Original Research Article

The effect of extraction method on yield and Bioactivity of *Adenanthera pavonina*

Abstract

Adenanthera pavonina (Fabaceae) is often used in traditional medicine for treating various diseases. Previous studies have shown various bioactivities. In this work methanolic extracts of different plant parts (Stem-bark, leaves, root, seeds) prepared using two extraction methods, Soxhlet and Ultrasound assisted solvent extraction (UASE), were examined for antioxidant and cytotoxic activities. Antioxidant activity was assayed against stable DPPH (2,2-diphenyl-1-picryl-hydrazil) free radical. Cytotoxicity was screened against brine shrimp, *Artemia salina*. Yields of extracts varied with the plant part and the extraction method. The sonicator bark extract showed the highest antioxidant activity (89.5%), significantly exceeding that of the Soxhlet extract. The seeds and leaves exhibited weak antioxidant activity, while the root showed moderate activity. Both sonicator and Soxhlet bark extracts displayed low cytotoxicity, and the seeds and leaves showed no cytotoxicity. These findings underscore the pharmacological potential of *A. pavonina* extracts and emphasize the need for purifying its active compounds.

Key Words: Soxhlet extraction, Sonication, *Adenanthera pavonina*, Antioxidant activity, Cytotoxic activity

1.0 Introduction

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Plants have been used for their medicinal properties to treat various ailments since ancient times, making them one of the oldest and most complete medical systems in the world [1,2]. The significance of medicinal plants is growing as they are increasingly seen as vital for addressing current and future health challenges [3]. Today, there is a rising global demand for medicinal plants in the production of herbal medicines and pharmaceutical products, as they are often considered safer alternatives to synthetic drugs [4].

It is important to highlight that only a small fraction of natural sources has been extensively studied for their medicinal properties. The chemical distinctiveness of these natural products

often exceeds that of other sources. Investigating bioactive compounds from natural origins offers numerous opportunities, including the identification of known compounds with either established or yet-to-be-discovered activities, as well as the potential discovery of entirely new compounds and their therapeutic effects [5].

Phytochemical processing techniques, such as maceration and Soxhlet extraction, are used to isolate bioactive compounds like polyphenols with antimicrobial, antioxidant, anti-inflammatory, and antiviral properties [6,7]. Extraction is a crucial step that separates compounds from plant materials using solvents or other methods, with the choice depending on the target compounds and source material. Traditional methods include maceration, percolation, and decoction, while newer techniques like ultrasound-assisted solvent extraction (UASE), microwave-assisted solvent extraction (MASE), and supercritical fluid extraction (SFE) have gained popularity in recent years [5]. Each method has its advantages, depending on factors like target compound type, plant material properties, efficiency, cost, and safety. After extraction, bioactive compounds are further analyzed and refined for use in potent and effective products. However, yields and bioactivities can vary depending on the extraction method used [8].

This study seeks to compare the yield, antioxidant activity, and cytotoxicity of methanolic extracts of *Adenanthera pavonina* obtained through two extraction methods: Soxhlet extraction and ultrasound-assisted solvent extraction (UASE). Antioxidants are believed to act as protective agents, helping to reduce oxidative damage in the human body [9]. Plants are rich in radical-scavenging molecules such as flavonoids, phenolics, and other secondary metabolites with antioxidant properties [10]. In addition to their antioxidant effects, phenolic compounds offer a range of other functional benefits, including antimicrobial, anti-inflammatory, and antimutagenic properties [11].

A. pavonina is a medium to large-sized unarmed deciduous tree about 20 m in height with a greyish brown bark with longitudinal fissures distributed from tropical and subtropical Asia to North Australia and Polynesia [12]. The tree used in traditional medicine to treat various ailments. A red powder made from wood is used as an antiseptic paste. The ground seeds are used to treat boils and inflammations. A decoction of the leaves is used to treat gout and rheumatism [13]. Various plant parts of *A. pavonina* contain flavones, triterpenoids, alkaloids,

steroidal-glucosides, cysteine proteinase and fatty acids. Seeds contain non-protein amino acids γ-methylene glutamic acid, γ-methylene glutamine, γ-ethylidine glutamic acid and stigmasterol. Octacosanol, glucosides of β-sitosterol and stigmasterol are reported from leaves. Bark is found to contain stigmasterol glycosides [14]. Based on existing information about the medicinal properties of this plant, and our previous work [15] the present study aims to investigate the antioxidant and cytotoxic properties, of leaf, bark, seeds and root extracts of *A. pavonina.*

