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Original article

Phytochemical analysis, antibacterial and antioxidant potential of *Acronychia pedunculata* (L.) Miq.

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Abstract

The present study was aimed at phytochemical screening, antioxidant and antibacterial potential of *Acronychia pedunculata* (L.) leaf and stem extracts. The phytochemical analysis revealed the presence of sterols, terpenoids, flavonoids, saponins, tannins, carbohydrates, resins and glycosides, while proteins were absent in all the test extracts. Among the test extracts, maximum inhibition to test pathogenic bacteria (*E. coli, S. typhi, B. subtilis* and *S. aureus*) and antioxidant activity was most prominent in leaf methanol extract when compared to all the extracts of the plant. A maximum of 16.35 mm against *S. aureus* and 65.97% RSA was observed at 1 mg/ ml concentration in methanol leaf extract. The findings support and promote the pharmacological application of *A. pedunculata* extract and demand for purification of the active principle(s).

Key words: *Acronychia pedunculata* (L.) Miq., antibacterial, antioxidant, DPPH, RSA

1. Introduction

Plants have been used from ancient times for their medicinal properties to heal various disorders from time immemorial and are considered as one of the oldest available complete medical systems in the world (Sandhu and Heinrich, 2005; Dias *et al*., 2012). Medicinal plants are gaining importance as they are considered to be decisive in the treatment of present or future diseases of humans (Hassan, 2012). India is regarded as one of the biological hotspots of the world due to its varied geographical nature which serves as a host to some of the important medicinal plants of the world. In India, 2000 plant species have been explored in recent past for their medicinal properties (Mahendra *et al*., 2016). Presently, there is an increasing international market for medicinal plants for herbal medicine and pharmaceutical products as they are considered as much safer to present day synthetic drugs (Ferreira-Machado *et al*., 2004).

The present day antibiotics have their own drawbacks and are also losing their efficiency to combat against pathogens. Hence, the research areas have shifted their interest in obtaining newer compounds from plants which are safer and more efficient against the ever growing resistant pathogens. Antioxidants are considered as possible protective agents reducing oxidative damage to the human body (Yam *et al*., 2008). Radical scavenging molecules like flavonoids, phenolics and other secondary metabolites which possess antioxidant properties are rich in plants (Cai *et al*., 2003). Apart from having these, phenolic compounds have many other functional properties like antimicrobial, anti-inflammatory, antimutagenic, *etc*. (Hakkim *et al*., 2013). Due to the broad applicability of these natural products, plants have been exploited to the fullest extent for their phytochemicals having curative properties.

A. pedunculata is a small, evergreen aromatic tree distributed in South Asia from India and Srilanka to South China and Indonesia. The plant is known to contain higher medicinal properties as their leaves, bark, stem and fruits are widely used in the application against sores, scabies, and intestinal infections, due to their antifungal and antimicrobial properties (Lesueur, 2008). The oil obtained from this plant contains aromatic smell and, hence, it is also used in China for making perfumes (Rodrigo *et al*., 2007). Hence, based on the available data regarding the usage of medicinal values of this species, the present study was aimed at investigating the presence of secondary metabolites, antibacterial and antioxidant assets of leaf and stem extracts of *A. pedunculata*.

2. Materials and Methods

2.1 Plant collection

The fresh leaves and stem of *A. pedunculata* were collected from B.R. Hills, Chamarajanagar, Karnataka, India. The plant material was authenticated by a Taxonomist, Department of Studies in Botany, University of Mysore, Mysore. An herbarium (Voucher Specimen No. GJ706) of the plant is deposited in the Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, Karnataka, India.

2.2 Preparation of the plant extracts

The fresh leaves and stem of *A. pedunculata* were washed under running tap water, shade dried and powdered using a wearing blender. About 50 gm of dry leaf and stem powder was subjected to successive extraction with 300 ml each of petroleum ether, chloroform, ethyl acetate and methanol based on their polarity by keeping the same overnight in rotary shaker (150 rpm at 25 ± 2 °C). After extraction, each of the solvent extracts was filtered using Whatman No.1 filter paper and the filtrate was concentrated using rotary flash evaporator and stored at 4°C in an airtight glass bottle for further analysis (Trease and Evans, 1987).

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2.3 Phytochemical screening

Each of the extracts was subjected to qualitative phytochemical analysis for identification of various classes of phytoconstituents like alkaloids, flavonoids, triterpenes, saponins, carbohydrate, proteins, resin, glycosides, tannins (Harborne, 1973; Trease and Evans 1987).

