International Journal of Pharmacology and Clinical Research (IJPCR)
IJPCR | Vol.10 | Issue 1 | Jan – Mar - 2026
www.ijpcr.com
ISSN: 2349-5448
DOI : https://doi.org/10.61096/ijpcr.v10.iss1.2026.35-43

In Vitro Evaluation of The Anti-Fungal Activity of Hydroalcoholic Extract of *Aristolochia Bracteolata. Lam* Against Trichophyton Species

S. Selvakumar[*1], Vineka. M[2], Vinothini. S[2], Vishali. B[2], T. Venkata Rathina Kumar[3]

[*1]Assistant professor, Institute of Pharmacology, [2]Final year B. Pharm, [3]Principal,
Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai.
Affiliated to The Tamil Nadu Dr. MGR Medical University, Chennai-600032.

[*]Author for correspondence: Dr. S. Selvakumar, M. Pharm, Ph.D.,
Email: sankaranselvakumar@gmail.com

Published by:
27.03.2026

Abstract: Ringworm infection is one of the most common superficial fungal infections affecting the skin, hair, and nails. Among the dermatophytes, Trichophyton rubrum is considered the predominant causative organism responsible for ringworm infections worldwide. The increasing resistance to conventional antifungal drugs and their associated side effects have encouraged the search for alternative therapies derived from medicinal plants. The present study was designed to evaluate the antifungal activity of the hydroalcoholic extract of Aristolochia bracteolata against Trichophyton rubrum under in- vitro conditions. The plant extract was prepared using the Soxhlet extraction method and subjected to antifungal screening through disc diffusion, minimum inhibitory concentration (MIC), and time-kill assay techniques. The results demonstrated that the hydroalcoholic extract exhibited measurable antifungal activity against the test organism, showing concentration-dependent inhibition of fungal growth. The MIC analysis confirmed that higher concentrations of the extract significantly reduced fungal proliferation, while the time- kill assay revealed progressive reduction in fungal colony counts with increasing exposure time. The presence of bioactive phytochemicals such as flavonoids, alkaloids, and phenolic compounds may contribute to the antifungal effect. These findings suggest that Aristolochic bracteolata may serve as a potential natural source for the development of antifungal agents against dermatophytic infections.

Keywords: *Aristolochia bracteolata*, Antifungal activity, *Trichophyton rubrum*, Dermatophytes, Disc diffusion method, Minimum Inhibitory concenteration (MIC), Time kill assay.

INTRODUCTION:

Fungal infections are a major health concern affecting millions of people worldwide. Among these infections, dermatophytosis is one of the most common superficial fungal diseases that affects keratinized tissues such as the skin, hair, and nails. Dermatophytosis is caused by a group of fungi known as dermatophytes, mainly belonging to the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*. Among these

species, *Trichophyton rubrum* is considered the most prevalent pathogen responsible for chronic dermatophytic infections in humans.[1]

Although a variety of antifungal drugs are available for the treatment of fungal infections, their long treatment duration, potential side effects, and the increasing emergence of drug-resistant fungal strains remain major challenges. Because of these limitations, researchers are increasingly focusing on alternative therapeutic approaches, particularly those derived from natural sources.[2,3]

Medicinal plants have long been used in traditional medicine for the treatment of various infectious diseases. These plants contain a wide range of bioactive phytochemicals such as alkaloids, flavonoids, tannins, and phenolic compounds, which are known to exhibit antimicrobial and antifungal activities. One such medicinal plant is *Aristolochia bracteolata*, which has been traditionally used for the treatment of skin disorders, infections, and inflammatory conditions. Due to its therapeutic potential and the presence of various bioactive constituents, *Aristolochia bracteolata* is considered a promising natural source for the development of antifungal agents.[4]

RINGWORM INFECTIONS:

Ringworm infection, also known as dermatophytosis, is a common superficial fungal infection that affects keratinized tissues such as the skin, hair, and nails. In Tamil, ringworm infection is commonly referred to as "**Padarthamarai**" (**படர்தாமரை**), meaning a spreading circular lesion resembling a lotus shape. It is caused by a group of fungi known as dermatophytes, mainly belonging to the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*. Among these, Trichophyton rubrum is considered the most common species responsible for dermatophytic infections in humans worldwide. [5]

