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# Effect of Soxhlet and Ultrasound-Assisted Solvent Extraction Methods on the Bioactivity of *Adenanthera pavonina*

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## 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±0.10%), significantly exceeding that of the Soxhlet extract (70.30±0.54) and that of the control; α-Tocopherol-55.4%). The seeds and leaves exhibited weak

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antioxidant activity, while the root showed moderate activity. Both sonicator and Soxhlet bark extracts displayed low cytotoxicity; LC 50(ppm); 360 SE, 304 USAE, 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.

*Keywords: Soxhlet extraction; sonication; Adenanthera pavonina; antioxidant activity; cytotoxic activity.*

## 1. INTRODUCTION

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].

*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 [9]. 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 [10]. 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 and bark has been found to contain stigmasterol glycosides [11]. Based on existing information about the medicinal properties of this plant, and our previous work [12] the present study aims to investigate the antioxidant and cytotoxic properties, of leaf, bark, seeds and root extracts of *A. pavonina.*

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 [13]. Plants are rich in radical-scavenging molecules such as flavonoids, phenolics, and other secondary metabolites with antioxidant properties [14]. In addition to their antioxidant effects, phenolic compounds offer a range of other functional benefits, including antimicrobial, anti-inflammatory, and antimutagenic properties [15].

For the cytotoxicity assay *Artemia salina* (genus Anostraca) used due to various reasons. While

many Anostraca species exist, *Artemia salina* is easy to cultivate due to its high hatching success rate. The cysts, produced when their habitat dries and salt levels rise, hatch in 24–48 hours when exposed to water. Newly hatched *Artemia* (nauplii) are about 0.25 mm in size and molt around 17 times before reaching adulthood. The cysts are resilient, surviving extreme conditions up to 80°C, with hatching efficiency only decreasing at temperatures above 90–100°C. Nauplii can also tolerate abrupt changes in salinity. Despite their ability to withstand high salt levels, brine shrimp are sensitive to environmental changes, making them useful for toxicity screening. There is a positive correlation between brine shrimp lethality and human cell culture cytotoxicity, allowing the brine shrimp lethality test to identify cytotoxic compounds [7].

## 2. MATERIALS AND METHODS

The prepared plant materials were extracted with distilled methanol.

### 2.1 Collection of Plant Material

All four parts of *A. 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 (SE)

The dried and ground plant material (50 g) was extracted using Soxhlet apparatus 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].

The crude extracts were sterilized by autoclaving at 121 °C for 15 min before storing 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].

**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 |

*2.4.1.1 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.

*2.4.1.2 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.

*2.4.1.3 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 [16]:

$$\% \text{ Inhibition} = [(A_0 - A_t)/A_0] \times 100 \quad (1)$$

**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].

*2.4.2.1 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.

*2.4.2.2 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.

*2.4.2.3 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.

