

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

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,

Commented [WK1]: to reveal bioactive compounds

Commented [WK2]: State the purpose of the study in the final paragraph.

steroidal-glucosides, cysteine proteinase and fatty acids. Seeds contain non-protein amino acids γ -methylene glutamic acid, γ -methylene glutamine, γ -ethylidene 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*.

Commented [WK3]: support it with references

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.

Commented [WK4]: State why it is used and how it performs the evaluation.

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

Commented [WK5]: The calculation of SE and USAE's Percentage yield% and the formula method used should also be explained.

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

Commented [WK6]: Identify plant material as in SE

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. 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].

Commented [WK7]: Use abbreviation (SE)

Commented [WK8]: Specify the severity of soxhlet apparatus (xx kHz, xx% amplitude).

Commented [WK9]: can be deleted

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.

Commented [WK10]: Please specify how the sterilization of the obtained extracts is carried out.

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 μ L 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), CaCl₂ (4.7 g), MgCl₂ (1.9 g), MgSO₄ (6.3 g), and NaHCO₃ (0.18 g) [17].

Hatching brine shrimps

Commented [WK11]: All subheadings should be numbered.

Commented [WK12]: Method steps and formulas should be supported by references.

Commented [WK13]: The formula must be numbered.

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/H₂O. 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 μ L 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 / μ l	Final concentration* / ppm
150	500
75	250
30	100
15	50
7.5	25

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

150 μ L 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 μ L with artificial sea water using a dropper. Following volumes (300 μ L, 150 μ L, 75 μ L, 30 μ L, 15 μ L, and 7.5 μ L) from the 4%

Commented [WK14]: μ L

Commented [WK15]: μ L

DMSO/ artificial sea H₂O system were added as controls for the 1000 ppm to 25 ppm test solution concentrations respectively. Test was done in triplicate.

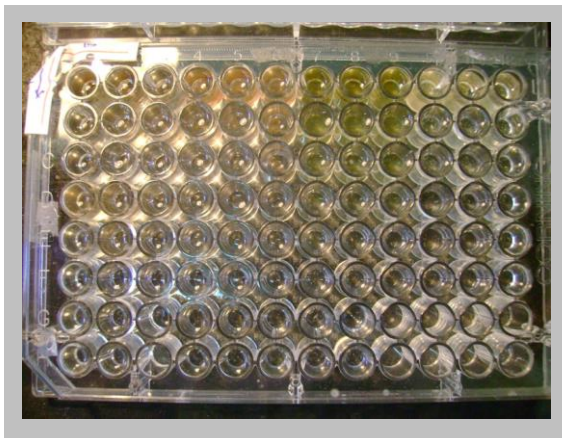


Figure 1.0 96 micro well plate, cytotoxicity assay of *A. pavonina* extracts
Key: From A-F concentration series (1000-25 ppm), G, H controls, 1-3 root, 4-6 Stem-bark, 7-9 leaves, 10-12 seeds.

Statistical analysis

Data are expressed as mean \pm 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

Commented [WK16]: Compare the results obtained with the studies in the literature. State the importance of the results and the reasons for the differences

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.

Commented [WK17]: calculation and formula should be explained in the method section

Commented [WK18]: Use the abbreviation SE

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.

Commented [WK19]: Use the abbreviation SE

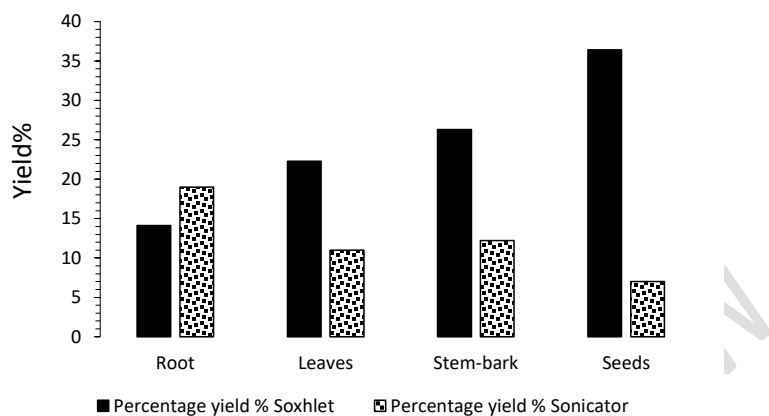


Figure 2.0. The yield of *A. pavonina* extracts from the three extraction methods

Commented [WK20]: two or three extraction ?

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.

