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Evaluating the combined effect of aqueous extracts of *Alternanthera sissilis* and *Tagetes erecta* on excision wound healing

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ABSTRACT

The present study evaluates the wound healing potential of combined aqueous extracts of *Alternanthera sissilis* and *Tagetes erecta* using an excision wound model in Wistar rats. The plants were authenticated, extracted by Soxhlet apparatus, and subjected to phytochemical screening and quantitative estimation of phenolics, flavonoids, and tannins. The extracts revealed the presence of diverse secondary metabolites, with *A. sissilis* showing a broader phytochemical profile. Acute toxicity studies confirmed safety up to 2000 mg/kg. The mixed extract ointment (5% w/w; 2.5% each) was applied topically and compared with vehicle control and standard povidone iodine ointment. Results demonstrated significant wound contraction in the mixed extract group (82.24% by day 20), superior to control (67.62%) but lower than standard (96.47%). These findings suggest that the bioactive constituents of *A. sissilis* and *T. erecta* contribute to enhanced collagen deposition, epithelialization, and tissue remodeling, supporting their traditional use in wound management. The study highlights the potential of combining plant extracts for cost-effective, natural wound healing formulations.

KEYWORDS:

Alternanthera sissilis; *Tagetes erecta*; wound healing; excision model; synergistic effect

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INTRODUCTION

Wound healing is a dynamic and complex biological process involving hemostasis, inflammation, proliferation, and remodeling phases, each regulated by growth factors, cytokines, and extracellular matrix components.[1] Despite advances in synthetic drugs and topical formulations, many agents are limited by delayed healing, cytotoxicity, or cost, which has encouraged the exploration of medicinal plants as safer alternatives.[2] Phytochemicals such as flavonoids, tannins, phenolics, and alkaloids are known to accelerate tissue repair by mechanisms including free radical scavenging, collagen synthesis stimulation, and angiogenesis promotion.[3]

Alternanthera sissilis (family Amaranthaceae) is a perennial herb traditionally used for its anti-inflammatory, antimicrobial, and antioxidant properties.[4] *Tagetes erecta* (family Asteraceae), commonly known as marigold, has been employed in ethnomedicine for wound healing, antiseptic, and anti-inflammatory activities.[5] Both plants are reported to contain diverse phytoconstituents such as flavonoids, saponins, glycosides, and phenolic acids, which contribute to tissue repair and regeneration.[6,7] However, limited scientific evidence exists regarding the synergistic potential of their combined extracts in wound healing.

Given the global emphasis on cost-effective, plant-based therapeutics, evaluating the combined effect of *Alternanthera sissilis* and *Tagetes erecta* is highly relevant. This study was therefore designed to investigate the phytochemical profile and wound healing efficacy of aqueous extracts of these plants using an excision wound model in Wistar rats. By comparing the healing response of the mixed extract with a standard drug (povidone iodine ointment) and vehicle control, the work aims to provide experimental validation for the traditional use of these plants and explore their potential as natural wound healing agents.

MATERIAL AND METHODS

Preparation of plant for extraction

The whole plant of *Alternanthera sissilis* and the leaves of *Tagetes erecta* have been collected from local area and authenticated using previous voucher at RB Science. The authenticated plant material were washed with water, dried under shade and powdered using a blender at low speed. The powdered leaves were stored in air tight container until taken for use.

Extraction of the plant material[8]

The powdered plant material was used for the extraction process. 102 g of *Tagetes erecta* powder was evenly packed in the extractor of the Soxhlet apparatus and extracted with water by hot continuous extraction process for about 7 h. 95 g of *Alternanthera sissilis* powder was evenly packed in the extractor of the Soxhlet apparatus and extracted with water by hot continuous extraction process for about 8 h. The solvent was filtered hot through to remove any impurity. The extracts were obtained by concentrating the solvent using rotary vacuum evaporation finally by evaporation on water bath. The resinous extracts were collected and placed in desiccators to remove the excessive moisture**.** The dried extracts were stored in desiccators for further processing.

Preliminary phytochemical screening[9]

All the extracts were evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in them. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as analytical responses to these tests.

