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Research Article

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Antibacterial Activity of Aqueous Root, Seed, Flower and Stem Bark Extracts of *Acronychia pedunculata* Grown in Sri Lanka

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ABSTRACT

In vitro antibacterial activity of six(06) different aqueous water extracts of Acronychia pedunculata found in Sri Lanka were investigated against Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 35218). Disk diffusion antimicrobial susceptibility testing method was used to determine antibacterial activity of each aqueous extract. The results showed that there was a marked antibacterial activity against S.aureus (ATCC 25923) strain. The highest activity observed for the combined aqueous sample which had all ingredients(roots, stem barks, leaves, flowers and seeds) in the same aqueous solution and the antibacterial activity was observed in a concentration dependent (r^2 = 0.97) manner. Lowest activity was recorded for seeds (r^2 = 0.87) while root (r^2 = 0.99) and stem bark (r^2 = 0.87) aqueous solution showed second most and third most antibacterial activity against S.aureus (ATCC 25923) on a concentration dependent manner. Gentamicin (10 µg/disk) and distilled water were used as positive and negative controls. When compared with Gentamicin inhibition zone (14.03 ± 0.03mm), the combined aqueous sample at a concentration of 1000µg/disk, showed some potent, significant antibacterial activity with an inhibition zone of 11.26 ± 0.03 mm ,where aqueous root sample has an inhibition zone of 10.90 ± 0.06 mm. These two aqueous samples (combined sample and root sample) could be used further experiments as lead compounds for the development of potent, novel antibacterial agents from Acronychia pedunculata. However there was no antibacterial activity observed for all aqueous extracts against E.coli (ATCC 35218) and this observed phenomena needs to be further investigated with other Gram negative bacterial strains.

Key words: *Acronychia pedunculata*, Aqueous extract, Antibacterial activity, Disk diffusion method, folk medicine, Sri Lanka.

INTRODUCTION

Acronychia pedunculata (Family: Rutacea) is a small tree with pale smooth bark and glabrous branches. This plant is also known as , Claw-flowered Laurel & Laka wood in English, Ankenda in Sinhala language and Kattukanni, Muttainari in tamil language. The plant is distributed in parts of India, Malaysia, Sri Lanka and Philippine Islands. This plant is common in Sri Lanka up to 5000 feet elevation. [1]

The leaves are arranged in simple, opposite or some alternate pattern where normal size of leaves vary between 7.5 cm -12.5 cm in length. Naturally plant flowers from February to April and flowers are in yellowish green in 1.8cm in length on long pedicels where flowers are arranged loosely in pyramidal, divaricately on long straight axilarry peduncles. The fruit is globular shaped in 1.2- 1.8 cm in length. The fruit has four chambers and has a seed per chamber. [1]

In traditional and folk medicine in Sri Lanka, the bark is used externally on swelling, fractures, sores and tonic for scabies and ulcers. The bark has also been used internally as purgative.[1,2] Extracts of *A.pedunculata* exhibit antibacterial activity against *Enterococcus faecalis* and *Pseudomonas aeruginosa*.[3] According to studies carried out by Pathmasiri and colleagues , methanolic extracts of stem and root bark exhibit significant cytotoxicity in human K3 tissue culture assay.[4] It is also worth stating here that dichloromethane extracts of stem bark of this plant showed inhibition of cyclooxygenase-2 (CoX-2)enzyme.[4]

Therefore this study was undertaken to investigate *in vitro* antibacterial activity of aqueous extracts of roots, seeds, flower, and leaves, stem barks of *A.pedunculata* separately and identify the most active aqueous extract/s with idea of using these extract/s for development of novel pharmacophores from plant sources against infectious bacterial pathogens.

MATERIALS AND METHODS

Collection & Authentication:
Whole matured plants with flowers and fruits were collected in Mallawapitiya area in Kurungegala district in Sri Lanka (GPS 7°28'21.7"N 80°23'23.8"E), in December 2015. The whole plant had been identified and authenticated by a botanist at National Herbarium at Peradeniya Sri Lanka.

A voucher specimen of whole plant of *A.pedunculata* (AP/ 01/2015), stem bark of *A.pedunculata* (AP/ 02/2015), seeds of *A.pedunculata* (AP/ 03/2015), roots of *A.pedunculata* (AP/ 04/2015), flowers of *A.pedunculata* (AP/05/2015), leaves (AP/06 / 2015) were deposited at the Department of Medical Laboratory Sciences, General Sir John Kotelawala Defence Sri Lanka.