Commented [WK21]: use abbreviation because it has been mentioned before

Commented [WK22]: Explain the reason for the differences arising from the extraction methods

Commented [WK23]: Edit as Table 4, no Table 4.0

Antioxidant activity should be given as %, please update the table

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%

Commented [WK24]: Was α -Tocopherol used as a control? Values should be given in the table.

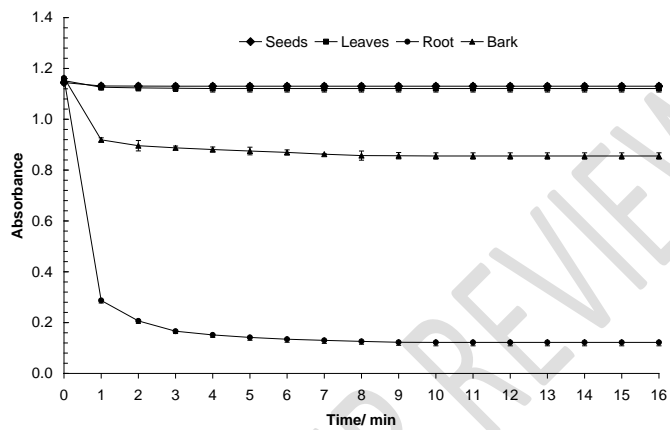


Figure 3. Decrease in absorbance of DPPH with *A. pavonina* sonicator extracts, over 16 minutes.

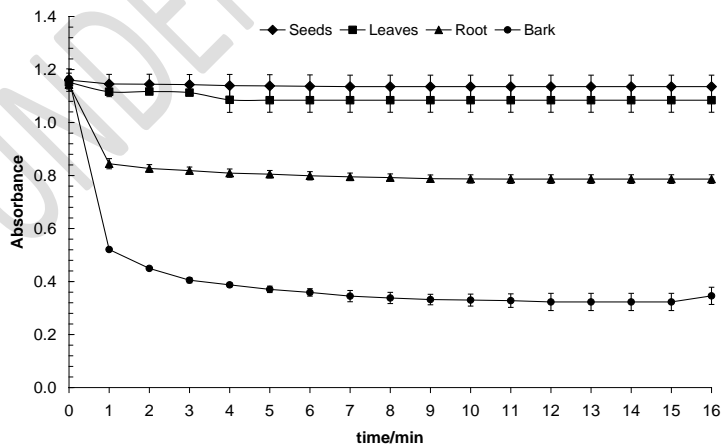


Figure 4. Decrease in absorbance of DPPH with *A. pavonina* Soxhlet extracts, over 16 minutes.

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].

Commented [WK25]: Letter the statistical analysis results on the table and graph. Also compare the values statistically. Review papers in South Asian Research Journal of Natural Products and organize statistical analysis show

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.

Commented [WK26]: use abbreviation because it has been mentioned before

Commented [WK27]: Explain the reason for the differences arising from the extraction methods

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

Extract	Concentration /ppm
---------	--------------------

Commented [WK28]: use abbreviation because it has been mentioned before (*A. salina*)

	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

Commented [WK29]: use abbreviation because it has been mentioned before (*A. salina*)

LC₅₀ for each extract was determined using probit analysis (Using SPSS® Release 11.00). LC₅₀ values of extracts of *A. pavonina* prepared by both Soxhlet and sonicator methods are given in Table 7.

Table 7. LC₅₀ values of *A. pavonina* crude extracts against brine shrimp *Artemia salina*.

	<i>Adenantha pavonina</i>			
	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.

References

1. Sandhu, D. S. and 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, M. D., Fosberg, F. R., Revised

Commented [WK30]: use abbreviation because it has been mentioned before

Commented [WK31]: Use abbreviations SE and USAE instead

Commented [WK32]: Explain the reason for the differences arising from the extraction methods

Commented [WK33]: The original value and novelty of the results should be explained in more detail

Commented [WK34]: References must be prepared in accordance with the rules.