Dermatophytic infections are commonly referred to as **tinea** infections, and they are classified based on the part of the body affected. For example, **tinea corporis** affects the body**, tinea pedis** affects the feet (commonly known as athlete's foot), **tinea capitis** affects the scalp, **tinea cruris** affects the groin region, and **tinea unguium** affects the nails. These infections usually appear as circular or ring-shaped lesions with redness, scaling, itching, and sometimes inflammation on the affected area of the skin.

Ringworm spreads easily through direct contact with infected individuals, animals, or contaminated objects such as clothing, towels, bedding, and surfaces. The infection is more common in warm and humid climates, where fungal growth is favored. Factors such as poor personal hygiene, excessive sweating, weakened immune system, and prolonged moisture exposure can increase the risk of developing tinea infections.[6]

TRICHOPHYTON SPECIES:

Trichophyton species are a group of filamentous fungi that belong to the dermatophyte family and are responsible for many fungal infections affecting humans. These fungi have the ability to invade keratinized tissues such as the skin, hair, and nails because they produce enzymes that can break down keratin. As a result, they cause a variety of infections collectively known as dermatophytosis or ringworm infections.[7]

The genus Trichophyton includes several species that are medically important, such as Trichophyton rubrum, Trichophyton mentagrophytes, and Trichophyton tonsurans. Among these, Trichophyton rubrum is considered the most common cause of dermatophytic infections worldwide. It is responsible for many forms of tinea infections including tinea corporis (ringworm of the body), tinea pedis (athlete's foot), tinea cruris (jock itch), and tinea unguium (fungal nail infection).

Infections caused by Trichophyton species are usually characterized by itching, redness, scaling, and ring-shaped lesions on the affected areas of the skin. These fungi spread easily through direct contact with infected individuals, animals, or contaminated surfaces such as clothing, towels, and floors. Warm and humid environmental conditions, poor hygiene, excessive sweating, and weakened immune systems can increase the risk of infection.[8]

PLANT PROFILE:

Botanical Description: *Aristolochia bracteolata* Lam. is a perennial herb belonging to the family Aristolochiaceae. It is commonly found in dry regions and grows as a prostrate or climbing herb.

Taxanomical classification:

KINGDOM	Plantae
DIVISION	Tracheophyta
CLASS	Magnoliopsida
SUBCLASS	Magnoliidae
ORDER	Piperales
FAMILY	Aristolochiaceae
SUBFAMILY	Aristolochioideae
GENUS	Aristolochia
SPECIES	A.bracteolata Lam[9]

Distribution: The plant is widely distributed in India, Africa, and other tropical regions. In India, it is commonly found in states such as Tamil Nadu, Kerala, Gujarat, and Karnataka.[11]

Vernacular name:

TAMIL	Aaduthinnapalai, Perumaruntu, Eshwaramuli
ENGLISH	Worm killer, Duchman's Pipe, Bracteated birthwort
MALAYALAM	Aduthinnappalai, Eeswaramooli, Karalekam
TELUGU	Ishvara-veru, Dulagovila
KANNADA	Adu Muttada gida, Kurigida, Kattehotte gida[10]

Phytoconstituents: The hydroalcoholic extract of Aristolochic bracteolata contains a variety of bioactive phytochemical constituents that may contribute to its antifungal properties. Preliminary phytochemical screening of the extract has revealed the presence of important secondary metabolites such as alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, steroids, and glycosides. In addition, certain specific compounds reported in the plant include aristolochic acids, aristo lactams, and essential phenolic derivatives.[12]

Among these phytochemicals, flavonoids and phenolic compounds are widely known for their antimicrobial and antioxidant activities, which may help inhibit fungal growth. Tannins possess strong astringent and antimicrobial properties that can interfere with fungal cell wall formation. Alkaloids and terpenoids are also known to disrupt microbial cell membranes and metabolic pathways, thereby contributing to antifungal activity.