Total Phenolic content[10]

To estimate the total phenolic content, 100 mg dried extract was macerated overnight with 10 mL of methanol and filtered. The solution was stored at 4°C in amber bottles and served as the stock solution (10 mg/mL) for subsequent analyses. For total phenolic content determination, 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After 2 min of incubation at room temperature 300 µL of 20% Na_2CO_3 aqueous solution was added and the mixture allowed to incubate in dark for 2 h. The absorbance of the colored solution was measured at 765 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Total Flavonoid content[11]

The total flavonoid content will be determined using Aluminum chloride method using quercetin as the standard flavonoid for reporting the results. 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25- 150µg/ml were prepared in methanol. 0.1 g of dried extract was extracted with 10 ml methanol, filtered, and make up the volume up to 100 ml. To 1 ml of extract or quercetin solution was added 1 ml of 5% sodium nitrite and the solution was incubated at room temperature for 10 min. To this was added 1 ml of 2% $AlCl_3$ methanolic solution and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm

Total Tannin content[12]

The total tannin content will be determined by spectrophotometric method using catechin as the standard tannin for reporting the results. The tannin contents or Proanthocyanidin were determined by method of Broadhurst et al., with slight modification, using catechin as a reference compound. A volume of 400µL of extract is added to 3 mL of a solution of vanillin (4% in methanol) and 1.5 mL of concentrated hydrochloric acid. After 15 min of incubation the absorbance was read at 500 nm. The condensed tannin was expressed as gE.Catechin/100g. Standard solutions of catechin (10-100 ppm) were similarly treated to plot the calibration curve.

Study of wound healing action

The wound healing action (excision wound) was studied on rats by topical application of the mixed extract to study synergism.

Animals

Healthy male Wistar male rats weighing 180-250g were used for the study. The animals were housed in cages during the course of experimental period and maintained at 12 day and night schedule with a temperature [17-26°C] maintained at standard experimental condition. The animals were fed with standard rodent pellet feed and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water.

Acute Toxicity Study[13]

A total of three animals were used which received a single oral dose (2000mg/kg) of the extracts of both the plants mixed in equal quantity. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a period of 14 days. Once daily observations were made for changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, perspiration, urinary incontinence, and defecation) and central nervous system (drowsiness, tremors and convulsion) changes. Mortality, if any, was also observed over the period of 2 weeks.

Preparation of test samples and standard drugs

The test samples were prepared by formulating the dried plant extract (equal quantity) in simple ointment base (cetostearyl alcohol, wool fat, white paraffin, and hard paraffin) as a 5 % w/w ointment. Commercially available Povidone Iodine Ointment (5 %) was used as standard drug for comparison of action.

Preparation of simple ointment base[14]

Hard paraffin (5 g) and cetostearyl alcohol (5 g) were taken in a porcelain dish maintained on water-bath at 70°C. Wool fat (5 g) and white soft paraffin (85 g) are added to this mixture and stirred until all the ingredients were in molten state and mixed. The mixture was stirred until cold and packed in suitable container.

Experimental procedure for wound healing by excision model

Experiment Design

The animals were divided in to 3 groups of 5 rat each and the experiment was designed as per table 5.1.

Table 1. Experimental design for excision model

Group	Nomenclature	Treament
Group I	Vehicle Control	Simple ointment base
Group II	Standard	Povidone iodine ointment (5% w/w)
Group III	Test	*Alternanthera sissilis and Tagetes erecta* extract ointment (2.5% w/w each)

All the test samples; vehicle and standard drug samples were applied topically on the wound of each of the animals daily, under sterile conditions.

Induction of wound[15]

On the day of inducing wound, each animal was anesthetized by the open mask method using short exposure to diethyl ether. The hair (fur) on the back of each rat was removed by shaving using an electric shaver. The area of the wound to be created was marked on the back of the animals with methylene blue using a circular stainless-steel stencil. A full thickness of the excision wound of 1.5 cm in width (circular area 2.25 cm^2) created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound left open. All the surgical procedures were carried out under sterile condition. After 24 h of wound creation, the ointments were applied gently to cover the wounded area once daily until complete healing. Wound area and wound contraction, were monitored on each day.

