RESEARCH PAPER

Traditional Medicinal Importance, Phytochemical Profile, and Antimicrobial Activity of *Aristolochia bracteolata* Leaf Extract

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Abstract:
Aristolochia bracteolata is one of the most widely used medicinal plants, in India it is used for the treatment of fever, inflammation, skin diseases, insect bites, and helminthic infections. The phytochemicals in the leaf extraction were determined using stranded protocols, and antimicrobial efficiency was determined by the well diffusion techniques. The results of this work concluded that the leaf extract consists of pharmacologically active compounds that might be useful against microbes as traditional medicine.
Keywords: Aristolochia bracteolata, Phytochemical screening, Antimicrobial activity, Traditional medicine, Leaf extract.
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INTRODUCTION:

Aristolochia bracteolata Lam. Is a medicinal plant belonging to the family Aristolochiaceae and is widely distributed in India, Sri Lanka, and tropical African regions (Burkill et al., 1985, Kokwaro, 2009: Taha et al., 2019). Traditionally, the plant has been used for the treatment of fever, inflammation, skin diseases, insect bites, helminthic infections, and various microbial disorders (Iwu, 1993:Duke 2019 and Rajasekhar et al., 2017). Different parts of the plant possess significant pharmacological properties including antimicrobial, antioxidant, anti-inflammatory, antiparasitics, and antipyretic activities due to the presence of biologically active phytochemicals such as alkaloids, phenolic compounds, tannins, saponins, and steroids (Kavitha et al., 2010: Sawarkar et al., 2019 and Nandhini et al., 2019). Medicinal plants serve as an important source of therapeutic agents and continue to play a vital role in modern drug discovery (Sofowara, 1993: Srivastava et al., 1996). Plant- derived phytochemicals exhibit a wide range of biological activities including antibacterial, antifungal, antioxidant, antiviral, and anti -inflammatory effects. Although several medicinal plants have been investigated for the synthesis of bioactive materials, limited information is available regarding the therapeutic potential of products synthesized using *Aristolochia bracteolata* leaf extract. Therefore, the present study was undertaken to prepare bioactive materials using the methanolic leaf extract of *Aristolochia bracteolata* and evaluate their antimicrobial, antioxidant, anti-inflammatory, and physicochemical properties.

MATERIALS AND METHODS:

Plant Material Collection and Extraction:

Fresh leaves of *Aristolochia bracteolata* were collected from the Seshachalam forests near Sathyavedu, Andhra Pradesh, India. The plant material was washed thoroughly with tap water; shade dried at room temperature, and powdered using an electric blender. For aqueous extraction, 20g of powdered leaf material was mixed with 200 ml of deionized water and heated at 100^0c for 20-30 min. The extract was filtered through Whattman No.1 filter paper and stored at 4^0C until further use. For methanolic extraction, 400 g of leaf powder was soaked in 400 ml methanol and incubated for 24 h at room temperature with intermittent shaking. The extract was filtered through Whattman No.1 filter paper and concentrated extract was stored at 4^0C (Harborne, 1998: Sasidharan et al., 2011).