The combined action of these phytochemical constituents present in the hydroalcoholic extract may be responsible for inhibiting the growth of dermatophytes such as Trichophyton species. Therefore, the presence of these bioactive compounds supports the potential use of Aristolochic bracteolata as a natural source for the development of antifungal agents against dermatophytic infections.[13]

Ethnomedicinal Uses: Aristolochia bracteolata has been traditionally used in various systems of medicine for the treatment of skin diseases, fungal infections, wounds, and inflammatory conditions. The plant is also used for managing intestinal worms, fever, and snake bites. Traditionally, the leaves are applied externally to treat ringworm and other skin infections. These uses indicate the potential antimicrobial and antifungal properties of the plant.[14]

MATERALS AND METHODS:

Plant Collection: Fresh aerial parts of Aristolochia bracteolata were collected from the Madurai region of Tamil Nadu, India. The collected plant material was authenticated and thoroughly washed with distilled water to remove dust and other impurities.

Preparation of Plant Extract:

SOXHLET EXTRACTION: The collected aerial parts were shade dried at room temperature for several days until complete drying was achieved. The dried plant material was then powdered using a mechanical grinder to obtain a coarse powder. The powdered material was subjected to hydroalcoholic extraction using a Soxhlet apparatus with a solvent mixture of 70% ethanol and 30% distilled water. The extraction process was carried out for several cycles until the solvent became colorless. The obtained extract was filtered and concentrated using a rotary evaporator to obtain a semi-solid hydroalcoholic extract, which was stored for further antifungal activity studies.[15]

Preparation of Fungal Culture: The fungal strain *Trichophyton rubrum* was obtained from a microbiology laboratory culture collection. The organism was cultured and maintained on Muller Hinton Agar + 2% Glucose plate medium under sterile laboratory conditions. The fungal culture was incubated at an appropriate temperature for adequate growth before conducting antifungal experiments.

Evaluation of Antifungal Activity

1. Disc Diffusion Method:

The antifungal activity of the hydroalcoholic extract was evaluated using the disc diffusion method. Sterile Muller Hinton Agar + 2% Glucose plates were inoculated with the fungal suspension of *Trichophyton rubrum*. Sterile filter paper discs were impregnated with different concentrations of the plant extract and placed on the inoculated agar plates.

The plates were then incubated at suitable temperature conditions for fungal growth. After incubation, the plates were observed for the formation of zones of inhibition, which indicated the antifungal activity of the plant extract.[16,17]

2. Minimum Inhibitory Concentration:

Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that inhibits visible growth of a microorganism after incubation. In the present study, MIC of the hydroalcoholic extract of *Aristolochia bracteolata* against *Trichophyton rubrum* was determined using the broth dilution method. Different concentrations of the extract were prepared in Sabouraud dextrose broth. Each tube contained plant extract, fungal culture, and broth medium, while control tubes contained culture without extract and media alone. The tubes were incubated at 30°C for 24 hours. After incubation, fungal growth was evaluated by measuring optical density at 620 nm using a spectrophotometer. A decrease in optical density indicated inhibition of fungal growth. The lowest concentration that inhibited fungal growth was considered as the MIC of the extract.[18,19]

3. Time-Kill Assay:

The time-kill assay was performed to evaluate the rate of fungal killing by the plant extract over time. In this method, fungal cultures were exposed to different concentrations of the extract and incubated under controlled conditions. At predetermined time intervals, samples were taken and plated on agar media to determine the number of viable fungal colonies. The reduction in colony-forming units over time indicated the fungicidal activity of the plant extract.[20]

RESULT AND DISCUSSION:

IN-VITRO ANTI-FUNGAL ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *ARISTOLOCHIA BRACTEOLATA:*

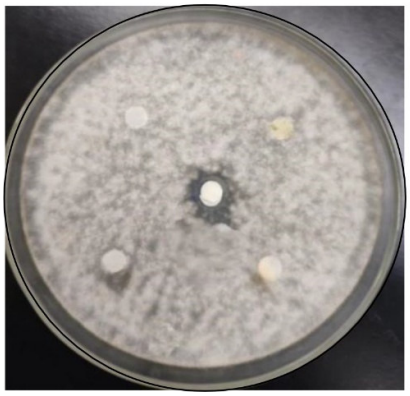
1. DISC DIFFUSION METHOD:

The results for the zone of inhibition of hydro alcoholic extract of *Aristolochic bracteolata* and standard drug Itraconazole were depicted in the following table.

