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Evaluation of *In Vitro* Wound Healing Activity in the Ethanolic Extract of *Aristolochia Bracteolata*

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Abstract: In our bodies, healing from wounds is a biological process that takes time. Medicinal plants heal wounds more quickly than the body's natural processes. One of the medicinal plants, *Aristolochia bracteloata,* is used to cure skin conditions, inflammation, and more. The plant's leaves are harvested and dried for two weeks in the shade. The leaves were thoroughly pulverised after being shade-dried, and extract was made using the soxhelt method with 70% ethanol as the solvent. Analyses of the extract's qualitative phytochemical composition were conducted. The plant extract contains saponins, glycosides, carbohydrates, phenols, courmarins, tannins, flavonoids, and anthocyanins, among other phytochemicals. Nevertheless, the leaf extract was discovered to be devoid of proteins, alkaloids, phytosterols, steroids, and terpenoids. The agar with the well diffusion method was used to test the extract's antibacterial and antifungal properties, and the extract displayed the zone of inhibition. The DPPH assay was used to test the antioxidant activity, and the extract showed free radical scavenging capabilities. The considerable acceleration of wound healing in the human cell line is investigated using the ethanolic extract of Aristolochia bracteolate. In L929 cells, the plant extract heals the lesion after 24 hours. In the wound healing scratch assay, the low concentration of the extract heals the wound more quickly than the high concentration of the extract.
Keywords: *Aristolochia Bracteloata*, Antioxidant Activity, Antimicrobial Activity, Wound healing scratch assay

1. Introduction

One of our body's major organs is the skin. Moreover, it serves as a sensory organ for touch and temperature perception and inhibits moisture loss. The skin can produce secretions that can kill bacteria, and the melanin pigment that is already present in the skin acts as a chemical pigment for protection against UV rays, which can harm skin cells. The skin's ability to regulate temperature is yet another crucial function. The blood vessels contract when the skin is exposed to cold temperatures. This permits blood to warm and regulates cutaneous warmth. The skin, a remarkable organ with multiple functions, always shields us from the outside world. An opening or break in the skin is referred to as a wound. Our bodies are shielded from germs by our skin. During surgery, if this skin is torn, pathogens may enter our bodies and spread infection. Deep wounds may impact the muscles, tendons, and ligaments. Our body's natural biological process

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of wound healing has four predetermined steps. Hemostasis, inflammation, proliferation, and remodelling. These four stages should take place in the correct order and amount of time for the wound to heal. Hemostasis is the process through which blood is transformed from its liquid state into a gel. The immune system is activated during the process of inflammation, which also regulates bleeding and fights infection. Rebuilding new tissues consisting of collagen and extracellular matrix is a process known as proliferation. The maturation stages known as remodelling are when the collagen changes from type III to type I and the wound completely closes. Many variables can interfere with one or more steps of the wound healing process, which can lead to faulty or subpar tissue restoration. Both acute wounds and delayed chronic wounds that show impaired healing typically haven't made it through all the necessary phases of recovery. Due to the incomplete, delayed, or uncoordinated wound healing process, wounds frequently enter a stage of pathological inflammation.[20][22][4][6][40]

These days, natural sources are being used to manufacture artificial wound healing agents. Many traditional healers, such as India and China, use wild herbs used to cure burns and wounds. Several plants were scientifically tested for their ability to treat wounds using various pharmacological techniques, however the majority of plants have not yet reached their full potential. In traditional medicine, *Aristolochia bracteolate* is crucial. To treat intestinal worms, insect bites, or skin itch, dried leaves are infused. Due to its trypanocidal and anthelmintic properties, it is referred to as "worm killer" in English. The plant can be found growing on riverbanks, in desert grasslands, and in woodlands at elevations between 50 and 740 metres above sea level. *Aristolochia bracteolate,* a climbing or prostrate perennial herb, has an offensive odour. From an underground rhizome, stems can develop to a height of 10 to 60 cm. The petiole is 0.5–4.5 cm long, and the leaves are oval and measure 1.5–8*1.5–7 cm. Flowers are trumpet-shaped, dark purple, tubular, and range in size from 0.5 to 5 cm. Oblong-ellipsoid capsules measure 1.5–2.5 cm. Moreover, it is used to treat wounds, arthritis, gout, achy joints, weight loss, intestinal pain, and snake bites. Because of its several pharmacological actions, it has been used for thousands of years in a variety of cultures as an indication. However, it was later found to be unquestionably hazardous. The herbal plant *Aristolochia bracteolata* has several different medical applications. So, in this investigation, microbiological and cell line tests were used to analyse the activity of *Aristolochia bracteolata.*[21][5]

2. Materials and Methods

2.1 Collection of plant samples: In the Coimbatore district, the plants were gathered at a roadside location close to a dwelling.

