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Phytochemical Screening and Wound Healing Activity of Different Leaf Extracts of *Rhynchosia rothii* in Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the present work to perform phytochemical screening and wound healing activity of different leaf extract of *Rhynchosia rothii.*
Place and Duration of the Study: The present work has been carried out at Oriental University, Indore between the duration of November-2020 to January-2021.
Methodology: *Rhynchosia rothii* dried leaf powder was subjected to continuous extraction with a soxhlet extractor using various organic solvents. Preliminary phytochemical screening with various qualitative chemical tests revealed the presence of Alkaloids, Tannins, Flavonoids, proteins, and mucilage present in *Rhynchosia rothii* leaf extracts. Wister rats of both sexes weighing 150-200 g were used for experimental purposes. The animals were kept in polypropylene cages at room temperature and with a light/dark cycle of 12:12 hours. All the test extracts at a different dose level and standard were administered topically once daily from day 0 to the day of complete healing or the 21st postoperative day, whichever occurred earlier.
Results: A significant increase in wound healing activity was observed in animals treated with alcoholic and aqueous extracts of *Rhynchosia rothii* leaves at a dose of 200 mg/kg body weight, compared to control treatments. Both methanolic and standard extracts of *Rhynchosia rothii* (200 mg/kg body weight) showed a dose-dependent increase in the rate of wound contraction. The

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methanolic extract of *Rhynchosia rothii* at a dose of 200 mg/kg body weight showed an increase in the percentage of wound contraction from 40.08% to 88.90% from day 3 to 12. The aqueous (standard) extract of *Rhynchosia rothii* at a dose of 200 mg/kg body weight showed an increase in the percentage of wound contraction from 46.02% to 100% from day 3 to 12.

Conclusion: From the previous results it was concluded that the Wound represents a serious health problem, both in terms of morbidity and mortality. Wound healing is a critical response to tissue integrity. Therefore, the wound healing property of *Rhynchosia rothii* can be attributed to the phytoconstituents present in it, which may be due to its individual or additive effect that accelerates the wound healing process. Since *Rhynchosia rothii* is grown in abundance in India and rest of other part of world, it could be a fairly good and easily available therapeutic agent due to its naturally occurring tendency for healing wounds as a healer, as well as controlling abnormal healing.

Keywords: Rhynchosia rothii; leaf extracts; excision wound; extraction; wound healing; plant extract; rat.

ABBREVIATIONS

CPCESA: Committee for the Purpose of Control and Supervision of Experiments on Animals

IAEC : Institutional Animal Ethics Committee.

1. INTRODUCTION

Traditionally, medicinal plants have played an important role in human life since ancient times [1]. Currently, natural compounds are the main source for modern drug discovery due to their therapeutic selectivity, minimal side effects, cheap source, and serve as primary molecules for drug discovery [2]. *Rhynchosia* (Fabaceae) species circulate widely in tropical and subtropical areas around the world. Some plants of this genus have been used in traditional medicine for the treatment of various ailments such as antibacterial, antidiabetic, abortive, healing, hepatoprotective, healing of boils, rheumatic pains, and skin infections [3]. This review collects traditional uses, isolated chemical compounds, and pharmacological activities of *Rhynchosia* species. A total of seventy-seven compounds of the genus *Rhynchosia* have been isolated so far, including flavonoids, isoflavonoids, flavan-3-oils, xanthones, biphenyls, simple polyphenols, and sterols. Interestingly, the genus Rhynchosia is a rich source of prenylated C-glycosylflavonoids and isoflavonoids [3-4].

Rhynchosia Volubilis species has been reported as an antiproliferative property and *Rhynchosia villosa* has shown inhibitory activity of the enzyme tyrosinase. In addition, Koreans use *R. Volubilis* as a local cosmetic that has the property of depigmenting skin blemishes, treating skin wrinkles, and anti-aging [3]. Additionally, *Rhynchosia beddomei* leaves were found to be antidiabetic. The essential oil isolated from *Rhynchosia heynei* and *Rhynchosia minima* leaves showed a broad spectrum of antimicrobial, antioxidant, and allelopathic activity [4]. Therefore, the development of drugs of natural origin is of great importance, based on the traditional and pharmacological uses of the genus *Rhynchosia* [3-4].

