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ESTIMATION OF ANTIMICROBIAL AND WOUND HEALING POTENTIAL OF *ABRUS PULCHELLUS* WALL LEAVES EXTRACT

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ABSTRACT

Abrus pulchellus Wall. is an underutilized, medicinal plant species with plenty of medicinal properties. The aim of present study was to investigate the antimicrobial and wound healing properties of *Abrus pulchellus* Wall. leaves. Leaves of *A. pulchellus* Wall. were extracted by Soxhlet extraction method with ethyl acetate and 70% ethanol. For this study five bacterial strains (*Staphylococcus aureus*, *S. epidermidis, Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Vibrio vulnificus*) and four fungal strains (*Candida albicans*, *C. sparapsilosis*, *C. tropicalis*, and *Aspergillus niger*) were tested using the agar disc diffusion method. Ointment of 70% ethanol extract was applied to assess the activity of wound healing in Wistar rats using an excision wound model. Animals were divided into 4 groups with 5 animals in each group. Betadine ointment (5% w/w) was used as a reference drug. The duration of epithelialization and wound contraction were measured to observe the healing process. The 70% ethanol extract showed significant antimicrobial activity against three microorganisms viz. *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Vibrio vulnificus* as compared to ethyl acetate extract. Compared to the control group, 70% ethanol extract (10% concentration) showed significant wound healing property ($p < 0.001$) in wound induced animal models whereas in 5% concentration it showed moderate wound healing property. The results of this investigation indicated that while *A. pulchellus* ethyl acetate extract lacked antibacterial action, 70% ethanol extract was found to have some antimicrobial activity and the same extract have significant wound healing activity. To identify the active phytoconstituents attributed to the antibacterial and wound-healing properties of the *A. pulchellus* further research is required.

Keywords: Agar disc diffusion, *A. pulchellus*, Wound healing, Antibacterial, Soxhlet apparatus

INTRODUCTION

Many native plants of Uttarakhand have demonstrated medicinal efficacy for a range of ailments, according to traditional herbal medicine practitioners **[1]**. In certain places of the world, they remain the main form of healthcare **[2]**. In the past ten years, attitudes towards the medicinal uses of ethnopharmacology have undergone a significant shift. Scientists have been studying plants to find potential wound healing qualities because they contain a variety of life-sustaining components **[1]**.

Wound healing is characterized by a predetermined set of reactions including immigration, multiplication, differentiation, and death of cell types inside the wound environment. To better understand wound healing, split it into four stages: acute inflammation, proliferation/granulation tissue development, and tissue remodeling. An extremely powerful part of the body's natural first response to injury is inflammation. There are two categories of inflammatory responses: vascular and cellular **[3]**. Early in the healing process, local vasodilation, blood and fluid extravasation into the extravascular space, and lymphatic drainage obstruction can produce classic signs of inflammation, including redness, swelling, and heat **[4]**. A new functional shield forms later, building on the initial inflammatory responses to damage. During this stage of the repair, cellular activity comes first **[5]**. Remodeling is the process of the matrix being deposited and then changed over time. During the proliferative phase of wound repair, granulation tissue rich in type III collagen and blood vessels replaces the fibrin clot that was formed during the initial inflammatory phase. This is followed by the formation of a collagenous scar that is primarily composed of type I collagen and has significantly fewer mature blood vessels **[6]**. Unveiled subcutaneous tissue serves as the perfect substrate for a wide array of microorganisms to contaminate and colonize, and the ideal conditions for microbial development are created when the corresponding tissue becomes devitalized (such as when it is ischemic, hypoxic, or necrotic) and the host's immune system reaction is weakened **[7]**. Different kinds of microorganisms enter and occupy a wound throughout time in the transition from contamination to colonization. Initial invasion of the wound region is frequently caused by gram-positive bacteria. Coagulase-negative *Staphylococci* (CoNS), the most prevalent kind, are derived as symbiotic organisms from the physiological milieu of unbroken skin around the wound. After a few days to a few weeks, depending on the patient's particular immunological habitat maintenance, rod-shaped gram-negative bacteria infiltrate the area and threaten the local species **[8]**. The patient's urogenital area, which is home to numerous species, including Enterobacteriaceae like *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* spp., or the patient's immediate surroundings, which frequently include Pseudomonadaceae, Acinetobacter, or yeasts, are the source of this contamination and subsequent colonization **[9]**. *A. pulchellus* Wall. (Family Fabaceae) commonly known as showy rosary pea, is a twining shrub **[10, 11]**. *A. pulchellus* Wall. bears a striking resemblance to the common rosary-pea (*Abrus precatorius*), except for bigger and more numerous leaflets, longer racemes of flowers, and incurved pods that grow longer, thinner, and packed with more seeds. Similar medical uses are also found for *A. pulchellus* Wall. and *A. precatorius* **[12]**. *A. precatorius* traditionally used to treat gonorrhea, wounds, inflammations, purulent eye infections, cough, bronchitis, and hepatitis. Additionally, it is used as an aphrodisiac and oral contraceptive **[13]**.

