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Evaluation of the antioxidant and antimicrobial activity of methanolic leaf extracts of Argyreia cuneata (Willd) Ker Gawl

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ABSTRACT

The rising prevalence of antimicrobial-resistant pathogens has underscored the urgent need for new compounds that can effectively combat infectious diseases. A promising source of these compounds lies in the secondary metabolites of species traditionally used for treating such diseases. One such species, *Argyreia cuneata*, a well-known medicinal plant, has demonstrated significant potential due to its antimicrobial chemical constituents. In this study, we evaluated the antimicrobial activities of methanolic extracts from *A. cuneata* leaves against various bacterial and fungal strains of therapeutic importance. The analysis revealed that the methanolic leaf extract contained phenolic compounds at a concentration of 2.57%, flavonoids at 1.30%, and exhibited antioxidant activity, as measured by DPPH, at 0.748%. The results indicated that the methanolic leaf extract exhibited robust antimicrobial activity, especially against *E. coli*. Furthermore, *Pseudomonas fluorescens* showed high susceptibility to both the methanolic leaf extract and ampicillin. In addition, ketoconazole demonstrated significant inhibitory effects against *Fusarium oxysporum* and *Aspergillus brasiliensis*. These findings suggest that the methanolic leaf extract of *A. cuneata* holds considerable promise as a source of antimicrobial agents. However, to fully harness its potential, further detailed investigation into its chemical composition is essential. This could pave the way for the development of new, effective treatments for combating infectious diseases.

INTRODUCTION

Plants must adapt to fluctuating ecological environments to survive. The oxidative environment exposes living organisms to a plethora of free radicals, including superoxide, hydroxyl radicals, nitric oxide, and peroxynitrite. Considerable evidence supports the pivotal role of free radicals in the genesis of diseases such as cancer, neurodegeneration, and inflammatory conditions (Halliwell, 2006, 2007; Ferguson, 2010). Consequently, the importance of antioxidants in neutralizing these free radicals cannot be overstated.

The antioxidant attributes of medicinal plants (Nandhakumar and Indumathi, 2013) primarily stem from phenolic compounds like flavonoids and phenolic acids, as well as from ascorbic acid and vitamin-E (Sawadogo et al., 2006). These antioxidants proficiently counteract the detrimental effects induced by oxidative stress triggered by free radicals (Gruz et al., 2011). Reactive oxygen species (ROS) or free radicals have been implicated in the pathogenesis of numerous diseases, including cancer and coronary conditions, owing to their toxic and mutagenic nature. Consequently, research into naturally occurring antioxidants has gained significant traction.

Medicinal plant's antimicrobial and antioxidant properties are being explored worldwide, primarily due to concerns about the toxicity of synthetic antioxidants and preservatives (Peschel et al., 2006). *Argyreia cuneata*, an endemic medicinal plant found in the southwestern regions, particularly in the Nallamalla forest, belongs to the family Convolvulaceae.

Materials and Methods

Plant Material Collection and Authentication

Argyreia cuneata leaves were collected from the Amrabad Tiger Reserve in Telangana for this study. The collected plant material was authenticated by the Central Council for Research in Ayurveda (AYUSH), Bangalore, Karnataka State, India. The leaves underwent a series of preparatory steps, including washing, shade drying, and pulverization.

Preparation of Extracts

The leaves that were collected were dried in the shade at room temperature. After drying, the plant material was finely powdered using a mechanical blender. The powdered leaves were meticulously transferred into a Soxhlet apparatus and subjected to extraction with methanol, maintaining temperatures within the range of 60 to 65°C for a duration of 5 to 7 hours. Subsequently, the resulting extract underwent

filtration using Whatman filter paper No. 1 and was further concentrated under reduced pressure at 40°C. The concentrated extract was carefully dried and subsequently stored in vials at 4°C, earmarked for future experimental endeavours.