A review of the literature revealed that the scientific study of the anti-inflammatory and healing activity of the *Rhynchosia rothii* plant was not previously conducted to evaluate the plant's traditional claim in wound management. The main objectives of the present research work are the biological screening of various extracts for wound healing activity using a rat excisional wound model to compare the therapeutic safety and efficacy of the plant.

2. MATERIALS AND METHODS

2.1 Chemical and Reagents

Petroleum ether (60-80°C), chloroform, ethyl acetate, methanol, and acetone were purchased local vendor. All analytical grade chemicals were used.

2.2 Preparation of Plant Extracts and Extraction Method

The fresh leaves of *Rhynchosia rothii* were collected and dried in the shade and pulverized with a mechanical grinder. 1 kg of the pulverized plant material was extracted with different solvents such as petroleum ether, chloroform,

ethyl acetate, ethanol, and water using a soxhlet apparatus. After the concentration and drying of each extract, extracts of chloroform, ethyl acetate, methanol, and acetone were selected for biological screening in various animal models [5]. All plant extracts were suspended in a simple ointment base and used for topical application to animals for wound healing activity [6-8].

The dried leaves powder of *Rhynchosia rothii* was subjected to continuous extraction with a soxhlet extractor using various organic solvents such as petroleum ether (60-80°C), chloroform, ethyl acetate, methanol, and acetone respectively. After the concentration and drying of each extract, the identification of the phytoconstituents was carried out utilizing various qualitative chemical tests. The solvents in the extracts were removed in a vacuum. The extraction values were calculated by weighing. The samples were stored at 4ºC [6].

2.3 Preliminary Phytochemical Screening of Various Extracts

The extracts of *Rhynchosia rothii* leaves obtained during the extraction process were subjected to preliminary phytochemical examination for the presence of various phytoconstituents using reported methods [7].

2.4 Experimental Animals

Wister rats of both sexes weighing 150-200 g were used for experimental purposes. The animals were kept in polypropylene cages at room temperature and with a light/dark cycle of 12:12 hours. The animals had free access to standard rat pellets and water under strict hygienic conditions. The animals were accustomed to the laboratory conditions for 48 hours before the experimental protocol to minimize non-specific stress if any. The animals were divided into groups of six animals each and fasted for 12 hours before the experiment. The animals were divided into 5 groups with 3 animals in each group. Group I was served as control group i.e. untreated group. Group II was treated with ethyle acetate extract, group III was treated with acetone extract, group IV was treated with methanolic extract, whereas group V was served as standard which was treated with Neosporin. The acute toxicity studies have been performed on plant extract by administering through oral route at different doses from 100 mg/kg b.w. to 2000 mg/kg b.w. After 21 days of the study, no any deaths were seen and therefore 200 mg/kg b.w doses were selected for further studies.

2.4.1 Selection of extracts used for wound healing studies

Various extracts were obtained from extraction process. Out of them following three extracts named as ethyl acetate extract, methanolic extract and acetone extract selected for wound healing study in rats. Standard Neosporin and untreated control extract were also selected for study.

2.4.2 Wound healing studies in rats: excisional wound model

The animals were anesthetized before and during wound creation with light ether anesthesia. The rats suffered excision injuries. The dorsal fur of the animals was shaved with an electric razor and the intended wound area was to be created as outlined on the animals' backs with methylene blue. A total excision wound thickness of 2.5 cm in width was created along the marks using serrated forceps, scalpels, and pointed scissors. Slides were placed on the wound and an impression of the wound was made with a marker [9]. The wound area was measured by placing the slide on graph paper. The treatments were performed by locally applying the test and standard formulations. The study was approved by CPCESA/IAEC [9-11].