2.4 Antibacterial activity

2.4.1 *Test organisms*

Both gram-positive bacteria (*Bacillus subtilis* (MTCC 121) and *Staphylococcus aureus* (MTCC 7443)) and two gram-negative (*Escherichia coli* (MTCC 7410) and *Pseudomonas aeruginosa* (MTCC 1688)) were collected from the Microbial Type of Culture Collection, Chandigarh and the cultures were maintained at 4°C in the Department of Botany before using for antibacterial activity.

2.4.2 *Agar-well diffusion assay*

The antibacterial activity of all the extracts (both leaf and stem) of *A. pedunculata* were evaluated by agar well diffusion method (Elecyinimi, 2007). About 15 ml of nutrient agar media was poured onto each petriplate and allowed to solidify. About 100 µl of test bacterial suspension (1.5×10^8 CFU/ ml) were swabbed onto the surface of NA media and uniformly spread using sterile glass spreader. Four wells of 6 mm diameter were made on each plate with the help of cork borer and the wells were loaded with 50 µl each of test samples (1 mg/ well) separately, while the concentration of standard was 100 µg/ well. The plates were incubated at 37°C for 24 h and the zone of inhibition was measured. The experiments were performed in triplicates.

2.5 Antioxidant activity

The radical scavenging activities (RSA) of the plant extracts against 2,2-Diphenyl-1-Picryl hydrazyl (DPPH) radical, purchased from Sigma-Aldrich, were determined by UV-vis spectrophotometry, described by Sultanova *et al*. (2006). All the extracts were subjected to RSA at the concentration of 200, 400, 600, 800 and 1000 µg/ ml from 5 mg/ ml of stock. Gallic acid was used as the standard. The test tubes with solutions were mixed thoroughly and allowed to stand for 30 min. at 25 ± 2°C. After incubation, the absorbance of each sample was measured at 517 nm and the experiment was performed in triplicates. The resulting discoloration and percent radical scavenging activity was calculated using the following formula:

$$\text{Percent RSA} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2.6 Statistical analysis

A simple statistical analysis was carried out to calculate the mean and the standard deviation. Each experiment was carried out in triplicate and average mean diameter of the inhibition zone was recorded (Sen and Batra, 2012).

3. Results

3.1 Phytochemical screening

The qualitative phytochemical analysis of both stem and leaf crude extracts of *A. pedunculata* revealed the presence of secondary metabolites such as sterols, terpenoids, flavonoids, saponins, tannins, carbohydrates, resins and glycosides (Table 1). It was noted that both stem and leaf extracts contained the presence of sterols, triterpenes, saponins, flavonoids, carbohydrates, resins and glycosides, while tannins were present only in stem extract and proteins were completely absent in all the test extracts.

Table 1: Table showing phytochemical screening of leaf and stem extracts of *A. pedunculata*

Phytochemicals	Test's	Leaf				Stem			
		PE	CH	EA	ME	PE	CH	EA	ME
Sterols	Salkowski	+	-	-	+	+	-	-	+
	Liberman-Burchard	+	+	+	+	+	+	+	-
Triterpenes	Salkowski	+	-	+	+	+	-	+	+
	Liberman-Burchard	+	+	+	+	+	+	+	-
Saponins	Foam	-	-	-	+	-	-	-	+
Alkaloids	Mayer's	-	-	-	-	-	-	-	-
	Dragondroff's	-	-	-	+	-	-	-	+
	Wagner's	-	-	-	-	-	-	-	-
	Hager's	-	-	-	-	-	-	-	-
Tannins	$FeCl_3$	-	-	-	-	-	-	-	+
	Gelatin	-	-	-	-	-	-	-	+
Flavonoids	Shinado	-	-	-	-	-	-	-	-
	$FeCl_3$	-	-	-	-	-	-	-	+
	Lead Acetate	-	-	-	+	-	+	-	+
Carbohydrates	Molisch's	+	+	+	+	+	+	+	+
	Fehling's	-	-	-	+	-	-	-	+
	Benedict's	-	-	-	+	-	-	-	+
Resins	Turbidity	-	-	+	-	-	-	+	-
	Acetic anhydride	-	-	-	-	-	-	-	-
Proteins	Biurete	-	-	-	-	-	-	-	-
	Ninhydrin	-	-	-	-	-	-	-	-
Glycosides	Keller-kiliani's	+	+	+	+	+	+	+	+

Note: + Present; - Absent; PE:Petroleum ether; CH:Chloroform; EA:Ethyl acetate; ME:Methanol

3.2 Antibacterial activity

The crude extracts of leaf and stem of *A. pedunculata* were subjected to screening for antibacterial activity against pathogenic bacteria by agar well diffusion method. The results of the antibacterial screening are presented in Table. 2. Among the test extracts, only methanol extracts of leaf and stem showed antibacterial activity against all the test pathogens (Figure 1). However, petroleum ether was active against *S. typhi* and *S. aureus*, chloroform extract against *E. coli* and *B. subtilis*, while ethyl acetate extract didn't inhibit B. subtilis. Among the methanol extract of leaf and stem, leaf extract offered a maximum zone of inhibition of 14, 14, 11 and 16 mm against *E. coli*, *B. subtilis*, *S. typhi* and *S. aureus*, respectively.