Estimation of Phenols

The estimation of phenols involved determining the total phenolic content of the methanolic leaf extract through the Folin-Ciocalteu assessment method (Al-Owaisi et al., 2014). Initially, 100 µl of the plant extract (1 mg/mL) was mixed with 500 µl of freshly prepared 10× diluted Folin-Ciocalteu reagent. This mixture was allowed to stand at room temperature for five minutes. Subsequently, 500 µl of 7.5% sodium bicarbonate solution was added to neutralize the mixture, which was then left at room temperature for thirty minutes, shielded from light. The absorbance at 765 nm was measured using a UV-vis spectrophotometer. Gallic acid served as the reference standard, with concentrations ranging from 0 to 200 µg/mL.

Estimation of Flavonoids

The total flavonoid content of the extracts was determined using the aluminum chloride colorimetric method, as outlined by (Al-Owaisi et al., 2014). For the test, 100 µl of plant extract (1 mg/mL) was combined with 750 µl of 90% methanol in a test tube. To this mixture, 50 µl of aluminum chloride (10%) and 50 µl of 1 M potassium acetate were added. The resultant mixture was allowed to react at room temperature for 30 minutes. After the incubation period, the absorbance of the solution was measured at 415 nm using a spectrophotometer. A standard curve was established using quercetin as the reference standard, facilitating the quantification of flavonoid content in the plant extracts.

DPPH radical scavenging assay

The antioxidant activity of the plant extracts was evaluated using the DPPH radical scavenging assay, following the methodology outlined by Mongalo et al. (2018). The extracts were prepared at various concentrations (5, 10, 15, 20, and 25 µg/mL) by combining 100 µL of each extract (dissolved in methanol) with 900 µL of 10 mg/L DPPH solution in methanol. Blank controls were established using DPPH and methanol, while Trolox was utilized as the standard. Following incubation, the absorbance was measured at 517 nm using a spectrophotometer. Each test was conducted in triplicate to ensure accuracy and reliability.

The capability to scavenge the DPPH radical was calculated using the below equation.

DPPH Scavenged (%) = ((AB − As) / AB) × 100

Where AB is the absorbance of the blank solution and AS is DPPH radical + plant extract.

Antimicrobial Testing

We evaluated the antimicrobial properties of methanolic leaf extracts of *A. cuneata* using various bacterial and fungal strains. All strains in the Microbial Type Culture Collection (MTCC) are sourced from Chandigarh, India, and were maintained at 4°C on nutrient agar and potato dextrose agar (PDA) mediums, respectively.

Antibacterial Activity

The bacterial strains included two Gram-positive bacteria, *Bacillus subtilis* (MTCC-3053) and *Staphylococcus aureus* (MTCC-96), as well as two Gram-negative bacteria, *Escherichia coli* (MTCC-424) and *Pseudomonas fluorescens* (MTCC-9768).

Antifungal Activity

The fungal strains tested were *Fusarium oxysporum* (MTCC-284) and *Aspergillus brasiliensis* (MTCC-1344). We assessed the antifungal activity using the dual-culture method. The fungi were cultured on PDA medium, and a 5-mm-diameter agar block was excised from a fungal culture that had been incubated for 96 hours. It was then positioned at the center of a fresh PDA plate. Paper discs soaked in methanolic leaf extract were positioned around the plate. After incubating for five days at 30 ± 2°C, we measured the inhibition zones to evaluate the antifungal activity.

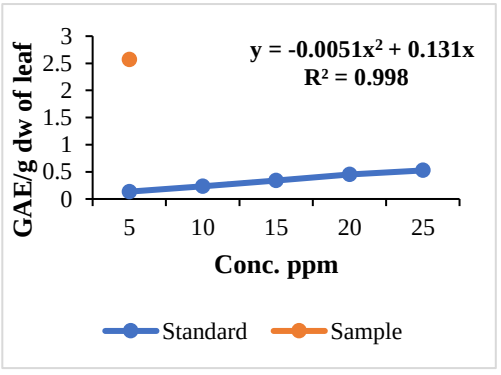
Results

Quantitative estimation of phenolic compounds and flavonoids was carried out in methanolic leaf extract of A. cuneata. The concentration of phenolic content in the leaf extract of *A. cuneata* was 2.57 mg / 100 mg and the concentration of flavonoids in the leaf extract was found to be 1.30 mg / 100 mg respectively (Chart-1(a and b); Table-1).