Phytochemical Screening:
The phytochemical analysis was performed to determine the bioactive compounds present in the leaf extract of *Aristolochia bracteolata*(Harborne, 1998: Trease, 2002).
Test for carbohydrates:Molisch's test: 1 ml of Molisch's reagent and a few drops of concentrated H_2SO_4 added to 2ml of plant leaf extract, formation of violet colour at the junction of two layers indicates the presence of carbohydrates.
Test for reducing sugars: Benedict's test: 1 ml of Benedict's reagent was added to 2ml of leaf extract and heated in the water bath for 5 minutes; the formation of brick-red colour indicates the presence of reducing sugars.
Test for Anthraquinone glycosides: Borntrager's test: A few drops of diluted H_2SO_4 were added to the 2 ml of leaf extract and heated for 5 minutes, filtered and cooled with an equal volume of dichloromethane; the formation of rose pink to red colour indicates the presence of Anthraquinone glycosides.
Test for Saponins: Froth test: To the 2 ml of leaf extract, 2ml of distilled water was added and shaken for 15 minutes. The formation of foam up to 1cm indicates the presence of Saponins.
Test for proteins: Biuret's test: 1ml of NaOH (10%) was added to 2ml of leaf extract and heated, later few drops of $CuSO_4$ (0.7%) were added, formation of a purplish violet colour indicates the presence of proteins.
Test for steroids: Libermann-Burchard test: A few drops of acetic anhydride were added to plant leaf extract, boiled and cooled later few drops of concentrated H_2SO_4 were added, The formation of brown ring at the junctions of two-layer and the upper layer turning green indicates the presence of steroids.
Test for tannins and phenolic compounds: Iodine test: A few drops of diluted iodine solution were added to the leaf extract of Aristolochia bracteolata. The formation of transient red colour indicates the presence of tannins and phenolic compounds.
Test for alkaloids: Wagner's test: 5 ml of diluted HCl was added to the plant leaf extract and filtered, later few drops of Wagner's reagent were added, and formation of reddish brown precipitate indicates the presence of alkaloids.
Test for flavonoids: Shinoda's test: Ethanol (95%), 0.5gms of magnesium and a few drops of concentrated HCl were added to the plant leaf extract, the formation of pink colour indicates the presence of flavonoids.
Antibacterial activity: The antibacterial activity of plant leaf extract was tested by using the well diffusion method against *E. coli, Klebsiella pneumonia*, (Gram negative), *S. aureus*, and *Bacillus subtilis* (Gram-positive). The bacterial cultures are obtained from the Department of Microbiology, S.V. University, and Tirupati. The bacterial cultures were spread evenly on the nutrient agar plate by using an L-rod then different concentration of plant leaf extract was loaded into the wells of the agar medium. Later the plates were incubated at 37°C for 24 hours. Amoxicillin was used as an Antibiotic. After incubation, the zone of inhibition was measured (Perez et al., 1990).
Antifungal activity: The antifungal activity of plant leaf extract was tested against *Aspergillus* species by using the well diffusion method. The fungal cultures are obtained from the Department of Microbiology, S.V. University, and Tirupati. The spore suspension of *Aspergillus* species was spread evenly on a potato dextrose medium by using L-rod. Later, wells were made in the PDA medium using a sterile borer then different concentrations of plant leaf extract were added. Fluconazole was used as an Antibiotic. The plates were incubated at room temperature for 5 – 7 days. After incubation, the zone of inhibition was measured (Valgas et al., 2007).

Results & Discussion:
Phytochemical Analysis:
The phytochemical analysis of the plant leaf extract revealed the presence of carbohydrates, saponins, proteins, steroids, alkaloids, phenolic compounds, tannins and the absence of anthraquinone glycosides, flavonoids and reducing sugars. The phytochemical analysis is performed in duplicates (Sofowara, 1993: Herborne. 1973: Okwu, 2001 and Rahilla et al., 1994). The results are shown in table-1 and Figure.1.
Table. No: 1 Phytochemical analysis of ***Aristolochia bracteolata***leaf extract

Phytochemicals	Test	Observation	Result
Carbohydrates	Molisch's test	Violet colour	+
Reducing sugars	Benedict's test	Colour change Negative	-
Anthraquinone Glycosides	Borntager's test	Colour change negative	-
Saponins	Froth test	Formation of foam	+
Proteins	Biuret's test	Purplish violet colour	+
Steroids	Libermann-Burchad's Test	Reddish brown green rings	+

Tannins & Phenolic compounds	Iodine Test	Transient red colour	+
Alkaloids	Wagner's test	Reddish Brown Precipitate	+
Flavonoids	Shinoda's test	Colour change negative	-

(+) – Positive and (-) – Negative

Figure 1: Phytochemical analysis of ***Aristolochia bracteolata***leaf extract

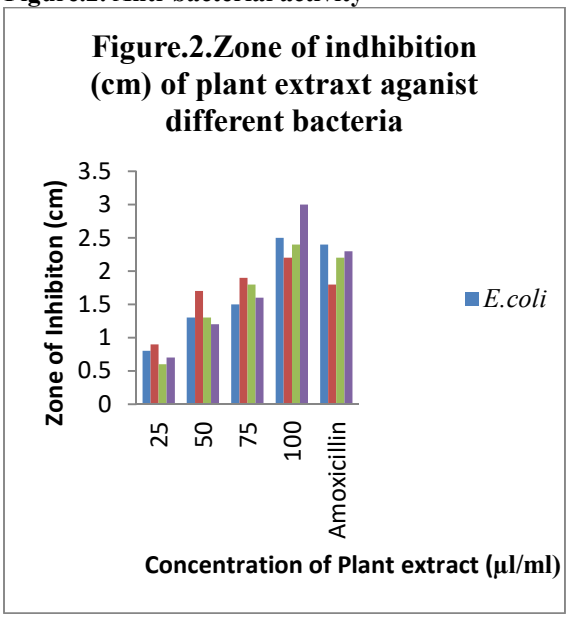
Antibacterial Activity:

The antibacterial activity of plant leaf extract against the bacterial strains was studied and the amoxicillin used as stranded, the results are shown in Table 2 and figure.2. The plant leaf extract of *Aristolochia bracteolata* exhibited excellent antibacterial activity against both Gram-positive and Gram-negative bacteria. At the lowest concentration of plant leaf extract the zone of inhibition for gram-positive bacteria was 0.8 to 1.5 cm and for gram-negative bacteria was 0.6 to 1.8 cm, at the highest concentration the zone of inhibition for gram-positive bacteria was 0.9 to 2.2 cm and for gram-negative bacteria was 0.9 to 3.0 cm. With increasing the concentration of plant leaf extract, the zone of inhibition also increased. The antibacterial activity of leaf extract was better than the control. Similar reports were seen in Physicochemical Characterization and Antibacterial Activity of *Aristolochia bracteolata* ((Ishaku et al., 2016).

Table. No: 2 Antibacterial activity of ***Aristolochia bracteolata*** leaf extract:

Concentration plant leaf extract (µl/ml)	Zone of inhibition(cm)			
	E.coli	*Bacillus subtilis*	*S.aureus*	*Klebsiella pneumoniae*
25	0.8	0.9	0.6	0.7
50	1.3	1.7	1.3	1.2
75	1.5	1.9	1.8	1.6
100	2.5	2.2	2.4	3.0
Amoxicillin (Stranded)	2.4	1.8	2.2	2.3

Figure.2. Anti-bacterial activity

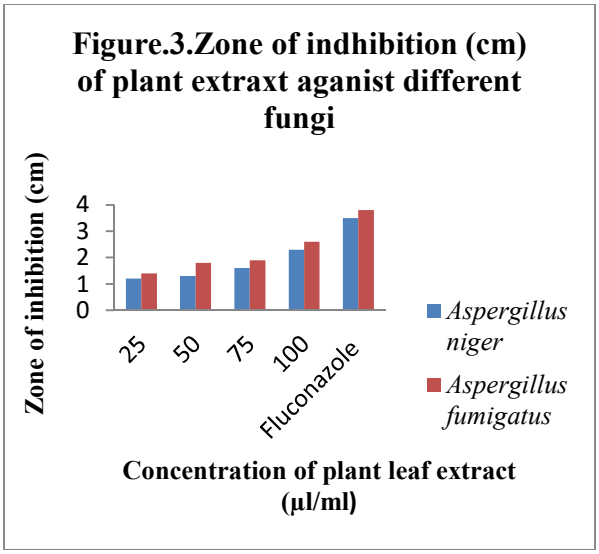


Antifungal Activity:

The antifungal activity of plant leaf extract was assessed against *Aspergillus niger* and *Aspergillus fumigatus* was showed in Table.3 and figure.3,Fluconazole is used as stranded. At the lowest concentration, the zone of inhibition was shown *Aspergillus fumigatus* and the highest fungal activity was shown in *Aspergillus niger* 3.8 cm against both fungal strains. The results indicate by referring antifungal activity of *Aristolochia bracteolata* that crude extract was enhanced the antifungal potential of the plant extract (Javaid et al., 2022).

Table No: 3 Antifungal activity of ***Aristolochia bracteolata*** leaf extract

Concentration plant leaf extract (µl/ml)	***Aspergillus niger***	***Aspergillus fumigatus***
25	1.2	1.4
50	1.3	1.8
75	1.6	1.9
100	2.3	2.6
Fluconazole	3.5	3.8



Conclusion: Based on this study results it is confirmed that the plant leaf extract of Aristolochia bracteolata consists of phytochemicals such as carbohydrates, proteins, saponins, alkaloids, tannins, and phenolic compounds. The leaf extract has the ability to inhibit microbes such as bacteria and fungi and it might be used as antibiotics and antifungal agents clinically up on further research and studies.

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