Measurement of wound contraction[15]

The progression of wound healing was judged by the periodic assessment of the contraction of excision wounds. Wound contraction was monitored by tracing the outline of the wound on tracing sheet and then using graph sheet to calculate the area of the

wound size. All animals in each group were monitored until complete healing of wounds occurred and the day at which each wound healed was recorded

$$\textbf{Percent wound contraction} = \frac{\boldsymbol{Healed\ area}}{\boldsymbol{Total\ area}}\ \boldsymbol{X}\ \mathbf{100}$$

RESULTS AND DISCUSSION

The phytoconstituents from the leaf of *Alternanthera sissilis* and *Tagetes erecta* were extracted using water as the extracting solvent and the extracts was obtained in 14.74 % and 5.03% w/w yield respectively. The extracts were tested for the presence of various categories of phytochemicals and the results are presented in Table 2. The findings of the phytochemical analysis suggest the presence of alkaloids, saponin glycosides, anthraquinones, phenolics, proteins and flavonoids in the *Tagetes erecta*. On the other hand, all class of metabolites were found to be present in the extract of *Alternanthera sissilis*.

Table 2. **Phytochemical screening of extracts**

Test	T. erecta	A. sissilis
Mayers Test	-	+
Wagners Test	-	+
Hagers Test	-	+
Dragendroff Test	-	-
Froth Test	+	+
Bontragers Test	-	+
Keddes Test	-	+
Keller-Kiliani Test	-	+
Gelatin Test	-	+
Ferric Chloride Test	+	+
Vanillin Hydrochloride Test	+	+
Alkaline reagent test	+	+
Shinoda test	+	+
Zinc hydrochloride reduction test	+	+
Ninhydrin Test	+	+
Liberman Burchard Test	+	+
Salkowski Test	-	+
Molisch Test	-	+

+suggests positive test result, - suggests negative test result

Quantitative estimation of phenolics, flavonoids and tannins

The total phenolic content of aqueous extract of *Tagetes erecta* and *Alternanthera sissilis* were 77.15 and 125.45 GAE mg/g, respectively. The total flavonoid content of aqueous extract of *Tagetes erecta* and *Alternanthera sissilis* were 69.78 and 85.88 QE mg/g, respectively. The total tannin content of aqueous extract of *Tagetes erecta* and *Alternanthera sissilis* were 81.23 and 53.81 CE mg/g, respectively.

Acute Toxicity Study

The acute toxicity test was performed by using the dried extracts at concentration of 2000 mg/kg to the test animal, administered orally. No animal died and hence the dose of upto 2000 mg/Kg was considered to be safe. As none of the animals died, the LD_{50} was considered to be more than 2000 mg/Kg and any dose less than 2000 mg/Kg would be considered for evaluation of wound healing action.

Wound Healing action

The mixed extract (equal amounts) were evaluated for *in vivo* wound healing effect by the excision model (n=5). The topical application of 5 % w/w of the mixed extract (2.5% each) resulted in an enhanced and statistically significant ($p < 0.001$) wound healing activity *in vivo*. At the initiation of the study, wound areas across all groups (vehicle control, standard, and mixed extract) were comparable (~311 mm²), confirming uniformity in wound size and validating the experimental design. On day 5, Vehicle control exhibited 26.32% contraction, representing the natural healing process. Standard treatment achieved 51.76% contraction, indicating a markedly accelerated healing response. Mixed extract showed 31.21% contraction, superior to control but less effective than the standard. This suggests that the mixed extract possesses wound healing activity, though its onset is slower compared to the standard. By Day 10, contraction in the standard group (~75%) was significantly higher than both control (~42%) and mixed extract (~61%). By Day 14, the standard group, maintained superiority (75.75%), while the mixed extract (61.26%) continued to outperform control (42.45%). These findings highlight that the mixed extract exerts a sustained effect, particularly evident in the intermediate phase, possibly through enhanced collagen deposition and epithelialization. On day 20 of study, Vehicle control reached 67.62% contraction, reflecting incomplete natural healing. Standard treatment achieved near-complete closure (96.47%), confirming its efficacy and Mixed extract demonstrated 82.24% contraction, significantly better than control, though still inferior to the standard. The extract's performance in the late phase suggests that its bioactive constituents contribute to tissue remodeling and wound closure, albeit less potently than the standard (Table 3, Figure 1 and 2).

