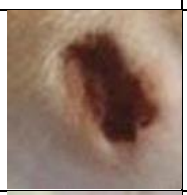
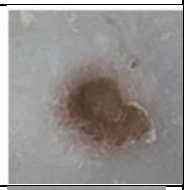
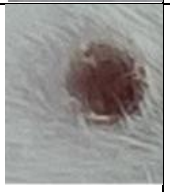
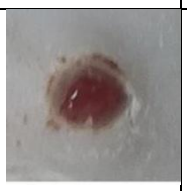
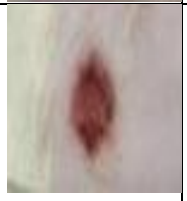
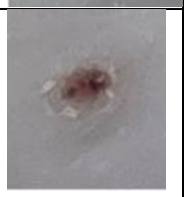
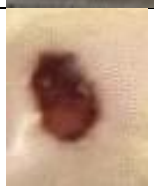
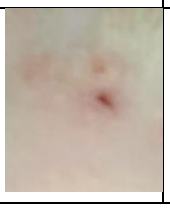
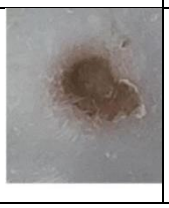
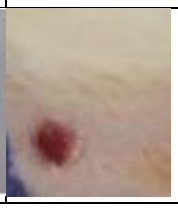
Day	Positive control	Standard Cipladine	TDAE 1%	TDAE 2%	TDME 1%	TDME 2%
0						
7th						
14th						
21th						

Table 6: Evaluation of wound healing activity of *Plumeria Rubra and Murraya koenigii L. Leaves*

Groups	Wound area (cm^2) Post-wounding days			
	0 day	7th day	14th day	21th day
Control (Group-I)	1.96 ±0.01	1.8±0.05	1.55±0.05	1.23±0.05
Standard (Group-II)	1.9±0.05	1.65±0.05**	0.68±0.02**	0.15±0.08**
CSA-1% (Group- III)	1.94±0.08	1.78±0.03*	0.86±0.05**	0.53±0.05**
CSA-2% (Group- IV)	1.94±0.08	1.71±0.07*	0.81±0.08**	0.41±0.08*
CSM-1% (Group-V)	1.91±0.08	1.75±0.05*	0.8±0.08**	0.35±0.01**
CSM-2% (Group-VI)	1.9±0.05	1.65±0.05*	0.69±0.01**	0.18±0.09**#

DISCUSSION

The use of medicinal plants is a significant alternative treatment for various diseases, recognized by the medical community for the scientifically verified efficacy and safety of several biologically active plants. This study focused on Plumeria Rubra and *Murraya koenigii* L. Leaves, emphasizing their traditional claims in Ayurveda, and evaluated their antimicrobial and wound healing properties. Authentication of the plants was conducted, examining morphological and microscopic features. Ash value, extractive values, and loss on drying were consistent with Ayurvedic Pharmacopoeia standards. Phytochemical analysis identified chemical constituents present in the extracts: methanol extracts contained glycosides, carbohydrates, and flavonoids, while acetone extracts revealed glycosides, tannins, phenolic compounds, and flavonoids. Thin Layer Chromatography (TLC) aided in the identification of these constituents. The antimicrobial properties were assessed using the agar cup and plate method, where the acetone extract at 50 mg/ml demonstrated the highest zone of inhibition compared to the standard Amoxicillin. Safety studies indicated the extracts were non-toxic at doses up to 2000 mg/kg. Wound-healing potential was investigated using an excision wound model in albino rats over 21 days, with wound area as the primary metric. The wound healing process consists of three phases: the initial inflammatory phase (0-48 hours), a proliferative phase (2 days to 3 weeks), and a remodeling phase (3 weeks to 2 years). Key mediators such as histamine

and serotonin are involved in the early wound healing responses. The study found that extracts from both plants exhibited significant wound healing effects, with CSM2% and CSM1% extracts showing marked reductions in wound area compared to the positive control group, signifying their potential therapeutic applications.

CONCLUSION

Plumeria Rubra and Murraya koenigii L. leaves were used for studying pharmacognostical, phytochemical and pharmacological evaluations. The results for ash and extractive values and loss on drying were within the range of that of the standard reference given in Ayurvedic Pharmacopoeia. In preliminary phytochemical analysis, *Plumeria Rubra* and *Murraya koenigii L.* Leaves Acetone extract showed the presence of carbohydrate, glycoside and flavonoids and Methanolic extract showed the presence of glycosides, carbohydrates, steroids, tannins, and flavonoids. The *in-vitro* antimicrobial study has shown that Acetone and Methanolic extract of *Plumeria Rubra* and *Murraya koenigii L.* Leaves does possess significant antibacterial activity with 50 mg/ml concentrations and with high concentration of methanol extract (50 mg/ml) it shows 10 mm zone of inhibition against *Escherichia coli* and 8 mm against *S aureus*. This *In-vivo* study has showed that Acetone and methanol extracts of *Plumeria Rubra and Murraya koenigii L. leaves* possess significant wound healing activities with 1% and 2% ointment, but the concentration of the methanol extract 2% ointment being more superior and showed significant to highly significant area when compared with standard Betadine against excision wound method in rats. Observations were made for the decrease in level of wound Area in rats. The finding of the present study demonstrated that *Plumeria Rubra and Murraya koenigii L. Leaves* extracts has potent wound healing activity and justify its use in traditional medicine to treat wound process.

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