2.0 Materials and Methods

The prepared plant materials were extracted with distilled methanol. Analytical TLC was performed with 0.1 mm thick 60F Kieselgel G. Merck plates. UV active spots on TLC were located with 365 nm, and 254 nm tubes of a Bio block UV lamp.

2.1 Collection of plant material

All four parts of *Adenanthera pavonina* were collected from a tree located in Nagolla area, Matale, Sri Lanka. Each plant material was carefully collected to exclude material contaminated with microorganisms like lichens, and fungus. Plant materials were authenticated by comparing with those at National herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka.

2.2 Preparation of the plant material for extraction

Each plant material was first washed under running tap water and dried under mild sunlight inside the laboratory to a constant weight. Each plant material was then cut into small pieces manually and used in the Soxhlet extraction (SE) with methanol. Materials, ground to a powder with a grinder, were used in Ultrasound assisted solvent extraction (UASE) with methanol.

2.3 Extraction methods

2.3.1 Ultrasound assisted solvent extraction (UASE)

A glass beaker containing ground plant material (50 g) and methanol (200 mL) covered with aluminum foil, was placed in a sonicator partially filled with water. The mixture was sonicated twice, with each session lasting 30 minutes. After each sonication, the mixture was filtered through cotton wool, and the filtrate was collected and evaporated under reduced pressure using a rotary evaporator [5].

2.3.2 Soxhlet extraction

The dried and ground plant material (50 g) was extracted using Soxhlet apparatus with methanol. extracted with methanol. The methanol volume and extraction duration are provided in Table 1. Each extraction before terminating, TLCs of the bulk-extract in the flask and the extractive coming out of the Soxhlet, which was almost colourless, were compared to ensure that no new compounds were present in the colorless extract. The extract was filtered through cotton wool, and the filtrate was collected and evaporated under reduced pressure using a rotary evaporator [5].

Table 1. Soxhlet extraction conditions used for *A. pavonina*

Plant part	Weight of dried plant material/g	Volume of methanol /mL	Duration of extraction /h
Root	50	700	13
Leaves	50	700	15
Stem-bark	50	500	16
Fruit	50	500	16

The crude extracts were stored at 4 °C in airtight glass bottles for further analysis.

2.4 Bioassays

2.4.1 Antioxidant activity

The radical scavenging activity (RSA) of the crude methanolic extracts was determined against 2,2-Diphenyl-1-Picryl hydrazyl (DPPH) using UV-vis spectrometry [16].

Preparation of DPPH solution

DPPH (4.0 mg) was dissolved in methanol and brought to the final volume of 100 mL to get 0.0001 M solution. The volumetric flask containing DPPH was covered with an aluminum foil to prevent the effect of light and kept in the refrigerator.

Preparation of test solutions

Each crude extract (2.0 mg) was dissolved in methanol (4 mL) to get 500 ppm solutions, in sterile Bijou bottles.

DPPH photometric assay

First, the absorbance of the prepared DPPH (0.0001 M) solution (3.0 mL) was measured at 515 nm (A_0). 40.0 µL of the test solution was added to the DPPH solution and absorbance (A_t) was measured at one-minute intervals over 16 minutes. Methanol (3.0 mL) was used as the blank. α-Tocopherol was used as the standard antioxidant. Antioxidant activity or RSA is expressed as the percentage inhibition and was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0 - A_t)/A_0] \times 100$$

2.4.2 Cytotoxicity

Cytotoxicity of the crude methanol extracts was determined using the brine shrimp (*Artemia salina)* assay. Artificial sea water used in this assay was prepared by dissolving the following compounds in distilled water (1.00 l): NaCl (24.73 g), KCl (0.66 g), $CaCl_2$ (4.7 g), $MgCl_2$ (1.9 g), $MgSO_4$ (6.3 g), and $NaHCO_3$ (0.18 g) [17].

