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In-vitro antimicrobial and antioxidant activity of *Argyreia cuneata* (Willd.) Ker Gawl. (Convolvulaceae)

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ABSTRACT

Objectives: *Argyreia cuneata* (Willd.) Ker Gawl. belongs to the family Convolvulaceae. The present study was performed to screen the potential of crude extract of various parts of *A. cuneata* to exhibit antimicrobial activity. **Methods:** Extraction of shade dried and powdered leaf, stem and flower of *A. cuneata* was carried out by maceration technique. Antibacterial and antifungal activity of extracts was evaluated by Agar well diffusion and Poisoned food technique respectively. Antioxidant activity was determined by DPPH radical scavenging, ABTS radical scavenging and ferric reducing assays. **Results:** All extracts were effective in inhibiting test bacteria and the susceptibility of bacteria to extracts was in the order: *Bacillus cereus* > *Shigella flexneri* > *Escherichia coli* > *Salmonella typhimurium*. Leaf extract and stem extract exhibited highest and least antibacterial activity, respectively. Extracts were effective in causing inhibition of seed-borne fungi viz. *Aspergillus niger* and *Bipolaris* sp to >50%. Leaf extract exhibited marked antifungal activity followed by flower extract and stem extract. All extracts were shown to exhibit concentration dependent scavenging and reducing activity. Antioxidant activity of extracts observed was in the order: leaf extract > flower extract > stem extract. **Conclusion:** Among various parts of *A. cuneata*, leaf extract exhibited marked antimicrobial and antioxidant activity. The plant can be employed as an effective antimicrobial and antioxidant agent in suitable form. Further studies may be undertaken to recover phytochemicals from the plant and to investigate the antimicrobial and antioxidant activity of isolated components.

Keywords: *Argyreia cuneata*, Maceration, Antimicrobial, Agar well diffusion, Poisoned food technique, Antioxidant

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INTRODUCTION

Argyreia cuneata (Willd.) Ker Gawl. belonging to the family Convolvulaceae is a suberect silky shrub with showy red flowers (Figure 1) and climbing tendency. The plant is popularly known by the name Purple morning glory and Purple convolvulus in English. The plant is found distributed in South India and common across the plains along the hedges. In Karnataka, the plant is found distributed in several districts viz. Bangalore, Shivamogga, Hassan, Chikmagalur, Davanagere, Kodagu and Uttara Kannada. Leaves are obovate-lanceolate, emarginate, broadly cuneate at base and glabrous above. Peduncle is 1-2cm with 3 or more flowers in axillary cymes. Corolla is funnel shaped, bright red in color. Fruit is a 4-seeded, leathery, dry ellipsoid berry [1,2,3]. Phytochemicals such as indole, isoquinoline, pyrrolidine, tropane alkaloids have been detected in the plant [4]. *A. cuneata* has ethnomedicinal significance and is used for the treatment of arthritis, diabetes, bone fracture, scabies, helminthic infections, rheumatism and to initiate labor pain and to ease delivery [5,6,7,8,9].

Figure 1: *A. cuneata* (Photograph by Prashith Kekuda)

The plant also has ethnoveterinary significance in terms of treating anorexia, swelling of throat and retention of fetal membrane [5,10]. *A. cuneata* is reported to exhibit pharmacological activities such as antidiabetic [11,12], analgesic [13], antipyretic [14], and wound healing activity [14]. The present study was conducted to evaluate antibacterial and antifungal activity of various parts viz. leaf, stem and flower of *A. cuneata*.

MATERIALS AND METHODS

Collection and extraction of plant material

The plant material was collected near Amruthapura, Tarikere, Karnataka during January 2017. The plant material was authenticated by Dr. Vinayaka K.S, Principal, KFGC, Shikaripura. Maceration process was performed for extraction of shade dried and powdered plant materials (leaf, stem and flower). Methanol was used as extraction solvent. Filtrates were evaporated to dryness at room temperature and crude leaf, stem and flower extracts were obtained [15].