2.2 Preparation of the plant extract: *Aristolochia bracteolata* fresh leaves were properly washed, chopped into little pieces, and allowed to air dry for two weeks in the shade. For later use, the shade-dried leaves were pulverised and kept at room temperature in an airtight container.

2.3 Soxhlet extraction of *Aristolochia bracteolate* *Aristolochia bracteolata* sample that had been finely powdered was put in a porous bag and placed in the soxhlet apparatus. As a solvent,

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ethanol is used in the extraction process. The round bottom extraction flask was filled with 150ml of solvent and set on the heating mantle. The apparatus's sample-holding section was put within the extraction chamber. All of these components were positioned vertically and the condenser was affixed to the top of the extraction flask. A series of Soxhlet extractions were performed. In two to three days, the extraction was finished. When the solvent has evaporated and the extract has been dried in a hot air oven, the solvent is removed from the extract. The presence of phytochemical components and other testes were checked in the solvent extract.

2.4 Phytochemical analysis of the plant extract: Employing the conventional method to screen *Aristolochia bracteolate* leaves for the presence of active phytochemicals. Several other biochemical analyses, including those of flavonoids, alkaloids, tannins, saponins, steroids, terpenoids, proteins, glycosides, phenols, xanthoproteins, and carbohydrates, were conducted.[12]

2.5 Determination of antioxidant activity DPPH Scavenging assay: To 2ml of 0.15mm DPPH, 1ml of an ethanolic extract of a research plant in various quantities (20–1200 ppm) was applied. The blank was made by mixing 2 ml of DPPH with 1 ml of 50% ethanol. The mixture was shaken and after 30 minutes of incubation,the absorbance was measured spectrophotometrically at 517nm. The inhibition percentage, which represents the radical scavenging activity, was computed as follows: **Radical scavenging activity (%)** = 1-(A sample/A blank) × 100 Where A is 517nm absorbance. It was also determined the IC50 (mg mL), or the quantity of plant extract needed to lower the initial concentration of DPPH radicals by 50%.[21][30]

2.6 Determination of Antimicrobial activity:

2.61 Anti-bacterial Examination:

Staphylococcus aureus and *Streptococcus aureus* have been used for antibacterial activity tests . Mueller Hinton agar was used to make culture plates, which were then covered with the chosen bacterial and fungal strains. Plant extracts were added, placed in the petri dish, and kept for 24 hours at 37°C in the incubator. After the incubation period of 24 hours, the zone has formed. Zone of inhibition were assessed.[17][29]

2.62 Anti-fungal Examination: *Cryptococcus neoformans* and *Tricophyts interdigitale* have been used for this examination. Mueller Hinton agar was used to make culture plates, which were then covered in the chosen bacterial and fungal strains. The petri dish was filled with the plant extract and left there for 24 hours at 37°C in the incubator. After the incubation period of 24 hours, the zone has formed. Zone of inhibition were assessed .

***2.7 Aristolochia Bracteolate's* ability to heal wounds *in vitro* using a scratch assay. *Invitro* scratch assay:** The *in vitro* scratch is a simple, affordable, and well-established method for determining cell migration in the lab. The basic procedure entails making a scratch in a cell monolayer, taking images at the start and at regular intervals during cell migration to close the scratch, and comparing the images to quantify the migration rate of the cell. When compared to other methods, this one is a good way to mimic cell migration to monitor intracellular events, if needed.

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3. Results and Discussion

3.1 Phytochemical Analysis

Table 1: Presence or Absence of phytiochemical coumpounds

PHYTOCHEMICAL	Presence (+) /Absence (-)
Alkaloids	-
Saponins	+
Glycosides	+
Carbohydrates	+
Phenols	+
Proteins	-
Phytosterols	-
Coumarins	+
Tannins	+
Flavonoids	+
Terpenoids	-
Steroids	-
Anthocyanins	+

The qualitative phytochemical analysis of the ethanol leaf extract of *Aristolochia bracteolata* revealed the presence of many different phytochemicals, including saponins, glycosides, carbohydrates, phenols, coumarins, tannins, flavonoids, and anthocyanins. However, the leaf extract was found to be devoid of a few phytochemicals, including proteins, alkaloids, phytosterols, steroids, and terpenoids.

