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Phytochemical and Antibacterial Studies of *Aristolochia bracteolata* Against Klebsiella pneumoniae

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Abstract
Medicinal plants are alternatives to conventional treatments being used for years to treat a variety of illnesses. To combat microorganisms that are frequently proven resistant to the existing conventional drugs, alternative antimicrobial herbs with improved efficiency are required. Thus, the phytochemical and antimicrobial activities of *Aristolochia bracteolata* roots were evaluated using standard techniques. Phytochemicals were analyzed using chemicals, thin-layer chromatography (TLC) and spectrophotometric methods. Fourier Transform Infrared Spectroscopy (FTIR) was carried out to reveal the functional groups present. The antibacterial potential of the medicinal plant was determined using the agar well diffusion method against *Klebsiella pnuemoniae*. Micro-dilution method was used to determine the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC). The phytochemical analysis revealed the presence of tannins, saponnins, steroids, terpenoids and flavonoids. FTIR analysis confirmed the presence of some functional groups such as alcohol, amines, amides, alkyne, alkene, alkanes as well as ketones. The MIC of the medicinal plant ranged between 20 and 50 (mg/ml) concentrations and MBC was determined at 35mg/ml concentration. This study has revealed that *Aristolochia* is a potent antimicrobial that is very effective against *Klebsiella pneumoniae* especially. This potency is not unrelated to the strong presence of phyto-compounds in the extracts and can useful in the production of novel drugs.

Keywords: Phytochemical, Antimicrobial *Aristolochia bracteolata, Klebsiella pneumoniae,* Medicinal

Introduction

Herbal medicines are an alternative to conventional treatments and have been used for years to treat a variety of illnesses (WHO, 2024). Traditional medicinal plants contain bioactive chemicals that are less poisonous, have fewer adverse effects, are less likely to cause resistance, and are linked to increased efficacy (Mir et al., 2020). Uwineza and Waskiewiez (2020) confirmed the anti-malarial, anti-cancer, and antibiotic characteristics of some traditional herbal medicines. Making sure that everyone has access to reliable, cost-effective medicine is one of the UN's Sustainable Development Goals (UN, 2015). However, the prevalence of subpar and fake drugs continues to be a problem, which is made worse by the emergent drug resistance problem. This poses serious issues for the field of public health and emphasizes the urgent need for ongoing initiatives to raise the standard and accessible medications around the world (Khuluza et al., 2017)

To combat microorganisms that are frequently chosen by the existing therapy regimen, comparative research on alternative antimicrobial medicines with improved efficiency is required (WHO, 2017). Alkaloids, phenylpropanoids, or flavonoids, and terpenoids, which include saponins, are significant plant antibacterial agents (Sampedro & Valdivia, 2014). The genus *Aristolochia* is the most numerous in the *Aristolochiaceae* family and has been extensively utilized in traditional Chinese medicine. With 550 species, the genus is also the most distinctive in the family (Gonzalez et al., 2018). This research aimed to prove the rationale behind the use of *Arislochia bracteolata* for the treatment of some illnesses and also to further establish its uses as means of cost-effective, available and affordable alternative therapy.

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Methodology

Collection of plant materials

The roots of *Aristolochia bracteolate* were collected from Ilorin South Local Government Area of Kwara State, Nigeria. The plant was identified and authenticated in a private laboratory in Ilorin Metropolis. The plant's roots collected were air dried at room temperature, crushed into small pieces and finely powdered.

Test organisms

The bacterial strain used for the experiments was a clinical isolate of *Klebsiella pneumoniae* collected from the University of Ilorin Teaching Hospital (UITH).

Preparation of bacteria inoculums

The young microbial inoculum/culture was pre-cultured in Mueller-Hinton Broth (MHB) overnight and prepared for 24 hours at 37 ^{0}C. Then the inoculum was adjusted at a concentration of 10^8 cells/ml using 0.5 McFarland standard equivalents (Bhalodia & Shukla, 2011). The absorbance of 0.600 at 450 nm was determined spectroscopically and then used as inoculum.