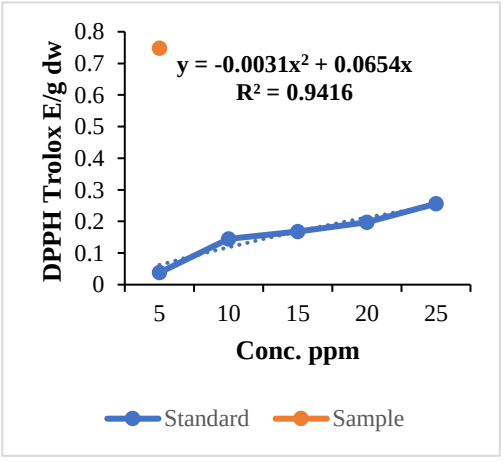
In the present investigation screening of antioxidant activity was done by using DPPH with the methanolic leaf extracts of *A.cuneata* and compared with the standard Trolox. The DPPH radical scavenging activity was found to be 0.748 µg / µl for leaf extract and Trolox at the concentration of 100 µg / µl (Table-1; Chart-1(c)). According to the above data, Trolox showed higher DPPH scavenging activity than the leaf extract at all concentrations.

Chart 1. Estimation of Total phenolics, Total flavonoid content and DPPH antioxidant activity in MeOH leaf extracts of *A. cuneata*.

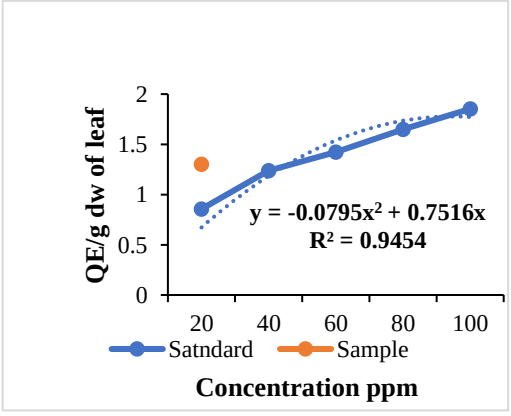
a)



b)



c)



TPC; b. TFC; and c; DPPH)

Table 1. Total Phenolic, Flavonoid content and Antioxidant capacity of the MeOH leaf extracts of *A. cuneata*.

Sample	Phenolics(GA mg/g dw)	Flavonoids (QE mg/g dw)	DPPH (TR mg/g dw)
MeOH leaf extract	2.570 ± 0.146	1.302 ± 0.06	0.748 ± 0.054

Antimicrobial

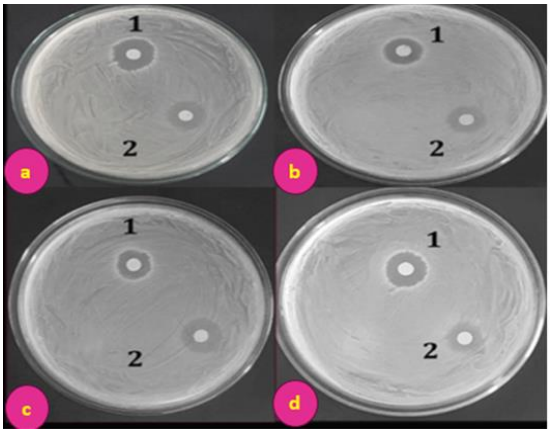
The antimicrobial results demonstrate varying degrees of susceptibility among different bacterial strains to the tested agents as methanolic leaf extract and ampicillin (Fig 1, Table 2 and Chart 2).