3.2 In vitro antioxidant activity of ethanolic leaf extract of *Aristolochia bracteolate:*

Table 2: DPPH Scavenging activity of ethanolic leaf extract of *Aristolochia bracteolata*

Sample concentration (µg/ml)	Percentage inhibition (%)
100	**8.29**
200	**15.54**
300	**34.45**
400	**43.10**
500	**63.21**

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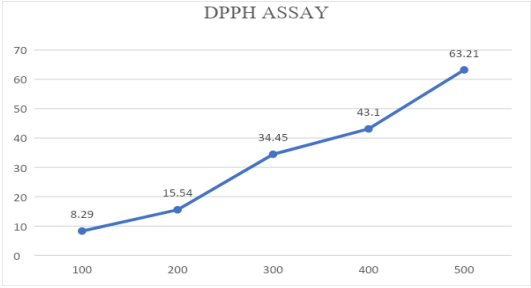
Percentage inhibition and the sample concentration value are plotted on the graph fig:1

Fig: 1 DPPH ASSAY



The rising antioxidant activity of *Aristolochia bracteolata* is depicted in this graph.

3.31 Antibacterial activity of ethanolic leaf extract of *Aristolochia bracteolate*: An approach known as well diffusion was used to measure antibacterial activity. Standard antibiotics created a zone of inhibition surrounding them. The inhibition zone's diameter is measured.

Fig 2: *Streptococcus aureus*

Fig 3: *Straphylococcus aureus*

3.32 Anti-fungal activity of the ethanolic leaf extract of *aristolochia bracteolata*

The well diffusion method was used to assess antifungal activity. Standard antibiotics created a zone of inhibition surrounding them.

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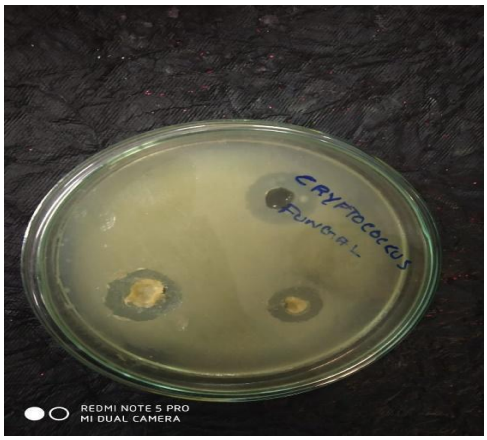

Fig 3: ***Crytococcus neoformans***

Fig 4: ***Tricophyst interdigitale***

The zone of inhibition is depicted in this image alongside a comparison of control elements. This shows the antimicrobial activity of plant sample. The zone formation was measured and tabulated in the following table 3.

Table 3: Antimicrobial activity in ethanolic extract of *Aristolochia bractelota*

MICRO ORGANISM	ZONE OF INHIBITION(MM)	CONTROL ANTIBIOTIC
straphylococcus aureus	**13**	**20**
streptococcus aureus	**9**	**10**

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crytococcus neoformans	**6**	**08**
tricophyst interdigitale	**8**	**12**

3.4 *INVITRO* WOUND HEALING EXAMINATION

The plant sample was examined in the cell line and resulted as:

Fig 5: *Invitro* wound scratch assay: Cell line: L929 Sample Name: *Aristolochia bracteolate*

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ss

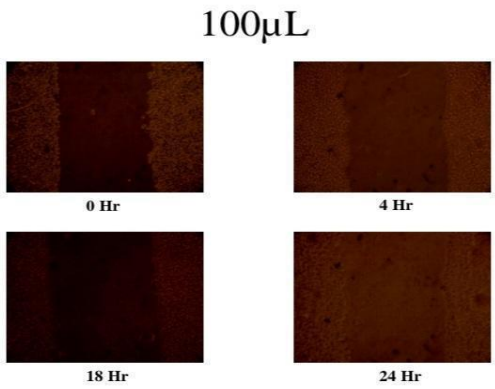

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Table 4: INVITRO WOUND HEALING ACTIVITY

TIME DURATION (h)	WOUND AREA (μm)	0h (%)	4h (%)	18h (%)	24h (%)
Concentration(μg/μl)					
25	1927	0	12	37	40
50	1699	0	19	39	41
75	1945	0	13	25	30
100	1879	0	9	12	20

In L929 cells, the wound is healed by the provided plant sample of *Aristolochia bracteolata* after 24 hours.

4. Conclusion

The wound heals more quickly from a 25g concentration than from a 100g concentration. where this 25 g concentration heals 40% in 24 hours, which is significantly more than the 100 g concentration's 20% healing rate. The ethanolic extract of *Aristolochia bracteolota* has the ability to promote wound healing. However, higher concentrations of plant extract do not heal wounds as well as smaller concentrations do.

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