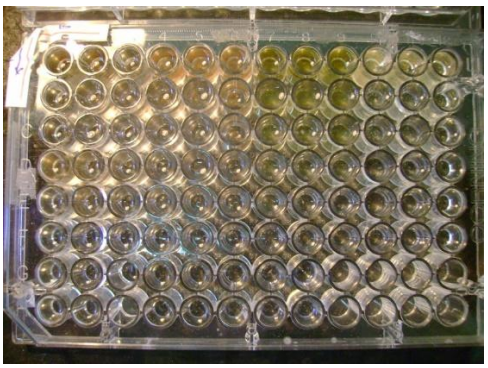

**Fig. 1. 96 micro well plate, cytotoxicity assay of *A. pavonina* extracts**

***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.

## 3. 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]. Pharmacological evaluation of medicinal plants should involve chemical analysis, as the presence of secondary metabolites suggests the plant's potential for pharmaceutical use. Various solvent extracts from different plant parts exhibit notable pharmacological effects, including antioxidant activity. Phenolic compounds, such as flavonoids and tannins, are closely linked to antioxidant activity in biological systems. Their redox properties enable phenolic compounds to act as reducing agents, hydrogen donors, and quenchers of singlet oxygen, thus contributing to the antioxidant effects of plant materials [21]. Previous studies have reported various bioactivities of various parts of *A. pavonia.* One recent study reported moderate antioxidant activity of ethyl acetate and ethanolic extracts of *A. pavonina* leaves and bark [21]. The plant materials have been extracted using the rotary evaporator. DPPH assay conducted on a lyophilized sample of the decoction prepared from the bark of *A. pavonina* showed IC 50 of 15.01 ± 0.57 μg/mL [22]. A recent study has shown comparable antioxidant activity of water extract of *A. pavonina* bark; IC50 15.8± 0.5 (Gallic acid, 4.5±0.3 μg/mL) [23]. Krishnaveni *et al* have reported the moderate antioxidant activity of *A. pavonina* leaves extracted with 70% ethanol-water mixture using maceration technique; IC 50 0.81 16.32 μg/mL (Ascorbic acid, 0.81 μg/mL) [23]. To the best of our knowledge, no studies have reported on the antioxidant activity of various plant parts of A. pavonina extracted using different methods [24].

### 3.1 Comparison of the Two Extraction Methods: SE 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 % | |
|---|---|---|
| | SE | USAE |
| Root | 14.1 | 19.0 |
| Leaves | 22.3 | 11.0 |
| Stem-bark | 26.3 | 12.2 |
| Seeds | 36.4 | 7.0 |

The extraction yield reflects the efficiency of the solvent and the extraction method in extracting specific components from the original material. It provides insight into the extractability of the plant under various conditions. 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 SE, facilitating the diffusion of solvents into materials with comparable polarities.

### 3.2 Antioxidant Activity

#### 3.2.1 Antioxidant activity of crude extracts of *A. 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 and Figs. 3 and 4.

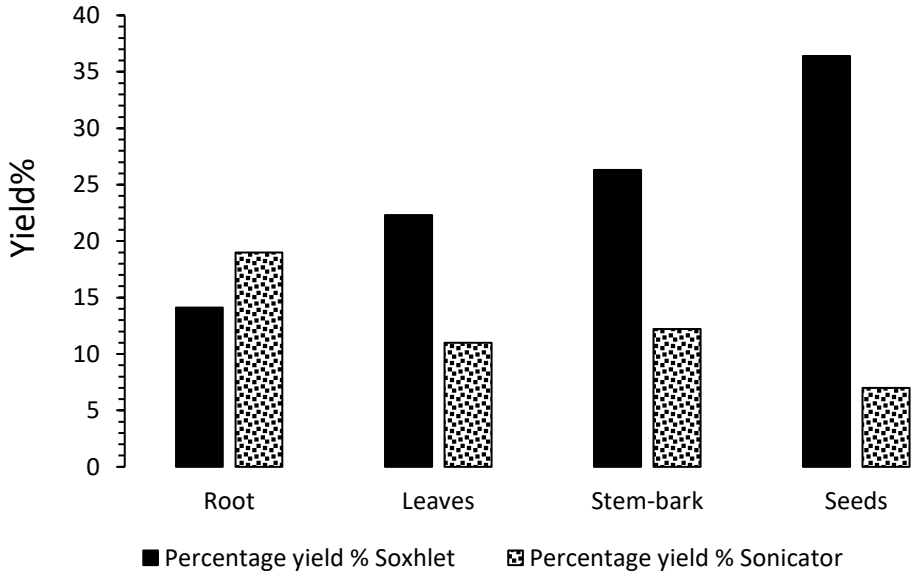


**Fig. 2. The yield of *A. pavonina* extracts from the two extraction methods**

**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$^{a}$ | 2.69±0.15$^{a}$ | 89.50±0.10$^{a}$ | 26.51±0.62$^{a}$ |
| Soxhlet extracts | 2.11±0.138$^{b}$ | 5.88±2.83$^{b}$ | 70.30±0.541$^{b}$ | 31.19±0.402$^{b}$ |
| α-Tocopherol | 55.4 | | | |

