THE APPRAISAL OF ANTIOXIDANT PROPERTIES OF TWO MALAYSIAN HERBS *Cosmos caudatus* AND *Piper sarmentosa*

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Abstract

Nowadays the impacts on the use of natural ingredients in modern medicine cannot be denied as they exhibit amazing antioxidant properties. Thus, the study aims to investigate the antioxidant properties of *Cosmos caudatus* and *Piper sarmentosum* leave extract using thin layer chromatography (TLC) dot blot assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The dot blot assay method was reported for the first time for both species. The crude extracts of *C. caudatus* and *P. sarmentosum* leaves extract were prepared through consecutive maceration using hexane, ethyl acetate, acetone and methanol. According to TLC dot blot assay, the methanol extract of *P. sarmentosum* inhibit the DPPH radical at a concentration of 0.30 mg/ml while acetone extract of *C. caudatus* inhibit the DPPH radical at a concentration of 0.012 mg/ml. The radical scavenging activities for both species show concentration dependent manner. Methanol extract of *P. sarmentosum* and acetone extract of *C. caudatus* show the lowest IC_{50} with 32.009 µg/ml and 14.12 µg/ml, respectively for DPPH assay. Therefore, the polar extracts of both species are known to be the most promising source of antioxidant agent compared to less polar extract. The results may be applicable to medical and pharmaceutical industries as they provide basic scientific data for the development of new natural medicines.

Keywords: antioxidant activity, *Cosmos caudatus*, herbs, *Piper sarmentosum*

Introduction

Most of natural antioxidants are derived from plant materials such as fruits, vegetables, herbs and spices. It is well known that *P. sarmentosum* (Kaduk) and *C. caudatus* (Ulam raja) leaves are the herbs from Malaysia that are high in antioxidants.

Generally, antioxidants are chemicals that neutralise free radicals by donating an electron to them, rendering them harmless thus minimising the oxidative damage they cause to biological processes. Typically, oxidative stress is associated with the presence of free radicals. When oxygen combines with specific chemicals, free radicals are formed, and they constitute a threat due to the potential harm they might cause when they connect to DNA, proteins, and the cell membrane. Antioxidants are crucial in preventing cell damage by neutralising free radicals, helping to protect the body cells from harmful disease like cancer (Baliyan et al., 2022).

The leaves of *P. sarmentosum* and *C. caudatus* are edible and widely used in pharmacological activities due to the presence of bioactive chemicals. Anticancer, antioxidant, antibacterial, and anti-inflammatory are only some of the bioactivities observed in these substances (Rahman et al., 2021). Regular consumption of these plants can reduce free radical exposure in the human body, lowering the risk of developing oxidative-related disorders.

Nguyen et al. (2021), reported that ethanol extracts of *P. sarmentosum* demonstrated a radical scavenging activity when tested against DPPH and ABTS radicals. However, none of them reported the scavenging activity from dot blot assay technique. Phenolic components in *C. caudatus* is reported to reduce oxidative stress since this chemical compound may play a direct part in oxidative stress defence and may be regarded as active metabolites involved in antioxidant activity (Cheng et al., 2015).

This study is essential to serve as a reference for future investigations into the phytochemical components, and antioxidant capabilities of *P. sarmentosum* and *C. caudatus.* A better understanding of the pharmacological capabilities of both species particularly the phytochemical ingredients in relation to its antioxidant properties could provide information on possible herbal medicines.

Materials and Methods

Plant extraction

The leaves of *P. sarmentosa* and *C. caudatus* were collected from Taman Herba UiTM Jengka Pahang, Malaysia. The samples were air-dried at room temperature for five days and then finely powdered using a grinder. The grinded leaves were macerated consecutively using organic solvents such as hexane, ethyl acetate (EA), acetone and methanol before followed by filtration. All the filtrates were subjected to evaporation to yield crude extracts.

Phytochemical Screening

The method for phytochemical screening was modified from previous work with a slight modification (Ilias Fazna et al., 2023). Three main phytochemicals such as alkaloid, terpenoid and phenolic were screened using developed TLC. The developed TLC was sprayed with certain detection reagents such as Dragendorff's, vanillin/H_2SO_4 and ferric chloride solutions. The developed TLC of extracts was also sprayed with 0.6% DPPH solution for screening the antioxidant activities qualitatively.