Antibacterial activity of *A. cuneata*

Agar well diffusion method was performed to evaluate antibacterial activity of leaf, flower and stem extracts of *A. cuneata* against bacteria viz. *Bacillus cereus*, *Escherichia coli*, *Shigella flexneri* and *Salmonella typhimurium*. Streptomycin was used as reference standard. Zones of inhibition were measured using a ruler [15].

Antifungal activity of *A. cuneata*

Poisoned food technique was employed to determine antifungal potential of leaf, flower and stem extracts of *A. cuneata* against two seed-borne fungi viz. *Aspergillus niger* and *Bipolaris* sp. Extent of reduction in mycelial growth of test fungi was determined using the formula:

Inhibition of fungal growth (%) = (C – T / C) x 100, where 'C' denotes the diameter of fungal colonies in control plates and 'D' denotes the diameter of fungal colonies in poisoned plates [15].

Antioxidant activity of *A. cuneata*

DPPH radical scavenging assay

Scavenging effect of various concentrations of leaf, flower and stem extracts of *A. cuneata* against DPPH free radicals was evaluated by employing the protocol of Raghavendra et al. [15]. Ascorbic acid was used as reference standard. Scavenging activity was determined by using the formula:

Scavenging of DPPH radicals (%) = (Ac – At / Ac) x 100, where 'Ac' and 'At' represents absorbance of DPPH control and absorbance of DPPH in the presence of extracts/ascorbic acid respectively. IC_{50} value was calculated.

ABTS radical scavenging assay

The protocol employed by Raghavendra et al. [15] was used to evaluate the potential of various concentrations of *A. cuneata* extracts to scavenge ABTS radicals. Ascorbic acid was used as reference standard. The scavenging activity was determined by using the formula:

Scavenging of ABTS radicals (%) = (Ac – At / Ac) x 100, where 'Ac' and 'At' represents absorbance of ABTS control and absorbance of ABTS in the presence of extracts/ascorbic acid respectively. IC_{50} value was calculated.

Ferric reducing assay

The efficacy of different concentrations of extracts of *A. cuneata* to exhibit reducing ability was investigated by Ferric reducing assay [15]. Ascorbic acid was used as reference standard. An increase in the absorbance with the increase in concentration of extracts or ascorbic acid indicated reducing potential.

Statistical analysis

Experiments were conducted in triplicates. Results are presented as Mean ± Standard deviation. IC_{50} values were calculated by using Origin (Data Analysis and Graphing) Software version 7.0 for windows.