- handbook to the Flora of Ceylon. Volume I, 469-471. (c) Rodrigo, S. K., & 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, T.S., Wang, M.L., Jong, T.T., McPhail, A.T., McPhail, D.R., Lee, K.H. X-ray crystal structure of Acrovestone, A cytotoxic principle from *Acronychia pedunculata*, *J. Nat. Prod.*, (1989); **52**, 1284-1289.
- Hassan, R.B. A. Medicinal plants (importance and uses). *Pharmaceutical Acta Sciences*, (2012); 3(10):139
 - Ferreira-Machado, S. C.; Rodrigues, M. P.; Nunes, A. P. M.; Dantas, F. J. S.; De Mattos, J. C. P.; Silva, C. R. and Caldeira-de-Araujo, A. Genotoxic potentiality of aqueous extract prepared from *Chrysobalanus icaco* L. leaves. *Toxicology Letters*, (2004);151(3):481-487.
 - Rodrigo, S. K. The effect of extraction method on yield and antimicrobial activity of *Acronychia pedunculata*, *Journal of Science-FAS-SEUL*, (2023); 04(10), pp. 18-30
 - Sia, Y., Chern, Z., Hii, S., Tiu, Z., & Arifin, M A. Antimicrobial, Antioxidant and Cytotoxic Activities of *Cosmos Caudatus* extracts. (2021);<https://scite.ai/reports/10.15282/http://dx.doi.org/10.15282/ijets.7.1.2020.1004>
 - Aziz, R.A., Sarmidi, M.R., Kumaresan, S. Phytochemical processing: the next emerging field in chemical engineering aspects and opportunities. *J. Kejurut. Kim. Malay*, (2003); **3**, 45–60.
 - Hayouni, E.A., 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 phoenicea* L. fruit extracts. *Food Chem.*, (2007); **105**, 1126– 1134.
 - Yam, M.F.; Basir, R.; Asmawi, M.Z.; Rosidah Ahmad, M. and Akowuah, G. A. Antioxidant and hepatoprotective activities of *Elephantopus tomentosus* ethanol extract. *Pharmaceutical Biology*, (2008); 46(3):199 206.
 - Cai, Y.; Sun, M. and Corke, H. Antioxidant activity of betalains from plants of the *Amaranthaceae*. *Journal of Agricultural and Food Chemistry*, (2003); 51(8):2288-2294.
 - Hakkim, F. L.; Arivazhagan, G. and Boopathy, R. Antioxidant property of selected *Ocimum* species and their secondary metabolite content. *Journal of Medicinal Plants Research*, (2013);2(9):250-257.
 - Liyanage, C.S. Studies on some medicinal plants and related plants of Sri Lanka, M.Phil. Thesis (PGIS), (2002).

13. Olumayokun, A. O.Echianu, C.A.. A.E. Aduragbemi, A. A. Makinde, J. M., Studies on *Adenanthera pavonina* seed extract. *Inflammopharmacology*. (2004), 12, 2,197-202.
14. The Wealth of India, A dictionary of raw materials and industrial products, Raw materials, Revised Edition. Vol I: A, 75-78.
15. (a) Rodrigo, S.K.; Jayasingha, U.L.B.; Bandara, B.M.R. Antifungal, antioxidant and cytotoxic activity of *Acronychia pedunculata* and *Adenanthera pavonina*. *Proc. Perad. Univ. Res. Sess.-Sri Lanka*, (2007); 12, 94–95. (b) Jayasinghe, P. K. I. D. E., Bandara, B.M.R., Ekanayaka, E. W. M. A., Thevanesam, V. 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*, (2002); 11, 105., (c) Ekanayake, U. G. M.; Dissanayake, D. M. S. N.; Rathuwadu, N.; Kumarasinghe, R. K. K. G. R.; Rodrigo, S. K.; Mantilaka, M. M. M. G. P. G. *J Fluor Chem*, (2020); 235, 109565. (d) Ali, M.S., Ahmed, F., Azhar, I., Pervez, M.K. A new five memberd lactone from *Adenanthera pavonina*, *J. Nat.Prod*, (2005); 19 (1), 37-40.
16. Roberta, R., Luciana, G.M., Luciana, C.C., Glaucia, P., Evaluation of the antioxidant properties of the Brazilizn Cerrado Fruit *Annona crassiflora* (Araticum), *Journal of Food Science*, **71**, 2, 102-107
17. Andrews, J.W., Determiration of minimum inhibitory concentration, *Journal of Antimicrobial chemotherapy*, (2001); 48, 5-16.
18. Quispe Candori, S., Foglio, M.A., Rosa, P.T.V., Meireles, M.A.A. Obtaining b-caryophyllene from *Cordia verbenacea* de Candolle by super critical fluid extraction. *J. Supercrit. Fluids*, (2008); **46**, 27–32.
19. Ishida, B.K., Ma, J., Bock, C. A simple rapid method for HPLC analysis of lycopene isomers. *Phytochem. Anal*, (2001); **12**, 194–198.
20. T. Dhanani, S. Shah, N.A. Gajbhiye, S. Kumar. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*, *Arabian Journal of Chemistry*, (2017);10, pp. S1193-S1199.
21. Ghosh, P.; Chowdhury H. R. Pharmacognostic, Phytochemical and Antioxidant Studies of *Adenanthera pavonina* L. *International Journal of Pharmacognosy and Phytochemical Research*, (2015); 7(1); 30-37

UNDER PEER REVIEW