S.NO	SAMPLE	ZONE OF INHIBITION IN mm				
		Trichophyton rubrum				
		Positive control	**Negative control**	**12.5 mg**	**25 mg**	**100 mg**
1	*Aristolochia bracteolata*	16mm	No zone	9mm	No zone	No zone

Note: PC-Positive control, NC-Negative control

1. 12.5 mg
2. 25 mg
3. 100 mg



DISCUSSION:

The results of the antifungal study revealed that the *Aristolochia bracteolata* sample exhibited zone of inhibition against the organism *Trichophyton rubrum*.

The above study revealed that the given sample, *Aristolochic bracteolata* of concentration 12.5mg exhibited zone of inhibition against the tested organism (*Trichophyton rubrum*), which indicates it can act as an antifungal agent.

2. MINIMUM INHIBITORY CONCENTERATION:

The results for the minimum inhibitory concenteration for the hydro alcoholic extract of *Aristolochia bracteolata* were depicted in the table.

S.NO	CONCENTRATION IN mg/ml	O.D VALUES
1	2 mg/ml	0.29
2	4 mg/ml	0.12
3	8 mg/ml	0.06
4	Blank control (only media)	0.00

DISCUSSION:

The present study demonstrated that *Aristolochic bracteolata* possesses concentration-dependent antifungal activity against *Trichophyton rubrum* as determined by the broth dilution method. A progressive reduction in optical density (OD) values was observed with increasing extract concentrations, with OD readings of 0.29 at 2 mg/ml, 0.12 at 4 mg/ml, and 0.06 at 8 mg/ml, while the blank control showed no turbidity (0.00). This decline in OD indicates effective suppression of fungal growth in a dose-responsive manner. The lowest concentration exhibiting marked inhibition was 8 mg/ml, which can be considered the Minimum Inhibitory Concentration (MIC). Although the extract shows promising antifungal potential, the relatively higher MIC value suggests moderate potency in crude form, warranting further phytochemical characterization, purification of active constituents, and additional antifungal evaluations for therapeutic relevance.

3. TIME KILL ASSAY:

The calculated CFU values were then tabulated for different concentrations of *Aristolochia bracteolata* extract at different time intervals to determine the killing kinetics of the test compound.

SAMPLE	6 hrs	12 hrs	24 hrs
Control	2 big spore colonies	TNTC (>3 × 10^5 CFU/ml)	TNTC
0.5 × MIC	~10 colonies (≈ 1 × 10^4 CFU/ml)	<5 colonies (≈ 5 × 10^3 CFU/ml)	No CFU
1 × MIC	3 colonies (≈ 3 × 10^3 CFU/ml)	<3 colonies (≈ 1–2 × 10^3 CFU/ml)	No CFU
2 × MIC	<3 colonies (≈ 1 × 10^3 CFU/ml)	No CFU	No CFU