Antioxidant activity

a. TLC Dot Blot Assay

This method was conducted to determine the lowest concentration of each extract of *P. sarmentosum* and *C. caudatus* by observing the first yellow colour against purple background appeared on the TLC. All extracts were twofold serially diluted ranging from 100 mg/ml to 0.024 mg/ml and were dropped onto TLC that contained a series of square blocks with a dimension of 1.5 x 1.5 cm.

The TLC surface was sprayed with 0.05% DPPH solution until it was fully covered. The appearance of yellow colour against purple background was observed for each single square block. The first square block with yellow colour indicates the lowest concentration of extract that was scavenged the DPPH radical.

b. DPPH Radical Scavenging activity

The method conducted was modified from previous work with a slight modification (Ilias Fazna et al., 2023). This method was utilised to evaluate the antioxidant activity of all extracts quantitatively. A series of concentrations of extracts ranging from 400 µg/mL to 12.5 µg/mL were prepared. About 3 mL of 0.004% DPPH solution was added to 1 ml of each extract concentration. Each mixture was left in the dark place for 30 minutes to initiate the scavenging reaction. After 30 minutes of incubation, the absorbance of all mixtures was recorded at 517 nm using a spectrophotometer. The experiment was repeated with the ascorbic acid as positive control. Generally, the reduction of purple colour of the mixture to yellow colour indicates the reaction between DPPH radical with antioxidant compounds in the extract samples.

Results and Discussion

Phytochemical Screening

Generally, phytochemicals refer to any chemical compounds naturally produced by plant sour ces as a result of a plant's immune system to protect them from fungi, bacteria and viruses as well as can be consumed by insects and other animals. The most common phytochemicals found in plants are alkaloid, terpenoid and phenolic compounds and some of them are believed to act as antioxidant. **Table 1** indicates the phytochemicals found in *P. sarmentosum* and *C. caudatus* leave. The leaves of *P. sarmentosum* contain all three phytochemicals mean while *C. caudatus* contain terpenoid and phenolic compounds. The result also indicates the leave from *P. sarmentosum* and *C. caudatus* are antioxidative. According to Ahda et al. (2023), the leaves of *C. caudatus* contain several of flavonoids compounds and they are antioxidative as well as *P. sarmentosum*.

Table 1 Phytochemicals from the extracts of leaves part of *P. sarmentosum* and *C. caudatus*

Phytochemicals	***P. sarmentosum***			***C. caudatus***		
	HE	EA	ME	HE	EA	AcE
Alkaloid	√	x	√	x	x	x
Terpenoid	√	√	x	√*	√	√*
Phenolic	√*	√*	√*	√	√*	√*

√ = present; x = not present; * = antioxidative (yellow color) when sprayed with 0.6% DPPH
HE:Hexane extract; EA:ethyl acetate extract; ME:methanol extract; AcE:acetone extract

Antioxidant activity

a. Dot blot assay

Antioxidant is known as a chemical substance that can retard oxidation reaction of body cells by removing free radicals and decrease oxidation stress. Free radicals attack important macro molecules leading to cell damage and homeostatic disruption.

High amounts of free radicals may lead to high risk of body exposure to harmful diseases such as cancer. Plant source is known to exhibit high antioxidant properties. Herbs such *P. sarmentosum* and *C. caudatus* possess unique antioxidant properties. **Figure 1** and **Table 2** illustrate the antioxidant properties of *P. sarmentosum* and *C. caudatus* leaves extract using TLC dot blot assay. In this method, the antioxidant activity of extracts for both species can be detected by observing the formation of yellow spot against purple background

appeared on TLC at different extract concentration after spraying with DDPH solution. The yellow colour indicates the reduction of DPPH radical by antioxidant from the extracts. Regarding *C. caudatus*, the most active extract for scavenging DPPH radical is acetone extract since the lowest concentration of extract which enable to scavenge free radical is at 0.024 mg/ml. As for *P. sarmentosum*, the value of 0.39 mg/ml is the lowest concentration needed for scavenging action towards DPPH free radical. Therefore, to effectively scavenge the free radicals, the use of more polar extracts as free radical scavengers are better compared to less polar extracts from both species. According to Khairunnisa et al. (2019), the synergistic action of phytochemicals existing in more polar extract to scavenge free radicals might be the reason for antioxidant action.