Preparation of aqueous extracts of *A.pedunculata:*
The roots, seeds, flowers, leaves, stem bark were removed from the plants and were air dried in shade for 2-4 days and were cut in to small pieces separately. In order to prepare aqueous extracts for each sample separately, twenty three grams (23 g) from each sample was boiled slowly in 92 ml of distilled water for approximately for 3 hours until the volume is reached to 18 ml. The prepared aqueous sample was left for cooling down and stored securely for later use.

There was another separate aqueous sample prepared by adding 23 g of roots, 05 g of seeds, 05 g of flower, 23g of leaf and 23 g of stem bark in to 316 ml of distilled water. This sample was boiled for about 5 hours until the final volume of the solution reached 62 ml. This sample was labelled as combined sample.

Antibacterial Activity Screening:
Prepared aqueous samples (labelled as roots, seeds, flowers, leaves, stem bark and all) were evaluated for antibacterial activity using disc diffusion methods as described by clinical and laboratory institute .[5] Distilled water was used as the negative control while Gentamicin (10 µg/disk) was used as positive control. The growth medium use for this experiment was Muller Hinton Agar and the antibacterial activities were determined against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 35218) at following concentrations of 500 µg of plant extract /disk,750 µg of plant extract /disk and 1000 µg of plant extract /disk. The experiment was carried out in triplicates for each sample and the diameter of the inhibition zone (in mm) for each extract against above mentioned strains were measured and recorded.

Statistical Analysis:
The data was statistically analysed by using Non Parametric-Kruskal-Wallis Test in SPSS (17^{th} Version) software package. Data expressed as mean inhibition zone diameter ± Standard Error of the Mean (SEM) and coefficient of determination (r^2 value). Significance level was set at $p < 0.05$.

RESULTS

The results are summarized in table 1. As shown, Gentamicin was used as the positive control at a concentration of 10 µg/disk for each extract and distilled water was used as the negative control. There was no antibacterial activity

observed against *E.coli* strains (ATCC 35218) for any of the prepared extracts at any concentration used for this investigation.

At 500 µg/disk concentration, there was no inhibitory zone observed for seed extract where mild inhibitory zones were observed for leaves (mean 5.03 mm), flowers (mean 6.06 mm) and stem bark (mean 6.10 mm) extracts. Root (mean 7.96 mm) and combined sample (mean 9.06 mm) showed moderate anti bacterial activity against *S.aureus* Strains (ATCC 25923). Same pattern of antibacterial activity was observed for all extracts at 750 µg/disk and 1000 µg/disk concentrations. However when compared with positive Gentamicin control at concentration of 10 µg/disk (mean inhibition of 13.98 mm) ,there was a strong antibacterial activity (mean inhibition 11.26 mm) was observed for the combined sample against *S.aureus* Strains, at the concentration of 1000 µg/disk. Further, the observed antibacterial activity for each extract was concentration dependent (r^2 values from 0.87 to 0.99, $p < 0.05$).

Table 1: Antibacterial Screening Results of Plant Extracts

Plant Extract	Antibacterial activity (inhibition Zone diameter – mm) *S.aureus* Strains (ATCC 25923)				*E.coli* Strains (ATCC 35218)		
	500 µg/disk	750 µg/disk	1000 µg/disk	r^2 Value	500 µg/disk	750 µg/disk	1000 µg/disk
Roots	7.96 ± 0.08	9.53 ± 0.15	10.90 ± 0.06	0.99	-	-	-
Seeds	0.00 ±0.00	2.1 ± 0.05	2.53 ± 0.03	0.87	-	-	-
Flowers	6.06 ± 0.03	7.00 ± 0.05	7.53 ± 0.03	0.97	-	-	-
Leaves	5.03 ± 0.03	7.10 ± 0.05	8.06 ± 0.03	0.95	-	-	-
Stem Barks	6.10 ± 0.05	8.10 ± 0.05	8.53 ± 0.03	0.87	-	-	-
Combined Sample	9.06 ± 0.03	9.86 ± 0.03	11.26 ± 0.03	0.97	-	-	-
Gentamicin	14.00 ± 0.02	14.03 ± 0.03	13.98 ± 0.04	N/A	15.00 ± 0.02	15.02 ± 0.03	15.04 ± 0.02
Distilled water	0.00	0.00	0.00	N/A	0.00	0.00	0.00

Data presented as mean Inhibition zone diameter ±SEM (n=3).
Sign (-) indicates no antibacterial activity. Sign (N/A) indicates not applicable.
Gentamicin was used as Positive control. Distilled Water was used as Negative control.