TNTC – Too Numerous To Count

INCUBATED PLATES IN TIME INTERVALS 6 HOURS

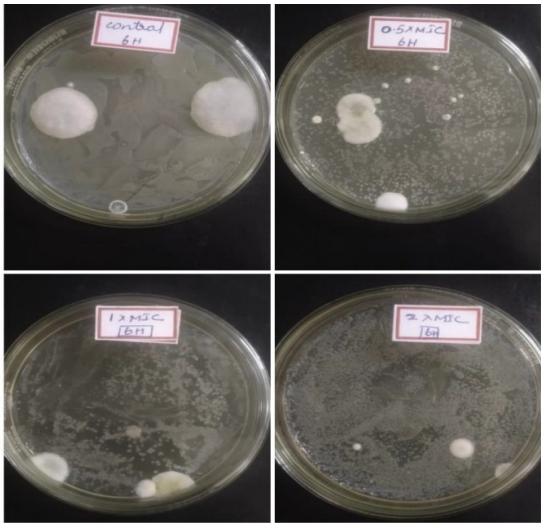
6 HOURS

CONTROL – TWO BIG MOULD SPORES

0.5 X MIC - 10 *Trichophyton* Colonies

1 x MIC - 3 *Trichophyton* Colonies

2 x MIC - Less than 3 *Trichophyton* Colonies



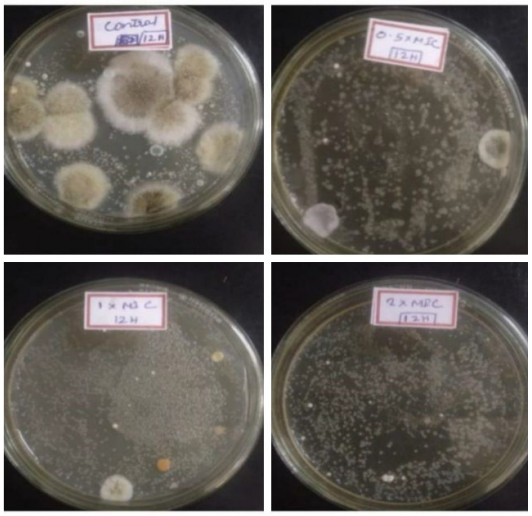
12 HOURS

CONTROL – TNTC

0.5 X MIC - Less than 5 *Trichophyton* Colonies

1 x MIC - Less than 3 *Trichophyton* Colonies

2 x MIC - No *Trichophyton* colonies



24 HOURS

CONTROL – TNTC

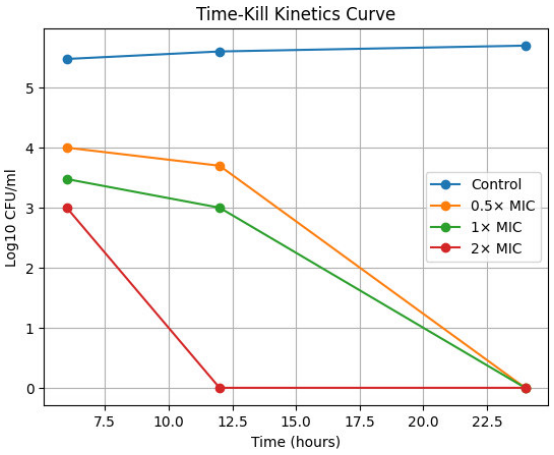
0.5 X MIC - No *Trichophyton* colonies

1 x MIC - No *Trichophyton* colonies

2 x MIC - No *Trichophyton* colonies

The Time-kill assay demonstrated a progressive reduction in viable fungal cells with increasing concentrations of the extract of *Aristolochia bracteolata* and longer incubation times. The control plates showed heavy fungal growth at all time intervals, indicating normal growth of the organism in the absence of the test compound. At **0.5 × MIC**, the number of colonies decreased gradually from 6 hours to 12 hours, and no viable colonies were observed after 24 hours. At **1 × MIC**, only a few colonies were observed at 6 hours, which further decreased at 12 hours, and no colony formation was detected at 24 hours. The highest concentration (**2 × MIC**) exhibited the strongest antifungal activity, showing very few colonies at 6 hours and complete inhibition of fungal growth after 12 hours. These results indicate a **time-dependent and concentration-dependent reduction in fungal viability**.

Time-Kill Kinetics Graph:



The time-kill kinetics curve was plotted to evaluate the antifungal activity of the extract of *Aristolochia bracteolate* against *Trichophyton rubrum.* The graph represents the relationship between incubation time (6, 12, and 24 hours) and the logarithmic colony forming units (Log CFU/ml).The control showed a continuous increase in fungal population over time, indicating normal growth of the organism. In contrast, the treated samples exhibited a progressive reduction in viable fungal cells with increasing concentration of the plant extract. The **0.5× MIC** showed gradual inhibition, whereas **1× MIC** demonstrated stronger suppression of fungal growth. The **2× MIC concentration showed rapid killing,** with no detectable colonies after 12 hours. This indicates that the antifungal activity of the extract is **both concentration-dependent and time-dependent**.