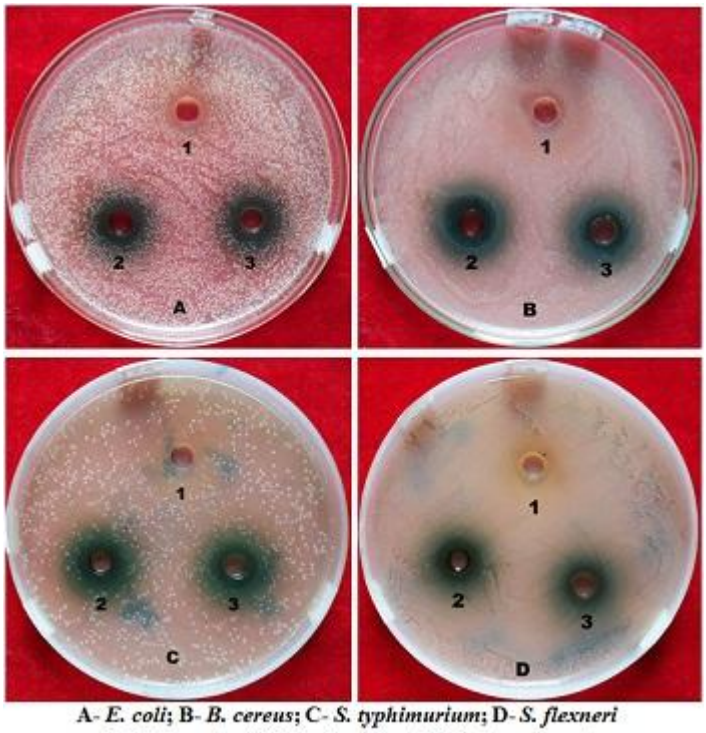
RESULTS AND DISCUSSION

Antibacterial activity of extracts of *A. cuneata*

Microbial infections are major cause of death worldwide. Indiscriminate use of antibiotics resulted in several negative impacts such as adverse effects on health, killing of non-target bacteria and emergence of resistant strains of pathogenic bacteria. Crude solvent extracts and purified compounds from higher plants are shown to be promising alternatives for antibiotics with activity even against antibiotic resistant bacteria [16-22]. In this study, we evaluated the potential of extracts of *A. cuneata* against gram positive and gram negative bacteria by agar well diffusion method. All extracts were shown to exhibit antibacterial activity. Among bacteria, *B. cereus* was found to exhibit greater susceptibility to solvent extracts. The susceptibility of test bacteria to extracts was in the order: *B. cereus* > *S. flexneri* > *E. coli* > *S. typhimurium*. Among solvent extracts, leaf extract displayed marked antibacterial activity followed by flower and stem extracts. Reference antibiotic caused higher inhibitory activity against test bacteria while DMSO did not cause inhibition of any of the test bacteria (Table 1; Figure 2). Studies have revealed the antibacterial potential of other *Argyreia* species such as *A. argentea* [23], *A. cymosa* [24], *A. speciosa* [25] and *A. osyrensis* [26].

Table 1: Antibacterial activity of *A. cuneata*

Test bacteria	Zone of inhibition in cm				
	Leaf extract	Stem extract	Flower extract	Antibiotic	DMSO
B. cereus	1.63±0.05	1.23±0.05	1.33±0.05	3.03±0.05	0.00±0.00
E. coli	1.40±0.00	1.20±0.00	1.30±0.00	3.30±0.00	0.00±0.00
S. typhimurium	1.33±0.05	1.13±0.05	1.20±0.00	3.20±0.00	0.00±0.00
S. flexneri	1.43±0.05	1.20±0.00	1.23±0.05	3.40±0.10	0.00±0.00



A- *E. coli*; B- *B. cereus*; C- *S. typhimurium*; D- *S. flexneri*
1- Stem extract; 2- Leaf extract; 3- Flower extract

Figure 2: Inhibition of test bacteria by extracts of *A. cuneata*

Antifungal activity of extracts of *A. cuneata*

The extensive and indiscriminate use of synthetic pesticides results in environmental pollution, toxic effects on non-target organisms and humans and development of resistance in fungal pathogens. The use of plants seems to be an effective alternative for synthetic fungicides. Crude extracts and purified compounds from higher compounds have shown to exhibit marked antifungal activity as revealed by a number of studies [18,27-33]. In the present study, we evaluated antifungal activity of *A. cuneata* extracts by poisoned food technique. All three extracts were effective in causing mycelial growth inhibition of *Bipolaris* sp. and *A. niger* (Table 2; Figure 3). Extracts were shown to inhibit the growth of both fungi to >50%. The extent of inhibition (%) of *Bipolaris* sp. by extracts was in the order: leaf extract (63.82%) > flower extract (59.57%) > stem extract (56.80%). In case of *A. niger* also, marked inhibitory activity was shown by leaf extract (61.48%) followed by flower extract (58.40%) and stem extract (57.86%). Studies have revealed the antifungal potential of other *Argyreia* species such as *A. nervosa* [34] and *A. argentea* [23].