MATERIAL AND METHODS

Plant collection and authentication

In the month of September and October, leaves of *A. pulchellus* Wall. were collected from Haldwani (Nainital), Uttarakhand, India and authenticated from the Northern Regional Centre of the Botanical Survey of India (BSI) in Dehradun having Accession No. 1306. The voucher specimen was deposited at B.S.I., Dehradun, in the Herbarium division for future reference.

Animals

Female Wistar rats weighing 160-180g were procured from the animal house of the Department of Pharmaceutical Sciences, Bhimtal, Kumaun University, Nainital. Animals were treated with care throughout the experimental procedures in accordance with the rules established by the Committee for the Control and Supervision of Experiments on Animals (CCSEA). The animals were housed in clean polypropylene cages at the animal house, which met basic living requirements. The temperature and humidity in the animal housing were kept at 25±2°C and 55±5% with an equal light and dark cycle (12:12). The rats were fed a regular diet of pellets and water **[14]**. The experimental methodology was approved by the Institutional Animal Ethics Committee of Kumaun University, Department of Pharmaceutical Sciences, Bhimtal Campus (KUDOPS/196).

Extraction of plant material

Plant material powder was extracted using a Soxhlet extractor, using organic solvents successively in increasing order of polarity (petroleum ether, ethyl acetate, and 70% ethanol). Petroleum ether was used for defatting of the plant material. The extracts were then subjected to rotary evaporator for removal of organic solvents and to obtain concentrated extract. Once the extracts were completely dried, their yields were quantified individually and kept at 4°C until they were needed for further investigation **[15]**.

The percentage yield was calculated as:

Percentage yield = Weight of extract/ Weight of plant material ×100

Phytochemical screening of different extracts

Extracts were analyzed for preliminary phytochemicals using a standard procedure after consecutive extraction. The plant extracts were screened for phytochemicals, including carbohydrates, alkaloids, tannins and phenolic compounds, steroids, proteins, flavonoids and saponins **[16]**.

Antimicrobial activity

The Institute of Microbial Technology (IMTECH) in Chandigarh, India provided the microorganisms. Bacterial cultures were *Staphylococcus aureus* (MTCC 3160), *S. epidermidis* (MTCC 435), *Pseudomonas aeruginosa* (MTCC 424), *Proteus vulgaris* (MTCC 426), *Vibrio vulnificus* (MTCC 15023) and fungal strains were *Candida albicans* (MTCC 183), *C. parapsilosis* (MTCC 1965), *C. tropicalis* (MTCC 9038), and *Aspergillus niger* (MTCC 8652).

Inoculum and culture media

The bacteria and fungi were kept on Tryptic Soya Agar (TSA; Hi-Media, India) and Potato Dextrose Agar (PDA; Hi-Media, India) respectively at 4°Cuntil they were employed in the study. Prior to use, the bacterial and fungal cultures were restored in Tryptic Soya Broth at 37°C and Potato Dextrose Broth at 28°C overnight respectively. To achieve a cell suspension of 10^6cfu/ml, the recently developed microbial cultures were suitably diluted in sterile broth medium.