Hatching brine shrimps

Brine shrimp eggs were kept in artificial sea water to hatch, for 48 hrs. The container was illuminated from a side. Aeration of sea water was carried out during the latter 24 h. After 48 h phototrophic nauplii were collected from the lighted side.

Preparation of test solutions

A 1000 ppm solution of each extract was prepared by dissolving 3.0 mg in 3.0 mL of 4% DMSO/H_2O. A 2000 ppm solution was prepared by dissolving 2.0 mg in 1.0 mL of the same solvent.

Assay

Assay was done in a 96 microwell plate. The volumes given in Table 2 were added to wells from the 1000 ppm stock test solution to get the desired concentration of the test solution in each well, and final volume of a well was adjusted to 300 µL by adding artificial sea water.

Table 2. Volumes added to a microwell from 1000 ppm stock solution of extracts to get the desired final concentration.

Volume added / µl	Final concentration* / ppm
150	500
75	250
30	100
15	50
7.5	25

* Final volume of each well was adjusted to 300 µl by adding artificial sea water.

150 µL from the 2000 ppm stock solution was added to get a final concentration of 1000 ppm. Then the brine shrimps were added with the help of a Pasteur pipette so that each well contained 10 of them. Finally, volume of each well was brought to 300 µL with artificial sea water using a dropper. Following volumes (300 µL, 150 µL, 75 µL, 30 µL, 15 µL, and 7.5 µL) from the 4% DMSO/ artificial sea H_2O system were added as controls for the 1000 ppm to 25 ppm test solution concentrations respectively. Test was done in triplicate.

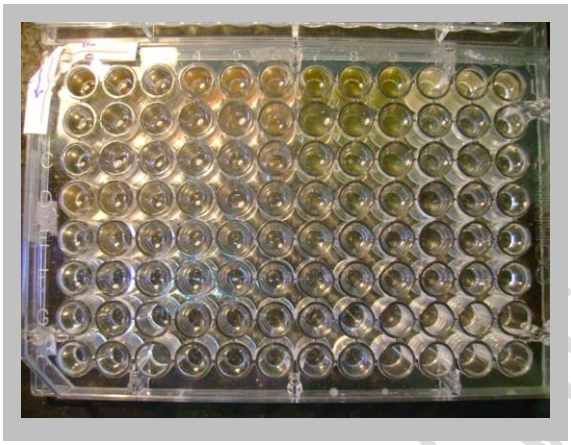

Figure 1.0 96 micro well plate, cytotoxicity assay of *A. pavonina* extracts
Key: From A-F concentration series (1000-25 ppm), G, H controls, 1-3 root, 4-6 Stem-bark, 7-9 leaves, 10-12 seeds.

Statistical analysis

Data are expressed as mean ± standard deviation (S. D). Statistical analysis involved a one-way analysis of variance (ANOVA). A value of P less than 0.05 ($p < 0.05$) was considered statistically significant.

Results and Discussion

Biologically active compounds are typically present in low concentrations in plants and other natural sources. An ideal extraction method should maximize yield while preserving the compounds' functional properties [18]. Studies have shown that extraction methods can significantly affect the biological activity of the resulting extracts, highlighting the importance of choosing the right technique [18,19]. In traditional methods like Soxhlet extraction, the effectiveness of solvents depends on factors such as compound solubility, mass transfer, and solute-matrix interactions, which influence heat and mass diffusion rates. Ultrasound-assisted solvent extraction (UASE) has gained popularity for its ability to enhance extraction efficiency, reduce extraction time, and minimize the use of toxic solvents. The high-frequency sound waves

in UASE disrupt plant cell walls, improving solvent penetration and releasing more extractable compounds [20].

3.1 Comparison of the two extraction methods: Soxhlet and USAE

Different parts of *A. pavonina* were extracted to methanol using Soxhlet and sonicator methods. First the percent yields were compared and the percentage yields corresponding to the two methods are summarized in Table 3. Extraction yield (mass of extract/mass of dry matter) was used as an indicator of the effects of the extraction conditions.