3. RESULTS AND DISCUSSION

3.1 Method of Extraction

The various organic solvent used for the extraction process. The extract (100g) was refluxed with the following solvents in the given sequence Petroleum ether, chloroform, acetone, ethyl acetate, and methanol. Thesolvents from the extracts were removed under a vacuum. The extractive valueswere calculated by weighing. The samples were stored at 4ºC. The extraction value for the plant in organic solvents is given in Table 1.

3.2 Preliminary Phytochemical Screening of *Rhynchosia rothii* Leaves

The healing properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids,

tannins, flavonoids, proteins, and mucilages. Therefore, preliminary screening tests can be useful in the detection of bioactive ingredients and can subsequently lead to drug discovery and development. Furthermore, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds [11]. Preliminary phytochemical screening with various qualitative chemical tests revealed the presence of alkaloids, tannins, flavonoids, proteins, and mucilages present in the *Rhynchosiarothii* leaf extracts and Glycosides, saponins, and amino acid were absent in the *Rhynchosiarothii* leaf extracts.

3.3 Wound Healing Activity: Excision Wound Model

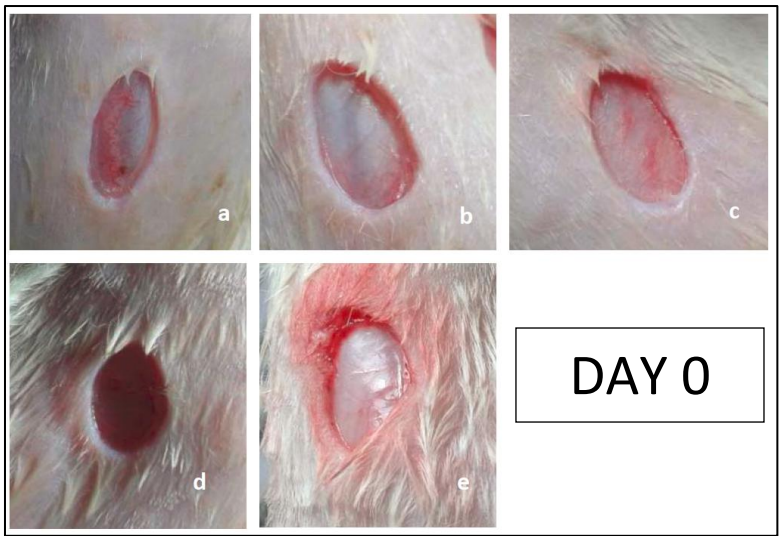
A significant increase in wound healing activity was observed in animals treated with alcoholic and aqueous extracts of *Rhynchosia rothii* leaves at a dose of 200 mg/kg body weight, compared to control treatments. Table 2 shows the effect of various *Rhynchosia rothii* extracts on wound healing activity in rats inflicted with an excision wound. In this model, extract-treated animals showed a faster decrease in wound size and shorter epithelialization time than control rats that received distilled water. The rate of wound contraction was lower in the control group of animals, while the percentage of wound closure was high in the aqueous extract group followed by the alcohol extract group, indicating the effect of the plant in promoting excision wound healing.

Control rats showed a time-dependent increase in wound contraction rate from 15.0% to 100% from day 3 to day 21, while complete wound closure. The aqueous extract (standard) of *Rhynchosia rothii* at a dose of 200 mg/kg body weight showed an increase in the percentage of wound contraction from 46.02% to 100% from day 3 to 12 (Fig. 1). *Rhynchosia rothii* methanolic extracts at a dose of 200 mg/kg body weight showed an increase in the rate of wound contraction from 40.08% to 88.90% from day 3 to day 12.

Rhynchosia rothii ethyl acetate extract at a dose of 200 mg/kg body weight showed an increase in wound contraction rate from 18.30% to 49.60% from day 3 to 12. *Rhynchosia rothii* acetone extract at a dose of 200 mg/kg body weight showed an increase in the percentage of wound contraction from 19.40% to 52.90% from day 3 to 12. Both methanol and standard extracts of *Rhynchosia rothii* showed a dose-dependent increase in the rate of wound contraction [12-13].