Table 2 Antioxidant activity of *P. sarmentosum* and *C. caudatus* leaves extract from TLC dot blot assay

Extract/standard	**Lowest concentration(mg/ml)** ***P. sarmentosum***	**Lowest concentration(mg/ml)** ***C. caudatus***
Hexane	0.78	0.39
Ethyl acetate	0.39	0.19
Acetone	-	0.024
Methanol	3.125	-
Standard ascorbic a cid	0.024	0.024

(-) not applicable

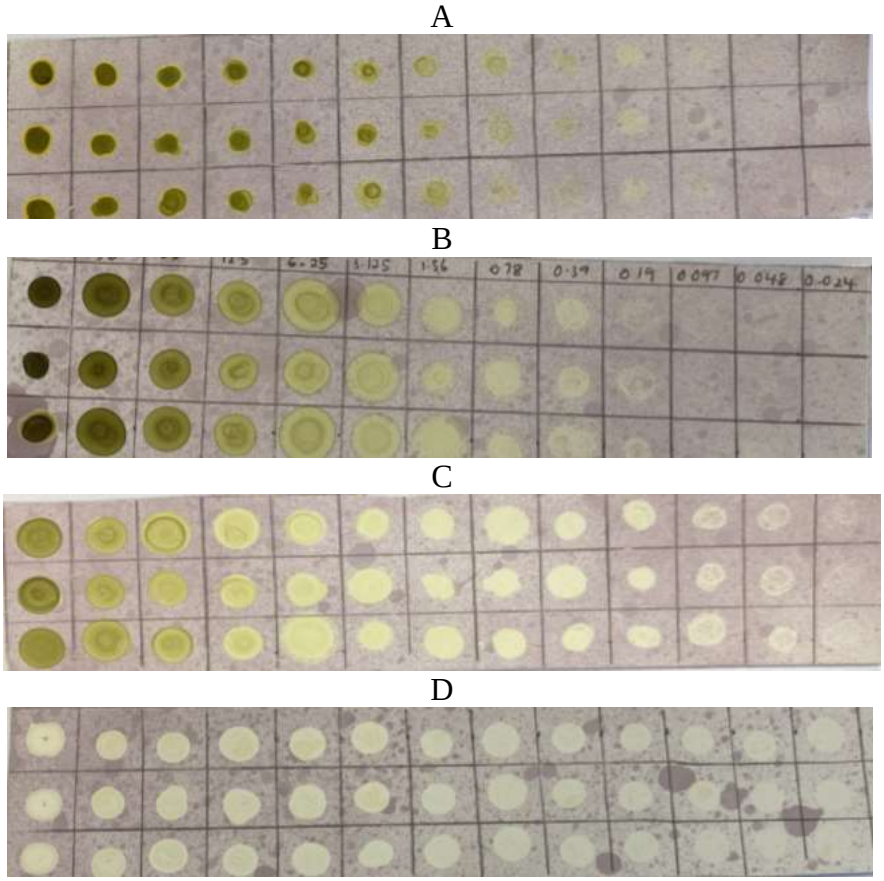

Figure 1 Examples of thin layer chromatograms of dot blot assay after sprayed with DPPH so lution. Concentration from left to right square blocks of TLC ranging from 100 mg/ml to 0.02 4 mg/ml

A: Thin layer chromatograms of hexane extract
B: Thin layer chromatograms of ethyl acetate extract
C: Thin layer chromatograms of acetone extract
D: Thin layer chromatograms of standard ascorbic acid

b. Quantitative DPPH Radical Scavenging activity

The DPPH radical scavenging assay is one of the quantitative approaches for evaluation of antioxidant properties to assess the potential of substances such as plant extract to serve as free radical scavengers. **Figures 2 and 3** depict the results of DPPH radical scavenging acti vity for both *P. sarmentosum* and *C. caudatus* leave extracts.