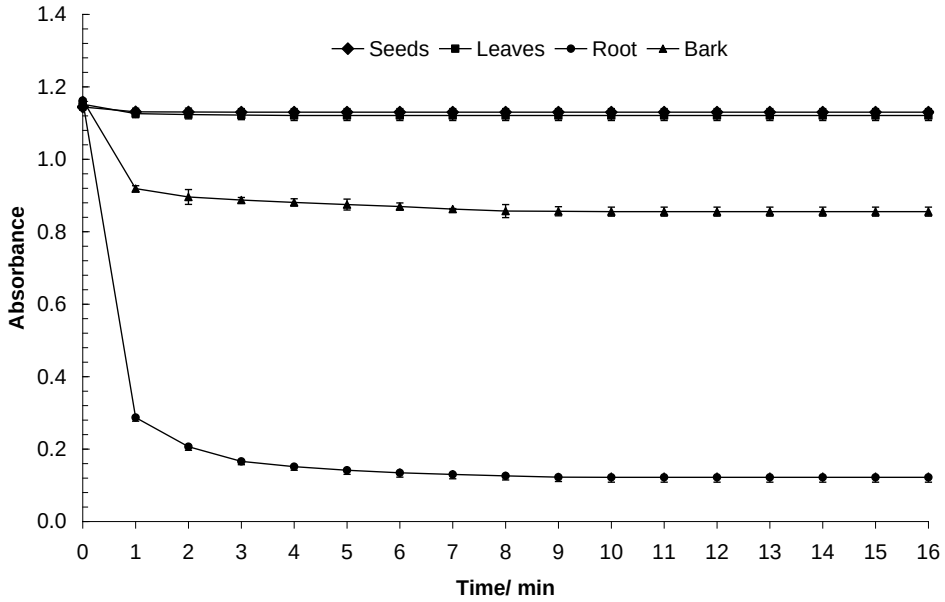


**Fig. 3. Decrease in absorbance of DPPH with *A. pavonina* sonicator extracts, over 16 minutes**

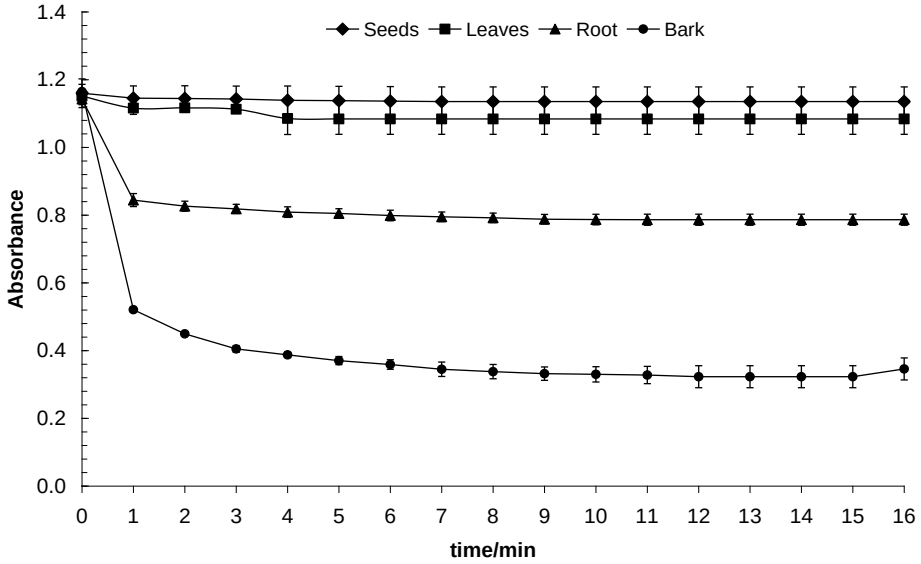


**Fig. 4. Decrease in absorbance of DPPH with *A. pavonina* Soxhlet extracts, over 16 minutes**

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

**Table 6. Cytotoxicity of *A. pavonina* SE given as percentage mortality against brine shrimp *A. 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 |

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

| | ***A. pavonina*** | | | |
|---|---|---|---|---|
| | **Root** | | **Stem-Bark** | |
| | **USAE** | **SE** | **USAE** | **SE.** |
| $LC_{50}$(ppm) | 184 | 108 | 360 | 304 |