Preparation of extracts

Ethanol Extract

The ground powdered plant material of *Aristolochia bracteolate* (100 g) was weighed and extraction was carried out on it using maceration technique. The powdered materials were soaked in ethanol for 24 hours at room temperature, it were then filtered with muslin cloth and Whatman filter paper respectively. The filtrate was then concentrated using a rotary evaporator at 40 – 50°C and the filtrate was kept in a well-tight sterile bottle/container under refrigerated conditions until required for use (Sohail et al., 2023).

Screening for antibacterial activity

The agar well diffusion method described by Daoud et al. (2015) was used to screen for the antibacterial properties of the extract. One millilitre (1 ml) of standardized bacterial inoculum was poured plated into molten-cooled Muller Hinton agar (MHA). Upon solidification, wells were made using a sterile cork borer (6 mm in diameter) into agar plates containing inoculums. Then, 1 ml of each extract (20% w/v) was added to respective wells. The concentration of extracts (20% w/v) has been selected based on our pre-experiments. The plates were allowed to stand for 30 min in order to allow extracts to diffuse well into the agar. Then, the plates were incubated at 37 ^{O}C for 18 hours. Antimicrobial activity was determined by measuring the zone of inhibition. Dimethyl sulfoxide (DMSO) at a concentration of 10% was employed as a negative control.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The method described by Okunye et al. (2023) was adopted to determine the minimum inhibitory and minimum bacteriocidal concentrations of the extracts. Bacterial strains were cultured for 24 hours at 37ºC on nutrient broth and then suspended in sterile distilled water to give a final inoculum concentration of $1.5 \pm 1.0 \times 10^3$cfu/mL. Dilutions ranging from 1.5 to 400 µg/mL of the extract were prepared in tubes including broth and DMSO 10% (v/v), in addition to one negative control (broth + DMSO 10% v/v + test microorganism) to ensure that the final concentration of DMSO in the assays does not interfere with the bacterial growth and one sterility control (broth + DMSO 10% v/v + extract). A 100 uL suspension of test microorganisms was added to individual tubes and incubated at 37ºC for 24 hours. The MIC of the extract was regarded as the lowest concentration that inhibited the visible bacterial growth and the MBC was regarded as the lowest concentration that prevented the growth of the organism after subculture onto freshly prepared plates. The results were recorded.

Phytochemical screening

Phytochemical screening for the presence of alkaloids, cardiac glycosides, terpenoids, triterpenes, flavonoids, saponins, and tannins was carried out using the methods described by Alhaithloul (2023).

Test for Alkaloid

About 2 ml of dilute Hydrochloric acid was added to the extract's filtrate. A few drops of Hager's reagent (picric acid in a saturated aqueous solution) were added to 2 ml of the mixture. The presence of a bright yellow precipitate confirms the presence of alkaloids

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Test for saponins
Twenty milligrams of the extract and fractions were boiled at 100 ^{0}C water in a water bath for five minutes and filtered. Ten millilitres of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for froth formation.

Test for flavonoids
Five millilitres (5ml) of dilute ammonia solution was added to 10 ml of the extract's filtrate followed by the addition of a few drops of concentrated H_2SO_4. Yellow colouration indicates the presence of flavonoids.

Test for tannins
A few drops of 1 % ferric chloride solution were added to 2 ml of the extract's filtrate. Production of a blue –black colour indicated the presence of tannins.

Test for terpenoids
About 0.5 g of the crude extract was mixed with 2 ml chloroform and 3 ml of H_2SO_4 was carefully added to form a layer. A reddish-brown colouration of the interface was an indication of terpenoids.

Test for Flavonoid
Two to three ml of extract filtrate were treated with a piece of magnesium ribbon and one ml of strong HCl was added. The presence of flavonoids is indicated by the pink-red or crimson colouring of the solution.