According to both figures, the trend in the percentage of radical scavenging activity shows concentration dependent manner for all types of extracts. For more polar extracts such as acetone extract of *C. caudatus* leave, the trend is quite obvious compared to less polar extract hexane. However, the result of radical scavenging activity *P. sarmentosum* illustrated in **Figure 2** is quite unique. Among three extracts, hexane extract reveals the highest percentage of scavenging (65%) at 400 mg/ml compared to methanol extract. This uniqueness might be due to the existence of phytochemicals in hexane extract of *P. sarment osum*.

According to the result in **Table 1**, all three phytochemicals found in hexane extract and phenolic are antioxidative. The scavenging effect from the combination of alkaloid, terpenoid and phenolic in hexane extract might contribute to the antioxidant activity. The scavenging action of free radicals by antioxidant can occur as hydrogen atom transfer (HAT), single electron transfer (SET) or sequential proton loss transfer (SPLET) mechanisms.

Table 3 presents the quantitative antioxidant activity of *P. sarmentosum* and *C. caudatus* leaves extract from DPPH radical scavenging assay. The IC_{50} value is the concentration of extracts that are required to scavenge 50% of the-DPPH radicals. The lower the IC_{50} values, the stronger antioxidant activity of the extracts. Acetone extract of *C. caudatus* has the lowest IC_{50} value at 14.12 μg/ml compared to other extracts, indicating the strongest antioxidant activity.

The phenolic content in the acetone extract might contribute to its potent antioxidant properties. On the other hand, the IC_{50} value of *P. sarmentosum* extracts are not significantly different between polar and non-polar extracts. However, both species have their own unique ability to express their antioxidant properties. In previous studies, it was found that *P. sarmentosum* extracts exhibited antioxidant properties, though these properties varied depending on the type of plant tissue being studied. Total flavonoid and phenolic content was found to be related with these antioxidant effects (Yeo et al., 2018).

The terpenoid also plays significant role in enhancing antioxidant properties. According to Seo et al. (2022), terpenoids demonstrated antioxidant properties, safeguarding cells against oxidative stress induced by reactive oxygen species (ROS). The presence of ROS causes cellular damage and the pathogenesis of a variety of diseases. In addition, terpenoid also can boost the extract's inhibitory impact and decrease the IC_{50} value by scavenging free radicals and reducing oxidative stress.