**Statistical analysis:** Antioxidant activity of extracts *A. pavonina,* prepared using both USAE and SE methods, were compared using "2-sample *t*-test" (Using Minitab 14.0). The SE of *A. pavonina* bark showed the highest activity, 89.5% and second highest activity by USAE of the bark, 70.30%. The root extract showed moderate activity while seeds and leaves exhibited low activity. Antioxidant activities corresponding to the SE extracts of *A. pavonina*

seeds and root are significantly ($p<0.05$) greater than those corresponding to USAE extracts. Antioxidant activity corresponding to the sonicator extract of *A. pavonina* bark is significantly greater than that of SE. Both Soxhlet and sonicator extracts of *A. pavonina* bark showed significant potential with radical scavenging ability, which is far better than previous findings [25]. The significant differences in antioxidant activity can be attributed to the extraction methods, different techniques can selectively extract different compounds from the plant material. These compounds, such as phenols, flavonoids, and other bioactive molecules, are responsible for the antioxidant properties [26]. Antioxidant compounds vary in their solubility depending on the solvent used. For example, non-polar solvents might extract lipophilic compounds, while polar solvents are better at extracting hydrophilic antioxidants. The choice of solvent influences which antioxidants are extracted and their concentration, however, in this work as only methanol has been used no variation due to the solvent. Different methods impact the efficiency of solvent penetration into the plant material and the release of bioactive compounds.

#### 3.2.2 Cytotoxicity of crude extracts of *A. 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.

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

$LC_{50}$ 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.

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. A significant difference in cytotoxicity was observed as different techniques can selectively extract different compounds from the plant material.

## 4. CONCLUSION

The choice of extraction method and solvent plays a crucial role in maximizing both extract yield and bioactivity. A comparative study has been carried out to evaluate the yield, antioxidant and cytotoxic activities of the extracts. This is the first study to examine the impact of extraction methods on the bioactivity of *A. pavonina*. 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.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Sandhu DS, Heinrich M. The use of health foods, spices and other botanicals in the Sikh community in London. Phytotherapy Research. 2005;19(7):633-642.
2. (a) Pathmasiri W. COX-2 inhibitors of natural origin: dereplication, isolation, and structure elucidation, Licentiate thesis; 2003. (b) Dasanayake MD, Fosberg FR, Revised handbook to the Flora of Ceylon. 2003;1:469-471. (c) Rodrigo SK, Guan H. Mechanistic study of nickel-catalyzed reductive coupling of ynoates and aldehydes. The Journal of Organic Chemistry. 2017;82(10):5230-5235. (d) Wu TS, Wang ML, Jong TT, McPhail AT,

McPhail DR, Lee KH. X-ray crystal structure of Acrovestone, A cytotoxic principle from Acronychia pedunculata, J. Nat. Prod. 1989;52:1284-1289.