Bacillus subtillis (MTCC-3053), the zone of inhibition with methanolic leaf extract was 0.5 cm (±0.1), and with ampicillin, it was 1.0 cm (±0.1). *Staphylococcus aureus* (MTCC-96), the zone of inhibition with methanolic leaf extract was 0.5 cm (±0.1), and with ampicillin, it was 0.7 cm (±0.1). *E. coli* (MTCC-424), the zone of inhibition with methanolic leaf extract was 0.6 cm (±0.1), and with ampicillin, it was 0.8 cm (±0.2). *Pseudomonas fluorescens* (MTCC-9768), the zone of inhibition methanolic leaf extract was 0.4 cm (±0.1), and with ampicillin, it was 0.6 cm (±0.1).

Table 2. Antibacterial activity

Bacterial strains	MeOH leaf extract	Ampicillin
Bacillus subtilis (MTCC-3053)	0.5± (0.1)	1.0± (0.1)
Staphylococcus aureus (MTCC-96)	0.5± (0.1)	0.7± (0.1)
Escherichia coli (MTCC-424),	0.6 ± (0.1)	0.8± (0.2)
Pseudomonas fluorescens (MTCC-9768)	0.4± (0.1)	0.6± (0.1)

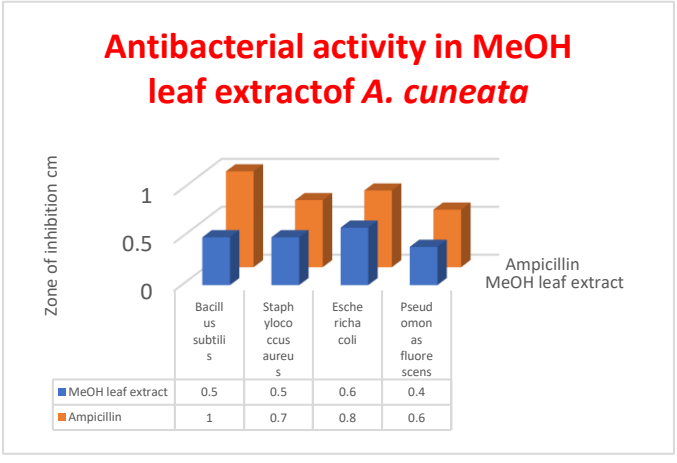
Figure 1. Antibacterial effect in MeOH leaf extract of *A. cuneat*



a) *Bacillus subtillis* **b)** *Staphylococcus aureus* **c)** *E. coli* and **d)** *Pseudomonas fluorescens*

1. Ampicillin (Standard)
2. MeOH leaf extract

Chart 2. Antibacterial activity of Methanolic leaf extract



The antifungal results showed that the methanolic leaf extract exhibited a 44% inhibition rate against *Fusarium oxysporum* (MTCC-284) and a 38% inhibition rate against *Aspergillus brasiliensis* (MTCC-1344). On the other hand, ketoconazole demonstrated a higher inhibition rate of 52% against *Fusarium oxysporum* and 46% against *Aspergillus brasiliensis*. These findings indicate varying degrees of antifungal efficacy between the two agents tested against the specified fungal organisms (Figure 2. and Table 3, chart 3).

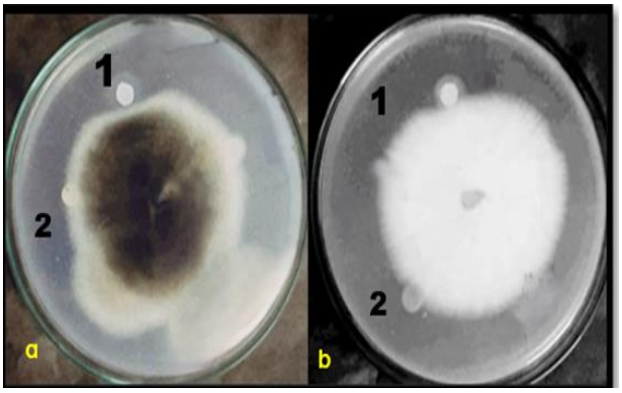


Figure 2. Antifungal effect in MeOH leaf extract of *A. cuneata*

a) *Fusarium oxysporum* (MTCC-284) and b) *Aspergillus brasiliensis* (MTCC-1344)