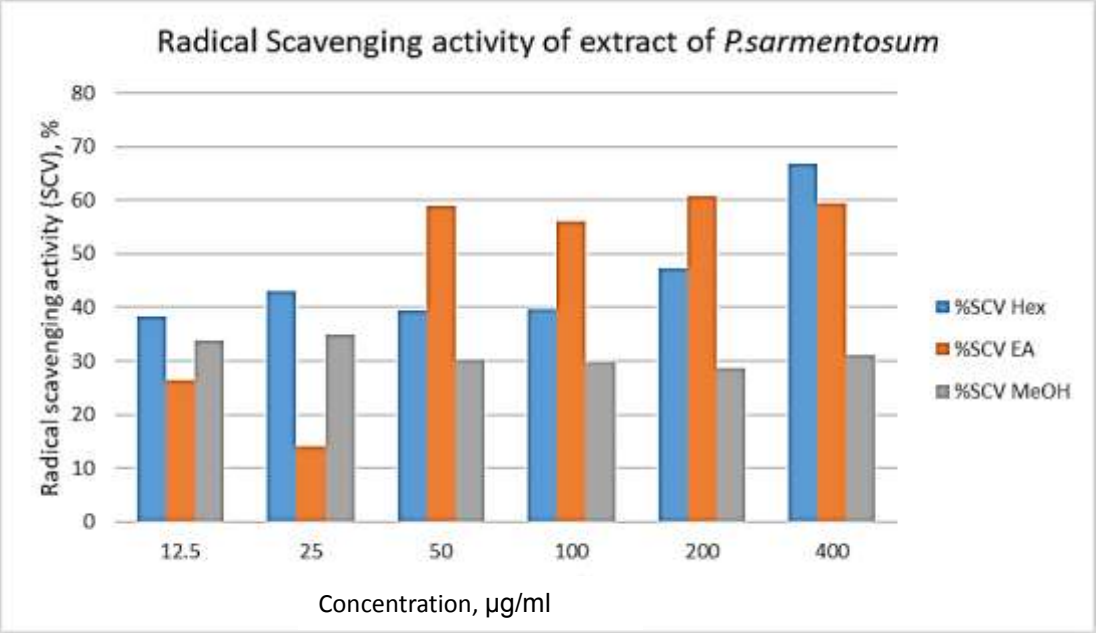

Figure 2 Radical scavenging activity of *P. sarmentosum* leaves extracts

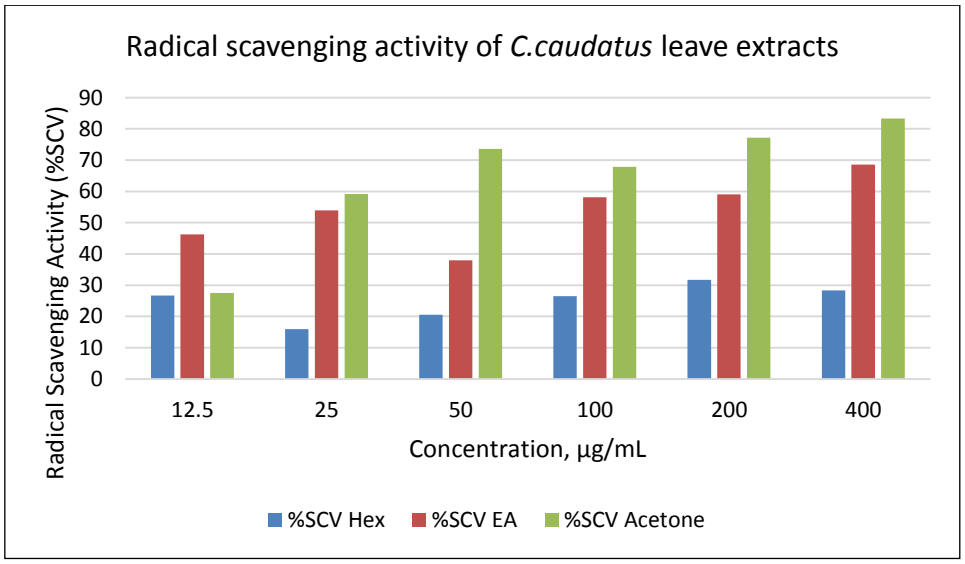

Figure 3 Radical scavenging activity of *C. caudatus* leaves extract

Table 3 Quantitative antioxidant activity of *P. sarmentosum* and *C. caudatus* leaves extract from DPPH radical scavenging activity

Extract/standard	**IC_{50} (µg/ml)** *C. caudatus*	**IC_{50} (µg/ml)** *P. sarmentosum*
Hexane	>400	40.108
Ethyl acetate	62.41	39.304
Acetone	14.12	-
Methanol	-	32.01
Standard ascorbic acid	3.83	3.83

(-) not applicable

Conclusion

The antioxidant activity from TLC dot blot assay of *P. sarmentosum* and *C. caudatus* leave extract was highest in methanol extract and acetone extract with the lowest concentration of 0.39 mg/mL and 0.024 mg/ml, respectively. The DPPH radical scavenging activity showed that the methanol extract from the leaves of *P. sarmentosum* exhibited the highest antioxidant activity with the lowest IC_{50} value of 32.01µg/ml and 14.12µg/ml for acetone extract of *C. caudatus*. The contribution of phytochemicals such as phenolic and terpenoid are purposely the remarkable reason for the antioxidant activity of *P. sarmentosum* and *C. caudatus* leaves extract.

Ethics Statement

The research does not require research ethics approval.

Authors Contribution

Writing – Original draft preparation, Shahrim, A.S and Ahmad Nazri, N.N; Literature Review, Daud, S; Methodology, Abdul Aziz, N; Writing – Review and editing, Harun, A."

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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