3. Hassan RBA. Medicinal plants (importance and uses). Pharmaceutical Acta Sciences. 2012;3(10):139.
4. Ferreira-Machado SC, Rodrigues M P, Nunes APM, Dantas FJS, De Mattos JCP, Silva CR, Caldeira-de-Araujo A. Genotoxic potentiality of aqueous extract prepared from Chrysobalanus icaco L. leaves. Toxicology Letters. 2004;151(3):481-487.
5. Rodrigo SK. The effect of extraction method on yield and antimicrobial activity of Acronychia pedunculata, Journal of Science-FAS-SEUL. 2023;04(10):18-30.
6. Sia Y, Chern Z, Hii S, Tiu Z, Arifin MA. Antimicrobial, Antioxidant and Cytotoxic Activities of Cosmos Caudatus extracts; 2012. Available:https://scite.ai/reports/10.15282/http://dx.doi.org/10.15282/ijets.7.1.2020.1004
7. Aziz RA, Sarmidi MR, Kumaresan S. Phytochemical processing: the next emerging field in chemical engineering aspects and opportunities. J. Kejurut. Kim. Malay. 2003;3:45–60.
8. Hayouni EA, Abedrabba M, Bouix M, Hamdi M. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and Juniperus phoenica L. fruit extracts. Food Chem. 2007;105:1126– 1134.
9. Liyanage CS. Studies on some medicinal plants and related plants of Sri Lanka, M.Phil. Thesis (PGIS); 2002.
10. Olumayokun AO, Echianu CA, Aduragbemi AE, Makinde AAJM. Studies on *Adenanthera pavonina* seed extract. Inflammopharmacology. 2004;12(2):197-202.
11. The Wealth of India. A dictionary of raw materials and industrial products, Raw materials, Revised Edition. 1949;1: A,75-78.
12. (a) Rodrigo SK, Jayasingha ULB, Bandara BMR. Antifungal, antioxidant and cytotoxic activity of *Acronychia pedunculata* and *Adenanthera pavonina*. Proc. Perad. Univ. Res. Sess.-Sri Lanka. 2007;12:94–95. (b) Jayasinghe PKIDE, Bandara BMR, Ekanayaka EWMA, Thevanesam V. (2002). Screening of Antimicrobial activity of Acronychia pedunculata (Ankenda) and *Adenanthera pavonina* (Madatiya) against bacteria causing skin wound diseases in humans, Proceedings of the Peradeniya University Research Sessions, Sri Lanka. 2007;11:105. (c) Ekanayake UGM, Dissanayake DMSN, Rathuwadu N, Kumarasinghe RKKGR, Rodrigo SK, Mantilaka MMMGPG. J Fluor Chem. 2020;235:109565. (d) Ali MS, Ahmed F, Azhar I, Pervez MK. A new five memberd lactone from Adenanthera pavonina, J. Nat.Prod. 2005;19 (1):37-40.
13. Yam MF, Basir R, Asmawi MZ, Rosidah Ahmad M, Akowuah GA. Antioxidant and hepatoprotective activities of Elephantopus tomentosus ethanol extract. Pharmaceutical Biology. 2008;46(3):199 206.
14. Cai Y, Sun M, Corke H. Antioxidant activity of betalains from plants of the Amaranthaceae. Journal of Agricultural and Food Chemistry. 2003;51(8):2288-2294.
15. Hakkim FL, Arivazhagan G, Boopathy R. Antioxidant property of selected Ocimum species and their secondary metabolite content. Journal of Medicinal Plants Research. 2013;2(9):250-257.
16. Roberta R, Luciana GM, Luciana CC, Glaucia P. Evaluation of the antioxidant properties of the Brazilizn Cerrado Fruit *Annona crassiflora* (Araticum), Journal of Food Science. 2006;71(2):102-107
17. Andrews JW. Determination of minimum inhibitory concentration, Journal of Antimicrobial chemotherapy. 2001; 48:5-16.
18. Quispe Candori S, Foglio MA, Rosa PTV. Meireles, M.A.A. Obtaining b-caryophyllene from Cordia verbenacea de Candolle by super crtitical fluid extraction. J. Supercrit. Fluids. 2008;46:27–32.
19. Ishida BK, Ma J, Bock C. A simple rapid method for HPLC analysis of lycopene isomers. Phytochem. Anal. 2001;12:194–198.
20. Dhanani T, Shah S, Gajbhiye NA, Kumar S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*, Arabian Journal of Chemistry. 2017;10:S1193-S1199.

21. Yumita A, Hanani E, Agustina A, Damayanti F, Priani KN, Fadila SN. Total phenolic content and antioxidant activities of leaves and bark extract of *Adenanthera pavonina* L. Natural product sciences. 2023;29(1):24-30.
22. Upamali1 BDN, Fernando1 MDM, de silva GDNK, Soysa SSBDP, Antioxidant potential of Sri Lankan native plant *Adenanthera pavonina*, 27th faobmb & 44th annual msbmb conference. 2019;237.
23. Wijesekara MA, Goonerathne LV, Soysa P, Perera DB, Jayasena S, Jayasiri A, Kottahachchi DU. *In vitro* screening of antioxidant and anti-inflamatory activities of plant extract *Adenanthera pavonina*. Proceedings of the 27th international forestry and environemental symposium of the department of forestry and environmental science, University of Sri Jayewardenepura, Sri Lanka. 2023;102.
24. Krishnaveni A, Danyalakshmi PGS, Kothai Andal D, Venkata Rathina Kumar T, Abdul Hasan Sathali A. Pharmacognostical, preliminary phytochemical screening, estimation of phyto constituents and its in-vitro antioxidant activity of *Adenanthera pavonina* linn (leaves). International Journal of Research in Pharmacology & Pharmacotherapeutics. 2022;11(2): 62-75.
Acailable:https://doi.org/10.61096/ijrpp.v11.iss2.2022.62-75
25. Ghosh P, Chowdhury HR. Pharmacognostic, phytochemical and antioxidant studies of *Adenanthera pavonina* L. International Journal of Pharmacognosy and Phytochemical Research. 2015;7(1): 30-37.
26. Shanthirasekaram K, Bulugahapitiya VP, Manawadu H, Gangabadage CS. Effect of extraction techniques on phytochemicals and antioxidants activity of garcinia quaesita leaves, Adv. Technol. 2022;(1):18-30.



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