Antimicrobial assay

0.1 ml of a diluted inoculum (10^6cfu/ml) of the test organism was disseminated on PDA/TSA plates using the glass spreader. Whatman paper discs (6 mm in diameter) that had been sterilized by dry heat sterilization were soaked in dimethyl sulfoxide (DMSO) used as a blank and extract dissolved in DMSO (70% ethanol extract and ethyl acetate extract separately) with 200 mg/ml concentration. The test system employed itraconazole discs (10 mcg/disc) for antifungal and cefotaxime discs (30 mcg/disc) for antibacterial activities used as a standard disc. Fungal plates were incubated for 48 hours at 28°C, whereas the bacterial plates were incubated for 24 hours at 37°Cin an incubator. Zone of inhibition (ZOI) against test organisms was measured to assess the antimicrobial activity. Triplicate experiments were carried out and the results were averaged **[17]**.

***In- vivo* wound healing activity**

Acute dermal toxicity studies

The *A. pulchellus* crude extract's acute cutaneous toxicity test was conducted in accordance with Organization for Economic

Cooperation and Development (OECD) guideline number 404 **[18]**. Three female rats with normal skin surfaces were randomly selected, housed in different cages, and allowed a week to acclimatize to their new environment. Approximately 10% of the body's hairy surface was removed when the dorsal part of the trunk was shaved 24 hours prior to the examination. 10% w/w of the extract formulation was applied to the shaved area every day for a full day. Every mouse was housed in its own habitat during the exposure period.

Following the removal of the residual test chemical at the end of the exposure period, the rats were observed daily for 14 days to check for any adverse skin reactions such as inflammation, irritation, or redness **[19]**.

Formulation of ointment

Two ointment formulations were made with varying quantities of extract: 5% (w/w) ointment, in which 5g of extract was included in 95g petroleum jelly, and 10% (w/w) ointment, in which 10g of extract was incorporated in 90g of petroleum jelly that had previously melted at 65°C on a water bath. The ointment composition was then thoroughly blended until homogeneity was achieved. For assessing the wound healing capacity of the extract in an excision wound model, betadine ointment (5% w/w) was used as the standard drug **[20]**.

Excision wound model

Grouped animals acclimatized to the working environment were anesthetized in an ether chamber. The rats back were depilated in the dorsal region, and a predefined 100 mm^2 full-thickness skin incision was made. The wound was cleansed with an ethanol-soaked cotton swab. Rat's wound was exposed to the open air without any clothing. The purpose of this model was to track wound contraction. Every day, the progressive changes in the wound region was observed. The proportion of the initial wound size that was reduced, used to illustrate wound contraction **[21]**.

Control Group: Animals treated with petroleum jelly.

Standard Group: Animals treated with 5% w/w betadine ointment.

Test Group 1 (T1): Animals treated with ethanolic extract ointment (5% w/w).

Test Group 2 (T2): Animals treated with ethanolic extract ointment (10% w/w).

If the wound size shrinks, it was recorded by drawing the injured margin on tracing paper. The percentage decrease in the wound following treatment pertaining to the wound at day zero was determined, and 100% was utilized in computation **[22]**. The following formula was used to determine the percentage wound contraction:

% Wound contraction = wound on day 0 – wound on day n / wound on day 0 × 100

Statistical analysis

Data analysis was carried out by using Graph pad 9.0 software. The results were represented as mean ± SEM (standard error of mean) for each group. Statistically significant of difference between the control and other experimental groups was assessed by one way ANOVA test. Statistical significance was determined at $p < 0.001$.

RESULTS AND DISCUSSION

Extractive values

For each gram of air-dried material, the amount of extractable matter was determined in milligrams. **Table 1** displays the results for the colour, appearance, and percentage yield of the ethanolic and ethyl acetate extracts.