Table 3. Yields of extracts prepared by Soxhlet and sonicator methods

Plant part	Percentage yield %	
	Soxhlet	**Sonicator**
Root	14.1	19.0
Leaves	22.3	11.0
Stem-bark	26.3	12.2
Seeds	36.4	7.0

As summarized in Table 3, yields of extracts vary with the type of plant material and also with the extraction method. Soxhlet method afforded higher yields of the leaf, stem-bark, and seed extracts than the sonicator method. The roots however gave a higher yield with the sonicator method. This clearly shows that there is an impact of the extraction method on the extractability of compounds from the different plant parts. The Soxhlet technique resulted in the greatest yields across various plant parts, possibly because heat is utilized during Soxhlet extraction, facilitating the diffusion of solvents into materials with comparable polarities.

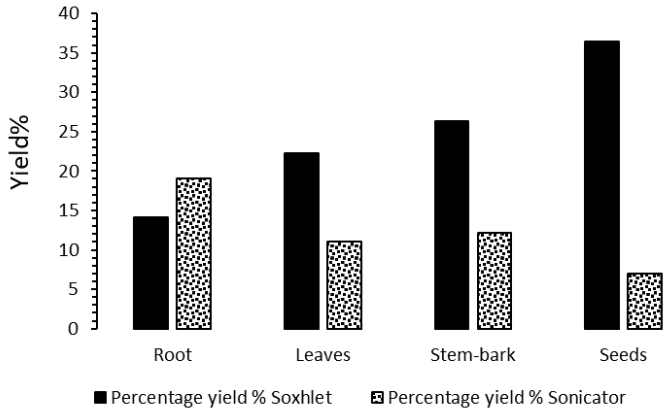

Figure 2.0. The yield of *A. pavonina* extracts from the three extraction methods

3.4 Antioxidant activity

3.4.2 Antioxidant activity of crude extracts of *Adenanthera pavonina*

The extracts of *A. pavonina* prepared employing the sonicator and Soxhlet methods were separately examined for their antioxidant activity by DPPH radical assay using α-Tocopherol as the standard antioxidant. Percentage antioxidant activity of each extract of *A. pavonina* prepared by both Soxhlet and sonicator methods are given in Table 4.0 and Figures 3 and 4.

Table 4. Percentage antioxidant activity of crude extracts of *A. pavonina* prepared by Soxhlet and sonicator methods in DPPH assay.

	Seeds	**Leaves**	**Stem Bark**	**Root**
Sonicator extracts	1.27±0.07	2.69±0.15	89.50±0.10	26.51±0.62
Soxhlet extracts	2.11±0.138	5.88±2.83	70.30±0.541	31.19±0.402

Activity of α-Tocopherol = 55.4%

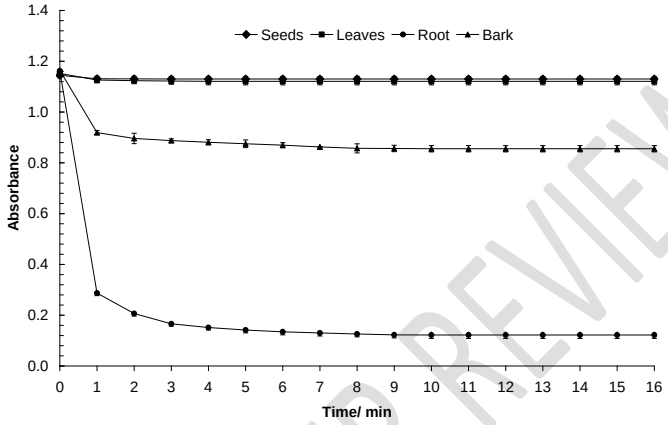

Figure 3. Decrease in absorbance of DPPH with *A. pavonina* sonicator extracts, over 16 minutes.