Table 2: Antifungal activity of *A. cuneata*

Treatment	Colony diameter in cm	
	***Bipolaris* sp.**	***A. niger***
Control	4.70±0.00	5.53±0.05
Leaf extract	1.70±0.00	2.13±0.05
Flower extract	1.90±0.00	2.30±0.00
Stem extract	2.03±0.05	2.33±0.05

Figure 3: Growth of test fungi in control and poisoned plates

DPPH radical scavenging activity of *A. cuneata*

The method involving scavenging of DPPH radicals is one of the most widely employed assays for evaluating antiradical activity of various kinds of samples including plant extracts [15,35-39]. In this assay, bleaching of DPPH radical color from purple to yellow by various concentrations of extracts and ascorbic acid was monitored at 517nm. Figure 4 shows the result of DPPH radical scavenging activity of *A. cuneata* extracts. All extracts were efficient in scavenging radicals in dose dependent manner. A scavenging potential of 50% and higher was recorded at concentration 12.50µg/ml, 25µg/ml and 50µg/ml in case of leaf extract, flower extract and stem extract respectively. Among extracts, leaf extract was more effective in scavenging radicals with IC_{50} value 14.22µg/ml followed by flower extract (IC_{50} value 25.35µg/ml) and stem extract (IC_{50} value 41.26µg/ml). Ascorbic acid was shown to scavenge DPPH radicals to higher extent (IC_{50} value 8.17µg/ml) when compared to *A. cuneata* extracts. It is evident from the results of the present study that the extracts of *A. cuneata* can possibly act as antioxidants owing to their scavenging ability against free radicals. Studies have shown the DPPH radical scavenging potential of various *Argyreia* species such as *A. nervosa* [40], *A. osyrensis* [26], *A. elliptica* [41], *A. argentea* [42], *A. roxburghii* [43] and *A. cymosa* [44].

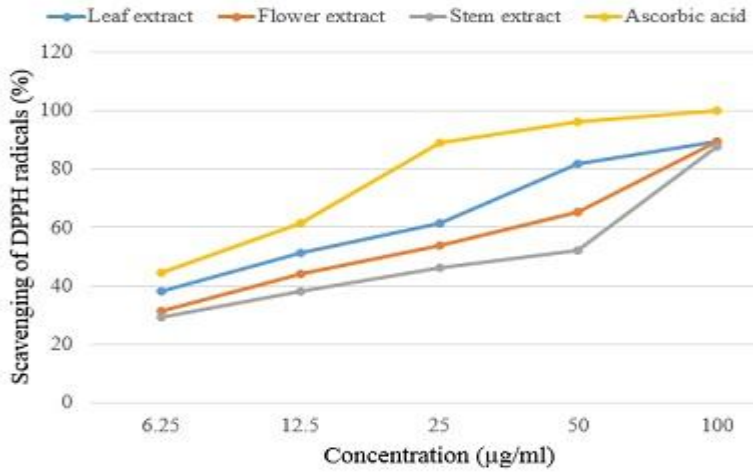


Figure 4: Scavenging of DPPH radicals by extracts of *A. cuneata*

ABTS radical scavenging activity of *A. cuneata*

Like DPPH radical scavenging assay, the scavenging of ABTS radicals is another widely used assays for determining antiradical activity of various kinds of samples including plant extracts [15,35,38,39,45,46]. Substances capable of donating electron (antioxidants) can reduce the blue-green colored ABTS radical solution into colorless form. In the present study, extracts of *A. cuneata* were shown to be effective in scavenging ABTS radicals in dose dependent manner and the result obtained is shown in Figure 5. All extracts were effective in scavenging ABTS radicals in concentration dependent manner. A scavenging potential of 50% and higher was recorded at concentration 12.50µg/ml in case of leaf extract while flower extract and stem extract revealed a scavenging potential of 50% and higher at concentration 25µg/ml. Among extracts, leaf extract was more effective in scavenging ABTS radicals with IC_{50} value 9.34µg/ml followed by flower extract (IC_{50} value 14.98µg/ml) and stem extract (IC_{50} value 22.47µg/ml). Ascorbic acid was shown to scavenge ABTS radicals to higher extent (IC_{50} value 5.83µg/ml) when compared to *A. cuneata* extracts. Although leaf, flower and stem extracts of *A. cuneata* displayed lower scavenging of ABTS radicals, however, it is evident that the extracts can be potent radical scavengers. Studies have shown the potential of other *Argyreia* species such as *A. cymosa* [44], *A. nervosa* [47] to scavenge ABTS radicals.