Phytochemical screening

Preliminary phytochemical screening for the identification of the phytoconstituents using ethyl acetate and ethanolic extract of *A. pulchellus* was carried out using chemical methods which exhibited the presence of carbohydrates, glycosides, saponins, tannins and flavonoids. Results of preliminary phytochemical screening are shown in **Table 2**.

Determination of antimicrobial activity

Antimicrobial activity was evaluated through disc diffusion method. Only ethanolic extract showed the antibacterial activity against three bacterial strains i.e. *Staphylococcus epidermis*, *Vibrio vulnificus* and *Pseudomonas aeruginosa* **(Figure 1)**. Both extracts (ethanolic and ethyl acetate extract) have not shown activity against fungal strains. Results of antibacterial activity are shown in **Table 3** and results of antifungal activity are shown in **Table 4**.

Acute dermal toxicity studies

There was no evidence of skin toxicity (inflammation, irritation, or redness) following a 24-hour application of the extract's 10% formulation. Following a 48-hour observation period and 14 days of cage side monitoring, no indications, symptoms, or death were seen in the animals.

Determination of wound healing activity

Table 5 below displays the animals progress in healing their wounds after being treated with *A. pulchellus* leaf extract with ointment (5% and 10% w/w), simple ointment (control group), and betadine (standard medicine). The extract including ointment in varying doses was shown to have a considerably higher wound-contracting ability than the control group (i.e., the group that received only simple ointment). Beginning on the sixth day, the animal groups given treatment with 10% (w/w) extract ointment had increased wound healing, which was equivalent to the animal groups given treatment with the standard treatment, i.e., betadine ointment. The group given treatment with 10% (w/w) extract ointment saw a significantly higher percentage of wound contraction and a shorter wound closure time. On the twelfth day, the animals in the 5% (w/w) extract ointment treated group began to exhibit increased wound constriction **(Figure 2)**.

Table1: Colour, Appearance and Percentage Yield (w/w) of Different Solvent Extracts of *A. pulchellus*

S. No.	Solvents	Colour	Appearance	Percentage Yield
1.	Ethyl acetate	Dark green	Sticky	4.16
2.	70% Ethanol	Dark green	Sticky	23.08

Table 2: Results of Phytochemical Screening

S. No.	Phytoconstituents	Ethyl acetate extract	70% Ethanol Extract
1.	Carbohydrates	+	+
2.	Alkaloids	+	+
3.	Tannins and phenols	-	+
4.	Steroids	+	+
5.	Protein	-	-
6.	Flavonoid	+	+
7.	Saponin	+	+

Table 3: Determination of Antibacterial Activity

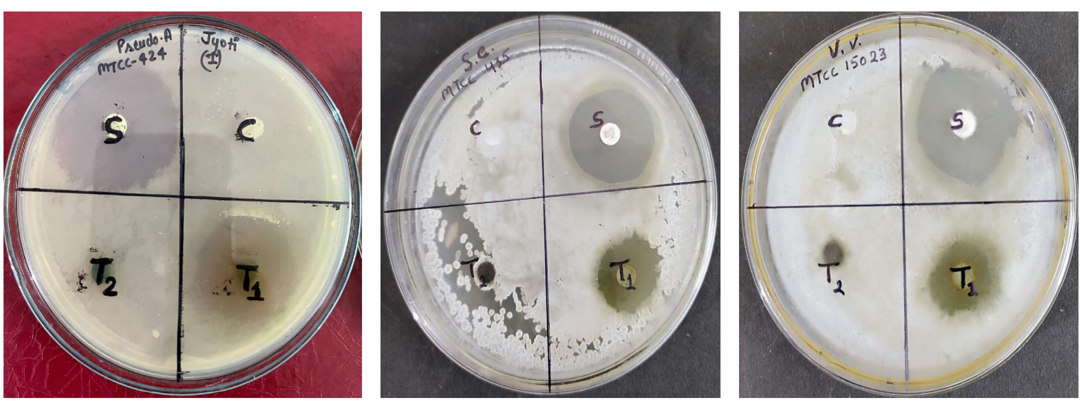
S. No.	Microorganisms	Zone of Inhibition (mm)			
		Standard	Control	T_1	T_2
1.	*Staphylococcus aureus*	30	-	-	-
2.	*Staphylococcus epidermis*	30	-	20	-
3.	*Pseudomonas aeruginosa*	35	-	28	-
4.	*Vibrio vulnificus*	28	-	18	-
5.	*Proteus vulgaris*	28	-	-	-