3.3 Antioxidant activity

DPPH was used to determine the antioxidant potential of leaf and stem extracts of *A. pedunculata*. The free radical scavenging activity showed noticeable scavenging activity of all the tested extracts (Figures 2 and 3). The results highlighted an dose dependent increase in the RSA of all the extracts. Among the extracts, methanol offered maximum free RSA of 29.18%, 36.22%, 43.91%, 58.28% and 65.97% in leaf, while 28.3%, 33.09%, 43.67%, 51.94% and 64.76% in 200, 400, 600, 800 and 1000 µg/ ml concentration, respectively. The methanol extract of leaf and stem showed highest free radical scavenging activity which was almost equal to the standard.

Table 2: Antibacterial activity of leaf and stem extracts of *A. pedunculata*

Extract	Solvent	Zone of inhibition (mm)			
		E. coli	*B. subtilis*	*S. aureus*	*S. typhi*
Leaf	Petroleum ether	00.00±0.00	00.00±0.00	09.25±0.17	07.50±0.50
	Chloroform	11.10±0.32	07.20±0.23	00.00±0.00	00.00±0.00
	Ethyl acetate	12.05±0.10	00.00±0.00	11.30±0.52	13.37±0.25
	Methanol	14.20±0.22	14.20±0.15	16.35±0.24	11.20±0.17
Stem	Petroleum ether	00.00±0.00	00.00±0.00	10.02±0.14	10.75±0.09
	Chloroform	10.07±0.10	09.15±0.12	00.00±0.00	00.00±0.00
	Ethyl acetate	10.17±0.10	00.00±0.00	10.35±0.40	08.10±0.25
	Methanol	13.17±0.38	14.02±0.25	14.87±0.60	11.25±0.10
Control (Streptomycin)		15.15±0.10	20.07±0.15	22.20±0.75	13.25±0.19

Values are Mean ± SE of three replicates

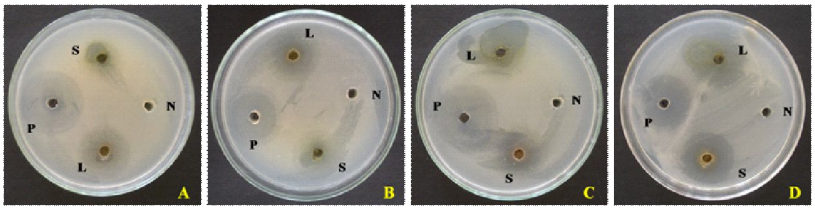

Figure 1: Antibacterial activity of *A. pedunculata* methanol extract against test pathogens by well diffusion method. A: *B. subtilis*; B: *S. aureus*; C: *E. coli*; D: *S. typhi*.