FTIR ANALYSIS
A dry root powder of *Aristolochia bracteolate* was taken. The dried powder was subjected to Fourier transform infrared spectroscopy measurement using the potassium bromide (KBr) pellet technique diffuse reflection mode at a resolution of 4cm-1. FTIR analysis was performed to detect the characteristic peaks and their functional groups using the Perkin Elmer Spectrophotometer system at the range of 400 to 4000/cm. Peak values for FTIR were recorded.

Results

Qualitative Phytochemical Analysis
The qualitative phytochemical analysis of the ethanol extract of *Aristolochia bracteolata* confirmed the presence of alkaloid, flavonoids, tannins, saponins, triterpenoids, steroids, phytosterols and carbohydrates (Table 1)

Quantitative Phytochemical Analysis
Table 2 shows the quantitative phytochemical analysis of the ethanol extract of *Aristolochia bracteolate*. The highest phytochemical concentration was found with Saponnin at 1.775mg/100g while the lowest concentration was found with Tannins having a concentration of 0.167 mg/100g. Flavonoids had a concentration of 1.088, Steroid had a concentration of 0.661 mg/100g and Terpenoids had a concentration of 0.527 mg/100g. The means of the result were significantly different at the $p \geq 0.05$ level

FTIR Analysis
The FTIR results for the ethanolic extracts of *Aristolochia bracteolata* revealed the presence of functional groups such as alcohol, amines, amides, alkynes, alkanes, unsaturated aldehydes and ketones, alkyl amides, alkyl halides, aromatic compounds, ethers and carboxylic acids having peaks at 3369.64/cm, 3350.35/cm, 3188/cm, 2929.87/cm, 1678/cm, 1041.57/ cm, 732/ cm, 803/cm, 1176.58/cm, 1033.85/cm respectively (Table 3).

Antimicrobial assay of *Aristolochia bracteolata*
The antibacterial assay of the ethanolic extract of *Aristolochia bracteolata* against *Klebsiella pneumoniae* showed that at 100 mg/ml of the extract, the inhibition was at 30.167 mm, at 50 mg/ml, it was 21.167 mm and at 20 mg/ml, it was at 15.833 mm. The means of the result were significantly different at the $p \geq 0.05$ level (Table 4).

Minimum inhibitory and minimum bactericidal concentrations (MIC and MBC)
The Minimum Inhibitory Concentration was 20 mg/ml while the Minimum Bactericidal Concentration was 35 mg/ml.

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Table 1: Qualitative Estimation of Phytochemical contents of *Aristolochia bracteolata*

S/N	Phytochemicals	*Aristolochia bracteolate*
1	Alkaloid	+
2	Flavonoid	+
3	Tannins	+
4	Saponnin	+
5	Terpenoids	+
6	Steroids	+
7	Phytosterols	+
8	Carbohydrates	+

Table 2: Quantitative Estimation of Phytochemical contents of *Aristolochia bracteolata*

Phytochemicals	*Aristolochia bracteolate*
Flavonoid	1.088 ± 0.645^{d}
Tannins	0.167 ± 0.001^{a}
Saponnin	1.775 ± 0.976^{e}
Terpenoids	0.527 ± 0.001^{b}
Steroids	0.661 ± 0.005^{c}

Values are expressed as mean of triplicates ± SEM and analyzed by one-way analysis of variance
Means with different uppercase letters indicate a significant difference at the significance level of 0.05

Table 3: Functional Groups Obtained from the FTIR analysis of *Aristolochia bracteolata* ethanol extract