Disc diameter = 6 mm.
Standard – Cefotaxime (30 mcg/disc), Control – DMSO, T1 – 70% ethanol Extract, T2 – ethyl acetate extract

Table 4: Determination of Antifungal Activity

S. No.	Microorganisms	Zone of Inhibition (mm)			
		Standard	Control	T_1	T_2
1.	*Candida albicans*	30	-	-	-
2.	*Aspergillus niger*	30	-	-	-
3.	*Candida parapsilosis*	28	-	-	-
4.	*Candida tropicalis*	27	-	-	-

Disc diameter = 6 mm.
Standard – Itraconazole (10 mcg/disc), Control – DMSO, T1 – 70% ethanol Extract, T2 – ethyl acetate extract



A (*P. aeruginosa*) B (*S. epidermis*) C (*V. vulnificus*)

Figure 1: Antimicrobial activity of 70% ehanol and ethylacetate extract of *A. pulchellus* leaves.
C- Control (DMSO), S- Standard antibiotic disc (Cefotaxime 30 mcg/disc)
T1- 70% ethanol Extract, T2- ethyl acetate extract

Table 5: Effect of extract of *A. pulchellus* on excision wound model in Wistar rats

S. No.	Groups	Wound area in mm				
		4^{th} day	8^{th} day	12^{th}day	16^{th} day	21^{th} day
1.	Control	1.1 ±0.016	0.9 ±0.016	0.7 ±0.022	0.7 ±0.033	0.3 ±0.040
2.	Standard	1.0 ±0.021	0.8 ±0.021	0.5 ±0.030	0.4 ±0.049	0.0±0.000***
3.	Test 1	1.0 ±0.016	0.9 ±0.021	0.5 ±0.021	0.4 ±0.021	0.0±0.022**
4.	Test 2	1.0 ±0.021	0.8 ±0.021	0.5 ±0.021	0.3 ±0.030	0.0 ±0.000***

Control- Petroleum jelly, Standard- betadine, Test 1- 5% ointment of ethanolic extract of *A. pulchellus* leaves, Test 2- 10% ointment of ethanolic extract of *A. pulchellus* leaves, **p< 0.01, *** p< 0.001