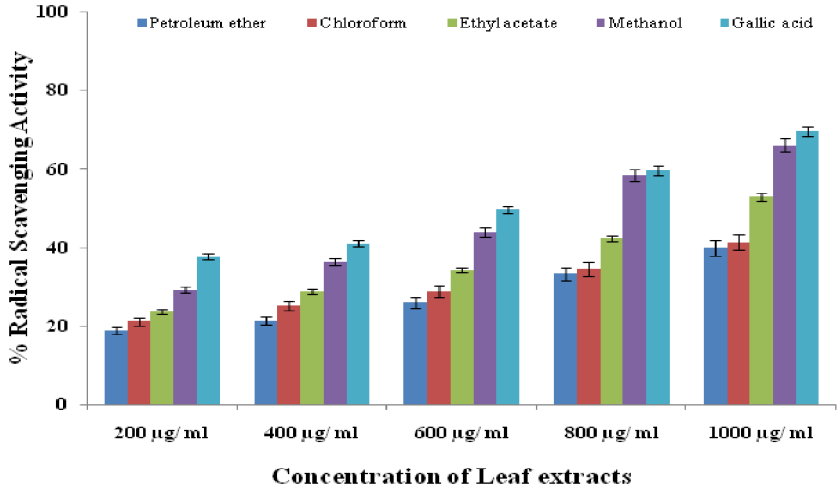

Figure 2: DPPH radical scavenging activity of leaf extracts of *A.pedunculata*

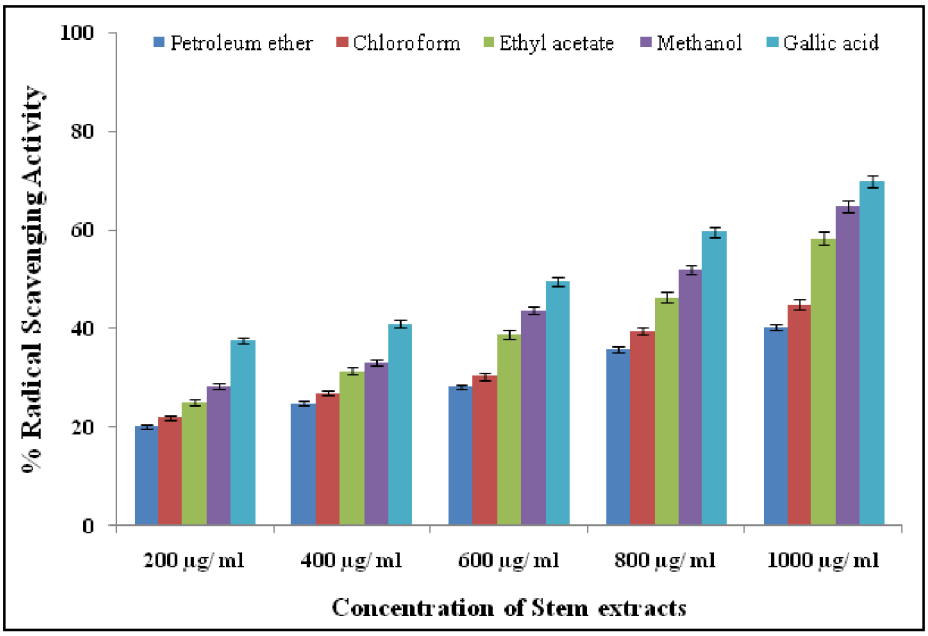

Figure 3: DPPH radical scavenging activity of stem extracts of *A.pedunculata*

4. Discussion

Plants have been a vital source of secondary metabolites which in turn have been used in various fields majorly being the pharmaceuticals. In the present investigation, phytochemical constituents, antibacterial and antioxidant properties of *A. pedunculata* were evaluated. The phytochemical screening of different solvent extracts of both leaf and stem extracts showed the presence of all the phytochemical constituents tested except proteins. It was also noted that, methanol extracts possessed a higher number of phytochemicals when compared to other extracts. The results are in agreement with the findings of Ara and DeClerch (2010) and Shakeri *et al*. (2011), wherein a large number of phytochemicals was observed in the methanol extract of *Adenanthera pavonia* and *Anabasis aphylla* when compared to other solvent extracts.

Further, the extracts were subjected to their antibacterial and antioxidant potential of the extracts. The results of the antibacterial activity revealed that between the extracts tested irrespective of the source, methanol extract presented inhibition against all the test pathogens, while other extracts exhibited inhibition three or fewer pathogens tested. Likewise, Mahendra *et al*. (2016), have reported inhibition to all the test pathogens in the methanol extract of both leaf and fruit extracts of *Argyreia osyrensis*. Also, the antibacterial activity carried out by agar well diffusion method of methanol extracts of leaf, stem, bark and root of *Datura metel* inhibited the growth of *Streptococcus hemolytic, S. dysenteriae, P. aeruginosa, E. coli, S. aureus, Klebsiella pneumonia* and *B. cereus* (Aksharaiyi, 2014).

The results of antioxidant activity of the plant extracts showed a dose-dependent increase in radical scavenging potential. Amongst the extracts tested, leaf methanol extract offered maximum RSA of 65.94% followed by stem extracts. The results are also in conformity with the findings of Devakumar and Sudha (2014) where they reported the antioxidant activity of *Argemone mexicana* leaf methanol extract which showed good scavenging activity than aqueous extract. Likewise, dose-dependent RSA was observed in the methanol extracts of *Viscum nepalense* and *Argyreia osyrensis* (Murali *et al*., 2011; Mahendra *et al*., 2016).

5. Conclusion

The present work reports the phytochemical constituents present in different solvent extracts of both leaf and stem extracts of *A. pedunculata*. Among the extracts tested for antibacterial activity, methanol extract offered significant inhibition to test pathogens when compared to others. The methanol extracts also offered significant antioxidant potential. Furthermore, attempts may be required to isolate and identify the bioactive compounds responsible for the antibacterial and antioxidant activity of methanol extract of *A. pedunculata*.

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Conflict of interest

We declare that we have no conflict of interest.

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