Table 3. Area of wound and % contraction of wound by excision model

Group	Vehicle Control	Standard	*Mixed* extract
Day	Area mm² (% contraction)		
Day 0	313.8 ± 2.683	311.8 ± 2.280	310.8 ± 2.280
Day 5	231.2 ± 4.025	150.4 ± 2.408	213.8 ± 4.604
	(26.32)	(51.76)	(31.21)
Day10	180.6 ± 4.393	75.6 ± 1.817	120.4 ± 4.159
Day 14	130.4 ± 3.975	21.2 ± 3.271	83.4 ± 4.561
	(42.45)	(75.75)	(61.26)
Day 20	101.6 ± 3.975	11.0 ± 2.550	55.2 ± 3.271
	(67.62)	(96.47)	(82.24)

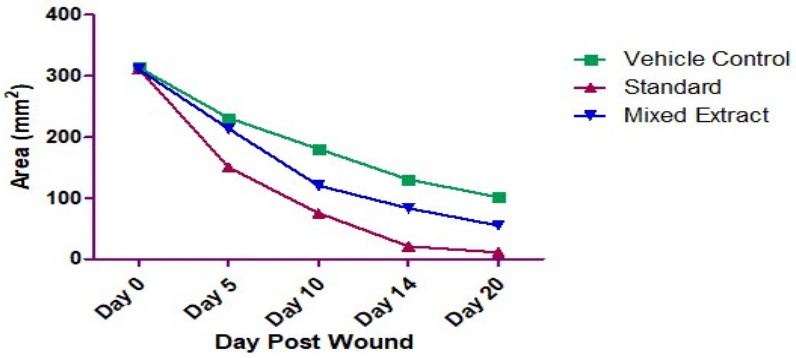

Figure 1. Wound healing efficacy of Mixed extract by *in vivo* excision model

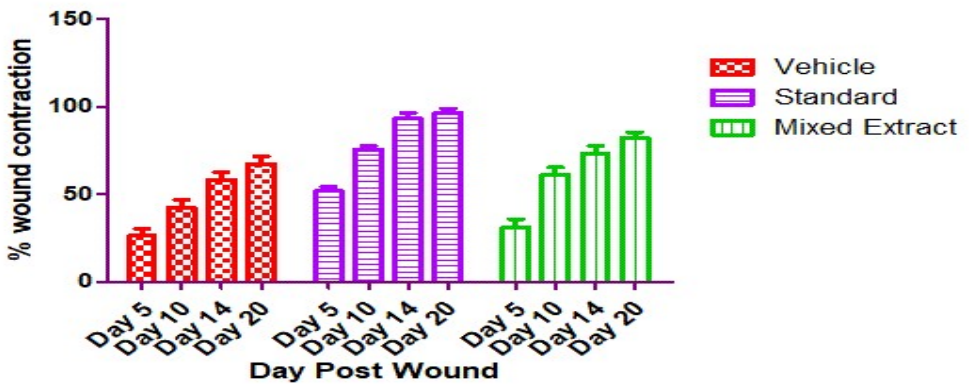

Figure 2. Percentage contraction of wound exhibited by mixed extract by *in vivo* excision model

CONCLUSION

The aqueous extracts of *Alternanthera sissilis* and *Tagetes erecta*, when combined, demonstrated measurable wound healing activity in the excision model, as evidenced by enhanced wound contraction compared to the vehicle control. The mixed extract achieved 82.24% contraction by day 20, which was significantly better than the control group (67.62%) but remained lower than the standard povidone iodine ointment (96.47%). These results indicate that while the combination of extracts contributes positively to wound healing, likely through the action of phenolics, flavonoids, and tannins, the efficacy is moderate and does not surpass the standard treatment. Importantly, the extracts were safe up to 2000 mg/kg in acute toxicity testing, supporting their potential for topical use. Overall, the findings validate the ethnomedicinal relevance of these plants and suggest that their combination may serve as a supportive, cost-effective natural formulation for wound management, though further optimization and mechanistic studies are needed to maximize therapeutic benefit.

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