S/N	Absorbance(cm^{-1})	Frequency(Cm^{-1})	Bond(Type of vib)	Functional Group
1	3500-3300	3369.64	OH group (alcohol)	OH stretching
2	3300-3200	3188	Ξ C − H Stretch	Alkynes
3	3350-3200	3350.35	N–H stretch 1◦, 2◦	Amines, Amides
4	3000-2700	2929.87	C–H stretch	Alkanes
5	1710-16651	1678	C = O stretch	Unsat aldehyde/ketone
6	1640-1550	1614.47	N-H bond	Amides
7	1250-1020	1041.57	C-N stretch	Aliphatic amines
8	850-550	732	C-CL stretch	Alkyl halides
9	900-6755	803	C-H "oop''	Aromatic compounds
10	1300-1000	1176.58	C-O stretch	Ethers
11	1000-850	1033.85	O-H bond	Carboxilic acid

Table 4: Antibacterial activity of the ethanol extract of *Aristolochia bracteolata*

		Zone of Inhibition(mm)/ Concentration (mg/ml)		
Organism	**Control (DMSO)**	**100**	**50**	**20**
Klebsiella pneumonae	-	30.167 ± 1.443^{c}	21.167 ± 1.041^{b}	15.833 ± 0.764^{a}

Key: DMSO = Dimethyl sulfur oxide, - = No activity
Values are expressed as mean of triplicates ± SEM and analyzed by one-way analysis of variance.
Means with different uppercase letters indicate a significant difference at the significance level of 0.05

Table 5: Minimum Inhibitory Concentration of the ethanolic extract of *Aristolochia bracteolata*

Organism	Concentrations	
	MIC(mg/ml)	MBC(mg/ml)
Klebsiella pneumonae	20	35

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Discussion

The presence of bioactive components in the ethanol extract of *Aristolochia bracteolata* may likely be responsible for the potent antimicrobial activity of this plant (Alhaithloul, 2023). According to Kolawole et al. (2023), alkaloids are well-known for their therapeutic properties of anesthetic, cardio-protective, and anti-inflammatory properties and more so, multiple biological properties, including anti-microbial, cytotoxic, anti-inflammatory, and anti-tumour properties, have been observed in the flavonoids; however, the capacity to function as potent antioxidants is the flavonoids most well-known property. FTIR analysis results confirmed the presence of some functional groups of various phytochemicals i.e., phenol, alkanes, alkenes, alkynes, aldehydes, ketones, alcohols, esters, carboxylic acids, amines and ethers in the plant may also be responsible for various pharmacological activities. These phytochemicals have various functional groups like O-H, C-O, C-H, C=C, CH3 and N-H. No bond was found in the region of 2220 to 2260 cm-1 indicating that no cyanide group was present in the samples. This implies that the plant has no toxicity due to the cyanide group. As there is an absence of cyanide group in the extracts, but still toxicological studies of the extracts are still required for in vivo studies. The findings from antimicrobial activity of ethanolic extracts of *Aristolochia bracteolata* in this study agreed with the report of Bhaaskaran et al. (2014) where the methanol extracts of *Aristolochia bracteolata* were effective against *Klebsiella Pneumoniae*. More so, the work of Bartha et al. (2019) and Bhaaskaran et al. (2014) revealed that the ethanolic extracts of *Aristolochia bracteolata* were effective against the isolates of *Klebsiella pneumoniae* and other pathogenic isolates used. The substantially lower MIC's and MBC's of *Aristolochia bracteolata* extracts which were 20 mg/ml and 35 mg/ml respectively for the test organism indicated that it has greater antibacterial properties even at low concentrations. The findings of this research correlated with that of Bartha et al. (2019) who confirmed low concentrations of *Aristolochia bracteolata* inhibiting the growth of *Klebsiella pneumoniae*.

Conclusion

This study revealed that *Aristolochia bracteolata* is a potent antimicrobial that is very effective against *Klebsiella pneumoniae*. This potency is not unrelated to the strong presence of phytocompounds in the extracts as well as the low MIC and MBC. *A. bracteolata* can then be further utilized to produce compounds needed to develop novel drugs.

Recommendations

1. Cultivation of *Aristolochia bracteolate* should be encouraged because of its antimicrobial properties.
2. Further studies should be carried out on the mechanism of antimicrobial activities of *Aristolochia bracteolate* plant extracts.

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