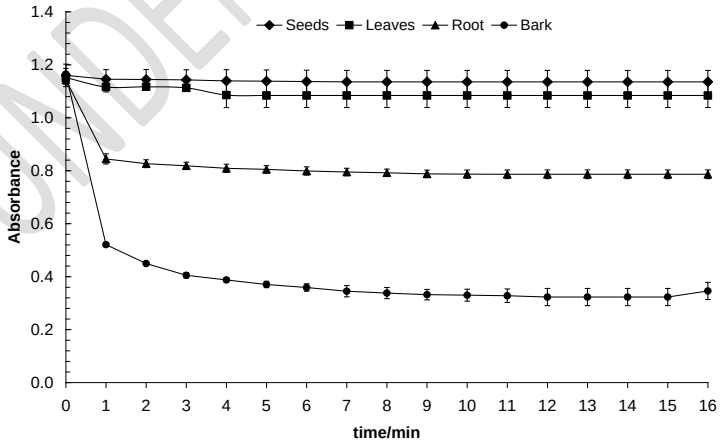

Figure 4. Decrease in absorbance of DPPH with *A. pavonina* Soxhlet extracts, over 16 minutes.

Statistical analysis

Antioxidant activity of extracts *A. pavonina*, prepared using both sonicator and Soxhlet extraction methods, were compared using "2-sample *t*-test". (Using Minitab 14.0). The sonicator extract of *A. pavonina* bark showed the highest activity, 89.5% and second highest activity by Soxhlet extract of the bark, 70.30%. The root extract showed moderate activity while seeds and leaves exhibited low activity. Antioxidant activities corresponding to the Soxhlet extracts of *A. pavonina* seeds and root are significantly ($p<0.05$) greater than those corresponding to sonicator extracts. Antioxidant activity corresponding to the sonicator extract of *A. pavonina* bark is significantly greater than that of Soxhlet extract. Both Soxhlet and sonicator extracts of *A. pavonina* bark showed significant potential with radical scavenging ability, which is far better than previous findings [21].

3.5.2 Cytotoxicity of crude extracts of *Adenanthera pavonina*

The extracts of *A. pavonina* prepared employing the sonicator and Soxhlet methods were separately examined for their cytotoxic activity using brine shrimp assay. The cytotoxicity of each crude extract of *A. pavonina* prepared by sonicator method is given in Table 5 as percentage mortality of brine shrimps.

Table 5. The cytotoxicity of *A. pavonina* sonicator extracts given as percentage mortality against *Artemia salina.*

Extract	**Concentration /ppm**					
	1000	500	250	100	50	25

Root	100	100	60	17	0	0
Stem-Bark	100	67	50	0	0	0
Leaves	0	0	0	0	0	0
Seeds	0	0	0	0	0	0

Cytotoxicity of each crude extract of *A. pavonina* prepared by Soxhlet method is given in Table 6 as percentage mortality of brine shrimps.

Table 6. Cytotoxicity of *A. pavonina* soxhlet extracts given as percentage mortality against brine shrimp *Artemia salina*.

Extract	**Concentration /ppm**					
	1000	500	250	100	50	25
Root	100	100	87	57	0	0
Stem Bark	100	77	37	0	0	0
Leaves	0	0	0	0	0	0
Seeds	0	0	0	0	0	0

LC_{50} for each extract was determined using probit analysis (Using SPSS® Release 11.00). LC_{50} values of extracts of *A. pavonina* prepared by both Soxhlet and sonicator methods are given in Table 7.

Table 7. LC_{50} values of *A. pavonina* crude extracts against brine shrimp *Artemia salina*.

	Adenanthera pavonina			
	Root		**Stem Bark**	
	soni.	**sox.**	**soni.**	**sox.**
LC_{50}(ppm)	184	108	360	304

soni.=sonicator extract, sox. =soxhlet extract.

Leaves and seeds did not show any cytotoxicity in the brine shrimp assay. Soxhlet and sonicator extracts of root showed moderate cytotoxicity. Stem bark showed weak activity.

Conclusion

According to the findings of this work yields of extracts and the bioactivities varied with the plant part and the extraction method. Soxhlet method afforded higher yield of stem-bark, seeds and leaves than the sonicator method. The sonicator bark extract exhibited the highest antioxidant activity (89.5%), significantly outperforming the Soxhlet extract. Seeds and leaves showed weak antioxidant activity, while the root displayed moderate activity. Both bark extracts showed low cytotoxicity, and seeds and leaves had no cytotoxicity. These results highlight the pharmacological potential of *A. pavonina* extracts and emphasize the need for purifying its active compounds.

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