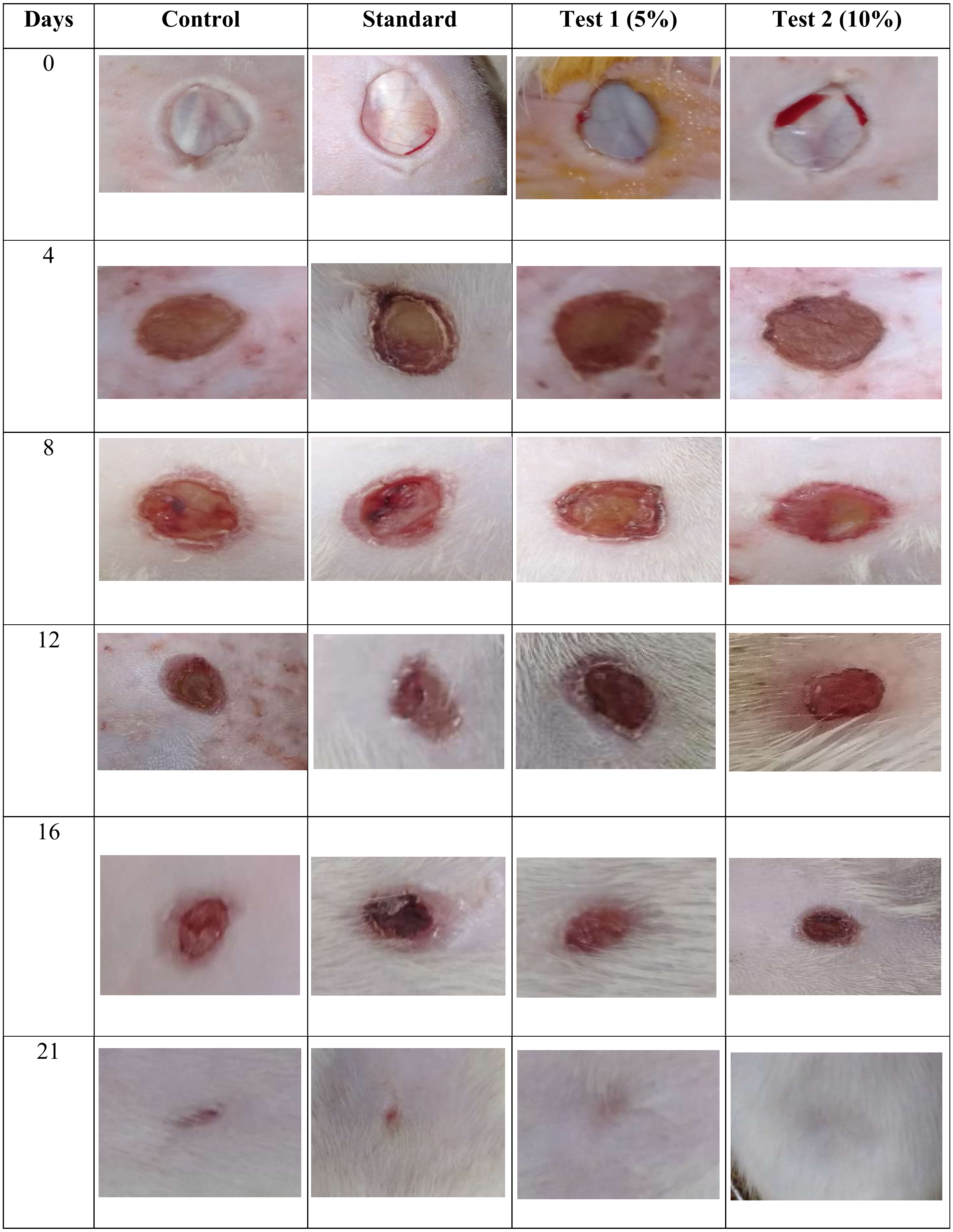


Figure 2: Physical examination of wound healing in control, standard, test1 and test 2 groups on different days Control – Petroleum jelly, Standard- Betadine ointment, T1- 5% ointment of 70% ethanol extract of *A. pulchellus* leaves, T2- 10% ointment of 70% ethanol extract of *A. pulchellus* leaves

CONCLUSION

The results of this investigation indicate that while *A. pulchellus* ethyl acetate extract lacked antibacterial action, its ethanolic extract was found to have antimicrobial activity against three different microorganisms: *Pseudomonas aeruginosa*, *Vibrio vulnificus*, and *Staphylococcus epidermidis*. *A. pulchellus* ethanolic extract increased angiogenesis, tensile strength, and the pace at which wounds contracted, all of which were indicators of wound healing activity. *A. pulchellus* was evaluated at doses (5% and 10%) for its impact on the different stages of wound healing. The *in vivo* studies on wound healing carried out on Wistar rats have indicated the significant potential of *A. pulchellus* ointment in wound healing. When compared to controls, *A. pulchellus* extract drastically accelerated the pace at which wound contracts and the epithelialization time ($p < 0.001$). The many stages of the healing process, including contraction, inflammation, fibroplasias, coagulation, macrophages, and epithelization, are interdependent. Treatment may influence the healing process in the process by mediating at least one stage of healing. The existence of flavonoids, steroids, terpenoids, and saponins in precise may be the cause of its antimicrobial and wound-healing properties. To identify the precise phytoconstituents which are accountable for the antibacterial and wound-healing attributes of the *A. pulchellus* plant, more research is necessary.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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