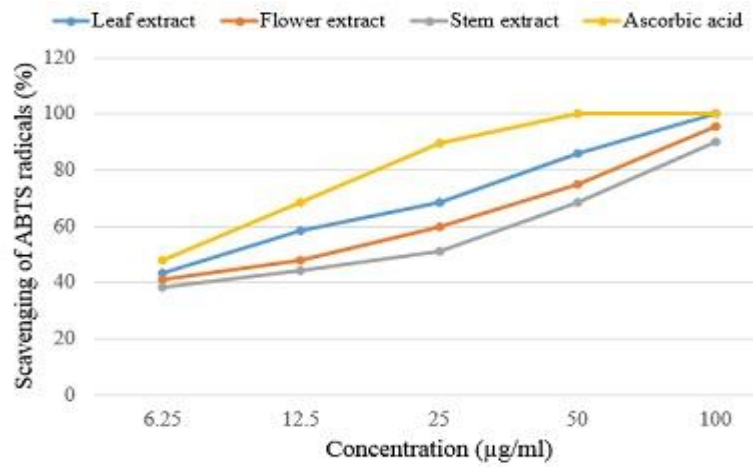


Figure 5: Scavenging of ABTS radicals by extracts of *A. cuneata*

Ferric reducing activity of *A. cuneata*

In this study, the reducing ability of leaf, flower and stem extracts of *A. cuneata* was evaluated by ferric reducing assay. The reducing ability of a substance is shown to be a significant indicator of its antioxidant capacity. The presence of reductones is responsible for reducing potential of the sample and these reductones reduce the Fe^{3+}/ferricyanide complex to the ferrous form resulting in the formation of Perl's Prussian blue complex. The intensity of the color complex is measured at 700nm in order to reveal the extent of reduction process [45,48-51]. In the present study, an increase in the absorbance with increase in the concentration of extracts of *A. cuneata* and ascorbic acid was observed indicating the reducing efficacy of extracts and ascorbic acid (Figure 6). The reducing potential observed was in the order: Ascorbic acid > leaf extract > flower extract > stem extract. It is clear from the results observed that the extracts of *A. cuneata* possess electron donating ability and hence the extracts can possibly terminate the chain reactions caused by free radicals. The reducing potential of other *Argyreia* species such as *A. argentea* [42], and *A. roxburghii* [43] has been reported.

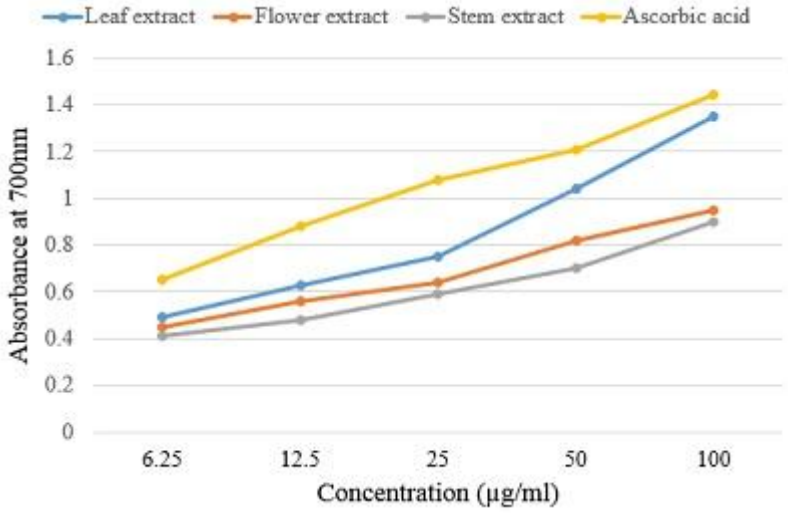


Figure 6: Ferric reducing activity of extracts of *A. cuneata*

CONCLUSION

Argyreia cuneata was shown to exhibit antimicrobial and antioxidant activity. Among various extracts, leaf extract exhibited marked activity followed by flower and stem extracts. The plant can be used against diseases caused by pathogenic bacteria and seed-borne fungi and oxidative damage.

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None

CONFLICTS OF INTEREST

None declared

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