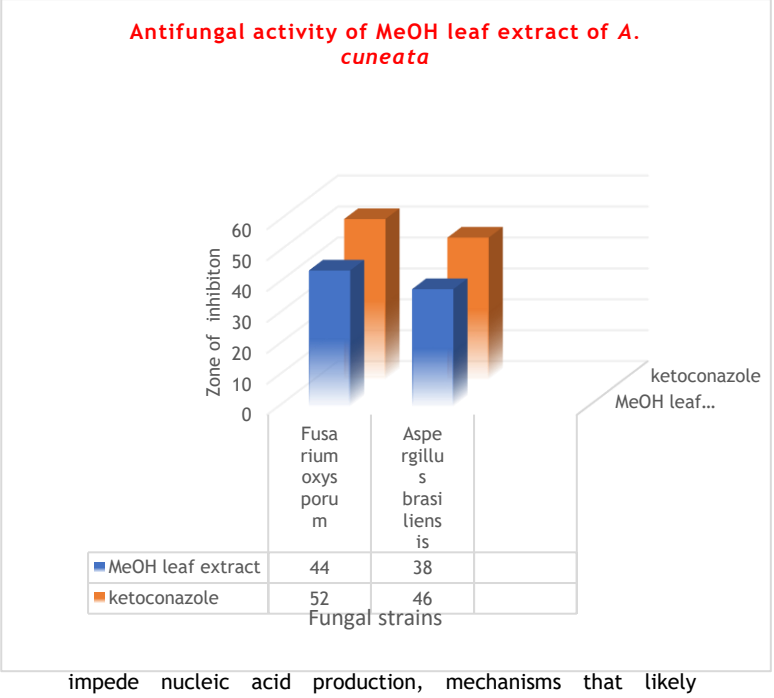
1. ketoconazole (Standard)

2. MeOH leaf extract

Table 3. Antifungal activity

Organism of fungal	Methanolic leaf extract	ketoconazole
Fusarium oxysporum (MTCC-284)	44%	52%
Aspergillus brasiliensis (MTCC-1344)	38%	46%

Chart 3. Antifungal activity of MeOH leaf extract



DISCUSSION

Our study analyzed the total phenolic and flavonoid content within the methanolic leaf extracts of *A. cuneata*, revealing notably high levels of these compounds. The considerable antioxidant and antibacterial activities observed in these extracts can be directly linked to their elevated phenolic and flavonoid content. Phenolic compounds hold significant importance in plants due to their exceptional ability to scavenge free radicals, largely facilitated by their hydroxyl groups. This ability makes them invaluable for quickly assessing antioxidant activity in various contexts (Yi et al., 2007). Moreover, these compounds play a crucial role in enhancing plants' tolerance to oxidative stress.

Flavonoids are known for their effectiveness in scavenging oxidizing molecules, including singlet oxygen and diverse free radicals implicated in numerous diseases (Bravo, 1998). They further exhibit the ability to inhibit reactive oxygen formation, chelate trace elements crucial for free-radical production, scavenge reactive species, and safeguard antioxidant defenses (Agati et al., 2012). This multifaceted role underscores their significance in combating oxidative stress and protecting biological systems from oxidative damage.

The leaf extracts of *A. cuneata* in methanol exhibit notable antibacterial properties, showcasing a pronounced efficacy against Gram-positive bacteria compared to Gram-negative strains. This heightened activity against Gram-positive bacteria can be attributed to the distinct composition of their cell membranes, particularly the outer peptidoglycan layer which functions as a comparatively inefficient permeability barrier. Such an observation aligns with similar studies on the antibacterial effects of strawberry tree leaves, which specifically target Gram-positive bacterial strains as reported by Orak et al. in 2011. These findings underscore *A. cuneata* as a promising source for the development of broad-spectrum antibacterial agents.

The augmented antibacterial efficacy is posited to stem from the increased flavonoid content present in *A. cuneata*. Flavonoids are renowned for their ability to disrupt metabolic processes and impede nucleic acid production, mechanisms that likely contribute significantly to their antibacterial activity as elucidated by Cushnie and Lamb in 2005. This suggests a potential avenue for the development of novel antibacterial drugs with a wide range of applicability.