JUSTIFICATION:

The bacterial colonies observed on the plates should not be mistaken for the growth of ***Trichophyton rubrum***. The bacterial growth is likely due to normal contamination from the sample or environmental exposure during handling. Additionally, the prolonged incubation period of 72 hours on **Potato Dextrose Agar** may have favored bacterial proliferation. Therefore, the observed bacterial colonies are considered incidental contamination and not related to the fungal growth being studied.

Standard interpretation

- Fungicidal activity → ≥ 3 $\log_{10}$ reduction in CFU/ml (99.9% killing) compared to the control.
- Fungistatic activity → Growth is inhibited but CFU reduction is less than 3 $\log_{10}$.

Based on the observation,

The time-kill assay demonstrated that the extract of ***Aristolochia bracteolata*** exhibited **fungicidal activity** against ***Trichophyton rubrum***, as a complete reduction in viable colony-forming units was observed at higher concentrations within 24 hours. The killing effect was both **time-dependent and concentration-dependent**.

DISCUSSION:

The time-kill assay results indicate that the extract of ***Aristolochia bracteolata*** exhibits significant antifungal activity against ***Trichophyton rubrum***. A progressive reduction in colony-forming units was observed with increasing extract concentration and incubation time. Complete inhibition of fungal growth was achieved at higher concentrations within 24 hours. Thus, the extract of ***Aristolochia bracteolata*** exhibited **fungicidal activity** against ***Trichophyton rubrum***, as a complete reduction in viable colony-forming units was observed at higher concentrations within 24 hours. Therefore, the plant extract demonstrates potential antifungal activity against dermatophytic fungi and may contain bioactive compounds capable of inhibiting fungal growth.

CONCLUSION:

The present study demonstrated that the hydroalcoholic extract of *Aristolochia bracteolata*

possesses notable antifungal activity against *Trichophyton rubrum*. The inhibitory effect observed in disc diffusion, MIC determination, and time-kill assay suggests that the plant contains bioactive compounds capable of suppressing fungal growth.

These findings highlight the potential of *Aristolochia bracteolata* as a natural source for developing antifungal agents to treat dermatophytic infections such as ringworm. However, further studies including isolation of active compounds, toxicity evaluation, and in-vivo investigations are necessary to confirm its therapeutic efficacy and safety.

REFERENCE:

1. Heitman J. Microbial pathogens in the fungal kingdom. Fungal Biology Reviews. 2011;25(1):48–60. https://doi.org/10.1016/j.fbr.2011.01.003
2. Weitzman I, Summerbell RC. The dermatophytes. Clin Microbiol Rev. 1995;8(2):240–259. https://doi.org/10.1128/CMR.8.2.240
3. Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. Mycoses. 2008;51 Suppl 4:2–15. https://doi.org/10.1111/j.1439-0507.2008.01632.x
4. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564–582. https://doi.org/10.1128/CMR.12.4.564
5. Hay RJ, Ashbee HR. Fungal infections. In: Griffiths CEM, Barker J, Bleiker T, Chalmers R, Creamer D, editors. Rook's Textbook of Dermatology. 9th ed. Oxford: Wiley-Blackwell; 2016. p. 36.1-36.99. https://doi.org/10.1002/9781118441152.ch36
6. Ramasubramania Raja R, Niranjan Babu M. Pharmacognostical, phytochemical and antifungal activity of Aristolochic bracteolata Lam. in ringworm infection. Res J Pharm Tech. 2011;4(7):1123–1126. Available from: https://rjptonline.org/HTMLPaper.aspx?
7. Nenoff P, Krüger C, Ginter-Hanselmayer G, Tietz H-J. Mycology – an update. Part 1: Dermatomycoses: causative agents, epidemiology and pathogenesis. J Dtsch Dermatol Ges. 2014;12(3):188–209. https://doi.org/10.1111/ddg.12245
8. Peres NTA, Maranhão FCA, Rossi A, Martinez-Rossi NM. Dermatophytes: host–pathogen interaction and antifungal resistance. A Bras Dermatol. 2010;85(5):657-667. https://doi.org/10.1590/S0365-05962010000500009
9. Bhagat S, Ahirwar B. Pharmacognostical and medicinal properties of Aristolochic bracteolata: A weed. Journal of Pharmacognosy and Phytochemistry.2025;14(1):410-414. https://doi.org/10.22271/phyto.2025.v14.i1e.15264
10. Shirwaikar A, Somashekar APS, Udupa AL, Udupa SL, Somashekar SS. Wound healing studies of Aristolochic bracteolata Lam. with supportive action of antioxidant enzymes. Phytomedicine. 2003;10(6-7):558-562. https://doi.org/10.1078/094471103322331548
11. Goji HM, Nadro MS. Phytochemical and in-vitro antioxidants potential of Aristolochic bracteolata root extract. Journal of Complementary and Alternative Medical Research. 2021;13(3):1-7. https://doi.org/10.9734/jocamr/2021/v13i330224
12. Girija DM, Kalachaveedu M, Subbarayan R, Jenifer P, Rao SR. Aristolochic bracteolata enhances wound healing in vitro through anti-inflammatory and proliferative effect on human dermal fibroblasts and keratinocytes. Pharmacognosy Journal. 2017;9(6S):s129-s136. https://doi.org/10.5530/pj.2017.6s.169
13. Hassen JNH. Phytochemicals: extraction, isolation & identification of bioactive Perim MC, Borges JDC, Silva EML, Araújo TAS, Silva ACO, Silva VC, et al. In vitro antibacterial and time-kill assay of ethanolic extract of Davilla nitida bark on multidrug-resistant bacteria isolated from diabetic foot lesions. Nat Prod Res. https://doi.org/10.31632/ijalsr.20.v03i03.005.
14. Bhagat S, Ahirwar B. Pharmacognostical and medicinal properties of Aristolochic bracteolata. J Pharmacogn Phytochem. 2025;14(1):410–414. https://doi.org/10.22271/phyto.2025.v14.i1e.15264
15. Al-Sayed E, Abdel-Sattar E, El-Halawany AM, Al-Yahya MA. Isolation, characterization and quantity determination of aristolochic acids, toxic compounds in Aristolochic bracteolata L. Pharmacogn Mag.

2011;7(27):232–237. https://doi.org/10.4103/0973-1296.84245

16. Mohamed MS, Idriss MT, Khedr AIM, Abd AlGadir H, Takeshita S, Shah MM, Ichinose Y, Maki T. Activity of Aristolochic bracteolata against Moraxella catarrhalis. Int J Bacteriol. 2014;2014:481686. https://doi.org/10.1155/2014/481686
17. Souza ESOL, Cortez AC, Melhem MS, Frickmann H, Souza JVB de. Factors influencing susceptibility testing of antifungal drugs: a critical review of document M27-A4 from the Clinical and Laboratory Standards Institute (CLSI). Brazilian Journal of Microbiology. 2020;51(4):1791–1800. https://doi.org/10.1007/s42770-020-00354-6
18. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008;3(2):163–175. https://doi.org/10.1038/nprot.2007.521
19. Cuenca-Estrella M, Moore CB, Barchiesi F, et al. Multicenter evaluation of the reproducibility of the proposed CLSI broth microdilution method for antifungal susceptibility testing of filamentous fungi. Antimicrob Agents Chemother. https://doi.org/10.1128/AAC.47.11.3470-3474.2003
20. Klepser ME, Ernst EJ, Lewis RE, Ernst ME, Pfaller MA. Influence of test conditions on antifungal time-kill curve results: proposal for standardized methods. Antimicrob Agents Chemother. 1998;42(5):1207-1212. https://doi.org/10.1128/AAC.42.5.1207