Table 1. Solvents and its Extractive Values

Solvent	Extractive Value (g)
Petroleum Ether	1.4
Chloroform	2.3
Acetone	3.1
Ethyl acetate	3.6
Methanol	5.2



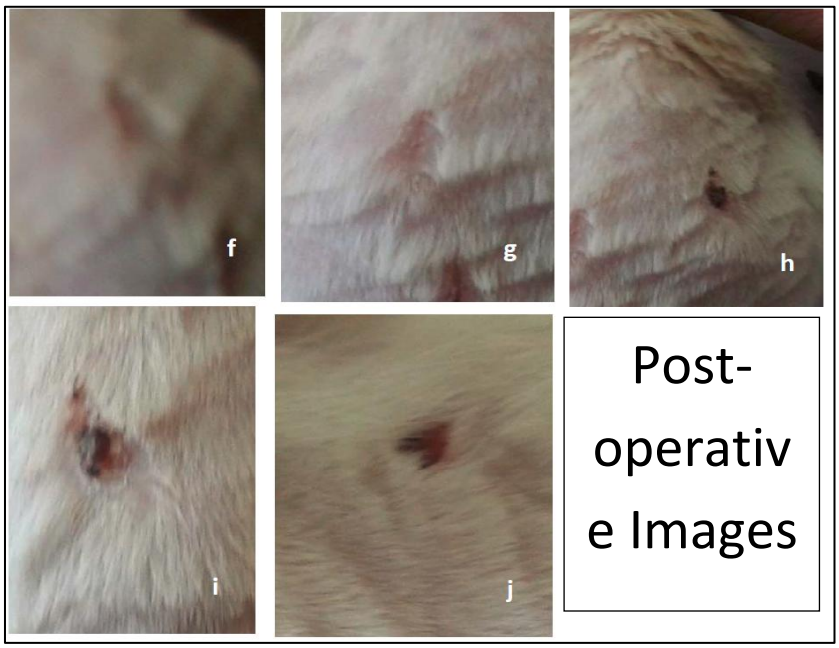


Fig. 1. a, f) Standard Neosporin, b, g) Alcoholic Extract, c, h) Ethyl Acetate Extract, d, i) Acetone Extract, e, j) Untreated Group

Table 2. Excision Wound Healing Model Data

Groups	Wound Area				% Wound Area					% Healed Area				
	Animal				Animal					Animal				
	Days	1	2	3	1	2	3	Avg	St. dev	1	2	3	Avg	St. dev
I: Untreated Control	1	11.5	13.0	12.5	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	9.0	11.0	11.5	78.3	84.6	92.0	85.0	6.9	21.7	15.4	8.0	15.0	6.9
	6	11.0	10.0	7.5	95.7	76.5	60.0	77.4	17.8	4.3	23.5	40.0	22.6	17.8
	9	5.0	5.0	6.0	43.5	38.5	48.0	43.3	4.8	56.5	61.5	52.0	56.7	4.8
	12	3.0	4.0	4.0	26.1	30.7	32.0	29.6	3.1	73.9	69.3	68.0	70.4	3.1
	15	1.0	1.0	3.0	8.7	7.7	24.0	13.5	9.1	91.3	92.3	76.0	86.5	9.1
	18	0.0	0.0	1.0	0.0	0.0	8.0	2.7	4.6	100.0	100.0	92.0	97.3	4.6
	21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	0.0
II: Ethyl Acetate Extract	1	14.0	12.5	11.5	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	11.5	9.5	10.0	82.1	76.0	87.0	81.7	5.5	17.9	24.0	13.0	18.3	5.5
	6	8.5	7.5	9.0	60.7	60.0	78.3	66.3	10.3	39.3	40.0	21.7	33.7	10.3
	9	7.5	6.0	7.5	53.6	48.0	65.2	55.6	8.8	46.4	52.0	34.8	44.4	8.8
	12	6.	5.	7.0	46.4	44.	60.	50.4	9.1	53.	56.	39.	49.6	9.1