DISCUSSION

This study examined *in vitro* antibacterial activity of aqueous extracts of *A.pedunculata*. This experiment was carried out with a view of scientific justification of its use in traditional and folk medicine in Sri Lanka. At the same time, there is a huge demand for novel, cheap, antibiotics due to the rapid emergence of novel antibiotic resistant microorganisms.[6,7,8]

On the other hand, there are only limited number of peer reviewed published reports on antibacterial activity of Sri Lankan medicinal plants.[9] In our previous studies we have shown that there are many Sri Lankan medicinal plants which possess significant antibacterial activity with a huge potential of development of novel antibacterial, anti-arthritic and anti-oxidant drugs from medicinal plants in Sri Lanka.[9,10,11,12,13] In general there are other extracts , such as hexane, methanol , chloroform are commonly being used for antibacterial screening tests.[14] we have decided to use water extracts for this experiment since aqueous extracts (hot or cold) are the most common form of extract being used in traditional and folk medicine.[21, 22]

There was no significant antibacterial activity observed against *E.coli* (ATCC 35218) strains for all aqueous extracts prepared. This was an interesting observation since there was no antibacterial activity observed even for the sample where roots, leaves, flowers, seeds and stem barks have been mixed together. Therefore this observed phenomena needs to be further investigated to see the effect of the extract on other Gram negative bacterial strains. These further investigations will provide a general idea about the its activity against other gram negative bacterial strains and based on those future results, it would be possible to classify its activity generally against Gram negative bacterial strains.

All aqueous extracts of *A.pedunculata* showed marked antibacterial activity against Gram positive *S.aureus* (ATCC 25923) strains. Aqueous seed extract showed the lowest antibacterial activity while 'all sample' showed the highest antibacterial activity. Aqueous extracts of roots and stem barks showed second most and third most activities respectively. The general order of potency of aqueous extracts, based on the results of this experiment, could be stated as combined sample > root > stem bark > leaves > flowers > seeds. The observed highest antibacterial activity for combined sample could be due to the synergistic activity of each compound/s from each plant material used in this experiment. Therefore it is worth to investigate each extract individually using various chromatographic techniques and identify active compound/s separately. Those identified compound/s could be further investigate for their *in vitro* synergistic activities later.

Gentamicin was selected as the positive drug as it inhibits the growth of wide variety of Gram negative and Gram positive microorganisms by inhibiting bacterial protein synthesis.[6] Gentamicin is also recognized as one of the most important medications needed in basic health system by World health Organization .[15] It is worth mentioning here that when compared to a pure compound as Gentamicin at 10 µg/disk concentration (inhibition zone of 13.98 mm) , the combined sample of 1000 µg/disk showed some potent, note worthy antibacterial activity (inhibition zone of 11.26 mm) against *S.aureus* (ATCC 25923) strains. Therefore it is fair to assume that a further chemically purified, combined aqueous sample would produce relatively higher antibacterial activity when compared to its crude aqueous sample and even to Gentamicin at 10 µg/disk concentration. This crude, combined aqueous sample could be further investigated to identify potential novel antibacterial compounds from natural resources.

The stem bark and roots contain polyphenol (Acrovestone), triterpene alcohols and acetophenones. [16, 17] These compounds might have played a vital role in observed high antibacterial activity in stem bark and root extracts and the results of this and previous studies collaborate with the use of these plants in traditional medicine.[18, 20] It is also worth stating here that oils from aerial parts of *A.pedunculata* possess a broad spectrum of antimicrobial activity against both Gram positive and Gram negative strains, particularly against *Salmonella enterica* and *Staphylococcus epidermidis.* [19]

Thirty four (34) compounds have been identified based on Gas Chromatography Mass Spectrometry (GC-MS) and Carbon-13 Nuclear Magnetic Resonance (^{13}C –NMR) studies .The major constituents were α-pinene and β-caryophyllene and these oils have shown to possess a broad spectrum of antimicrobial activity against both Gram positive and Gram negative microorganisms.[19]

CONCLUSION

In conclusion, the results of this study show for the first time, that aqueous extracts (mainly combined sample ,roots and stem bark) of *A.pedunculata* found in Sri Lanka, posses marked antibacterial properties against *S.aureus* (ATCC 25923) strain. The observed relatively higher activity (when compared to Gentamicin) for the combined sample must be further investigated as this could be used as a potential lead compound for the development of potent, novel antibacterial agents from *Acronychia pedunculata*. However, the activity of aqueous extracts against Gram negative microorganisms needs to be further investigated. At the same time this study rationalizes the use of this plant in traditional and folk medicine treatments in Sri Lanka. At the same time this plant could also be used for screening processes in identifying novel, potent antibacterial compounds from natural resources.

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