The use of different extraction solvents revealed that the phenolic and flavonoid content in *A. cuneata* exceeded previous findings for similar species. Our results from the methanolic extraction support the study conducted by Wintola and Afolayan in 2017, which found that the ethanol extract contained the highest concentration of flavonoids. This highlights the influence of extraction solvent polarity on the polarity of flavonoid compounds, particularly emphasizing the increased polarity observed in methanol. Additionally, a study by Singh and Jain in 2018 found comparable levels of flavonoids and phenolics in methanolic extracts from various plant species, emphasizing their important role in antioxidant activities. These combined insights provide valuable information on the complex relationship between extraction solvents, phytochemical composition, and functional properties. They also offer opportunities for improved extraction methods and potential applications in antioxidant-based therapeutics.

The significant phenolic and flavonoid levels in *A. cuneata* suggest potential health benefits, such as antioxidant, anti-inflammatory, and anticancer properties. Although the antioxidant activity of the methanolic leaf extract was lower than Trolox, it indicates that *A. cuneata* leaf extract could be a valuable natural antioxidant source. Further research should

isolate individual phenolic and flavonoid compounds to understand their contributions to overall antioxidant activity. Additional antioxidant assays, like ABTS and FRAP, could provide a more comprehensive evaluation of the antioxidant potential. Exploring the synergistic effects of combining these compounds with other antioxidants might enhance properties.

The antimicrobial study showed varying efficacy of the methanolic leaf extract and ampicillin against different bacterial strains. Previous studies reported similar antimicrobial activities for plant extracts and conventional antibiotics. For example, Jain et al., (2010) found significant antibacterial activity in methanolic leaf extracts from various plants, aligning with our findings. The comparable zones of inhibition between the methanolic leaf extract and ampicillin suggest potential as an alternative or adjunct antimicrobial agent. The antimicrobial activity could be due to bioactive compounds like phenolics, flavonoids, and tannins, which disrupt bacterial cell walls and inhibit protein synthesis (Deepti et al., 2012). Further research should isolate and identify these active compounds and understand their specific mechanisms of action. Combining methanolic leaf extracts with conventional antibiotics might enhance antimicrobial efficacy and reduce antibiotic resistance, as demonstrated by Patel et al., (2021).

Previous studies documented the antifungal properties of various plant extracts and conventional antibiotics. For instance, Gupta et al., (2024) reported methanolic extracts from different plants inhibited *Fusarium* species, aligning with our 44% inhibition of *Fusarium oxysporum*. Deep et al., (2020) noted ketoconazole inhibitory effects against multiple fungal strains, corroborating our fungal pathogens. While the methanolic leaf extract has notable antifungal activity, its efficacy is somewhat lower than ketoconazole . This suggests that plant-based extracts could be complementary antifungal agents but may require higher concentrations or combination therapies to match conventional antibiotics' potency. The antifungal activity might involve bioactive compounds like flavonoids, alkaloids, and terpenoids, which disrupt fungal cell walls and inhibit spore germination (Aqil et al., 2010). Further phytochemical analysis and isolation of specific active compounds could enhance antifungal properties. Combining methanolic leaf extracts with established antifungal agents like ketoconazole could achieve improved efficacy and reduce synthetic antibiotics' dosages, mitigating side effects and resistance issues.

CONCLUSION

The methanolic leaf extract of *A. cuneata*, rich in phenolic compounds and flavonoids, demonstrates significant antioxidant activity, underscoring its potential as a natural antioxidant source. Its ability to scavenge DPPH radicals and comparable antimicrobial efficacy to ampicillin and ketoconazole against various bacterial and fungal strains highlighted its dual health benefits. These findings support the potential applications of *A. cuneata* in nutraceuticals, functional foods, and antimicrobial therapy. Further research is needed to optimize its use and fully understand its bioactive mechanisms, paving the way for its integration into health-promoting and therapeutic applications.

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Competing interests

The authors declare that they have no competing interests

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Availability of data

All the data produced presented in this paper

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