Groups	Wound Area			% Wound Area				% Healed Area					
	Animal				Animal				Animal				
		5	5			0	9			6	0	1		
	15	3.5	3.5	2.5	25.0	28.0	21.7	24.9	3.1	75.0	72.0	78.3	75.1	3.1
	18	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	0.0
	21	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	0.0
	1	12.0	11.0	13.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	8.5	9.5	11.0	70.8	86.4	84.6	80.6	8.5	29.2	13.6	15.4	19.4	8.5
	6	7.5	8.0	12.0	62.5	72.7	92.3	75.8	15.1	37.5	27.3	7.7	24.2	15.1
III: Acetone Extract	9	6.0	6.0	7.5	50.0	54.5	57.7	54.1	3.9	50.0	45.5	42.3	45.9	3.9
	12	4.5	5.5	7.0	37.5	50.0	53.8	47.1	8.5	62.5	50.0	46.2	52.9	8.5
	15	3.5	4.5	4.0	29.2	40.9	30.8	33.6	6.4	70.8	59.1	69.2	66.4	6.4
	18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	0.0
	21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	0.0
	1	12.0	10.5	12.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	7.5	6.0	7.0	62.2	57.1	58.3	59.2	2.6	37.8	42.9	41.7	40.8	2.6
IV: Methanolic Extract	6	6.0	3.0	7.5	45.8	33.3	54.2	44.4	10.5	54.2	66.7	45.8	55.6	10.5
	9	5.0	2.0	3.5	41.7	19.0	33.3	31.3	11.4	58.3	81.0	66.7	68.7	11.4
	12	2.5	0.0	2.0	16.7	0.0	16.7	11.1	9.6	83.3	100.0	83.3	88.9	9.6
	15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	0.0
	18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	0.0
	21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	0.0
	1	11.4	12.1	12.4	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	6.3	6.4	6.6	55.3	52.9	53.2	53.8	1.3	44.7	47.1	46.8	46.2	1.3
V: Standard	6	5.2	4.1	3.2	45.6	33.9	25.8	35.1	10.0	54.4	66.1	74.2	64.9	10.0
	9	1.1	1.3	1.2	9.6	10.7	9.7	10.0	0.6	90.4	89.3	90.3	90.0	0.6
	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	0.0
	15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	0.0
	18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	0.0
	21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	0.0

4. CONCLUSION

The wound represents a serious health problem, both in terms of morbidity and mortality. Wound healing is a critical response to tissue integrity. It mainly depends on the repairing capacity of the tissue, the type, and extent of damage, and the general state of health of the tissue. Ideally, a therapeutic agent selected for wound treatment should enhance one or more stages of healing without producing harmful side effects. The traditional Indian medicine system has many plants with versatile medicinal properties, which require detailed research for the development of effective drugs.

Herbal products are potential wound healing agents and are highly preferred for their wide availability, non-toxicity, absence of unwanted side effects, and efficacy as crude preparations. Continuing with the development of drugs from plants to medicines, *Rhynchosia rothii* leaf extracts were selected for the wound healing effect [11-13].

Preliminary phytochemical analysis of *Rhynchosia rothii* revealed the presence of alkaloids, tannins, flavonoids, proteins, and mucilages. Several studies, including our previous work with other plant materials, have demonstrated the presence of similar phytochemical components responsible for promoting wound healing activity in rats. Therefore, the wound healing property of *Rhynchosia rothii* can be attributed to the phytoconstituents present in it, which may be due to its individual or additive effect that accelerates the wound healing process. Since *Rhynchosia rothii* is grown in abundance in India and rest of other part of world, it could be a fairly good and easily available therapeutic agent due to its naturally occurring tendency for healing wounds as a healer, as well as controlling abnormal healing [13]. From the above results we will formulate the herbal nanoparticles to be use for the treatment of wounds and inflammation.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental protocol was approved by the Institutional Animal Ethics Committee of the Deshpande Laboratories Pvt. Ltd. Madhya Pradesh (An ISO 9001:2008 Certified Drug Testing Laboratory) (CPCSEA Approved: 1410/c/11/CPCSEA).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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