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SCREENING FOR PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITY OF FLOWER EXTRACTS OF *QUISQUALIS INDICA* LINN.

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Abstract

Rangoon creeper, scientifically known as *Quisqualis indica* Linn. belongs to the family of Combretaceae and has been used as a traditional medicine for a long time. This plant treats many illnesses, such as fever and boil, and relieves headaches. However, the scientific data to support the effectiveness of this plant as a medicinal source still needs to be discovered. Therefore, this study aims to identify phytochemical constituents and determine the antioxidant properties of flower extracts of *Q. indica* L. Maceration technique was employed to extract the sample using three different solvent polarities: n-hexane, ethyl acetate, and methanol. The extractive yield of methanol showed the highest percentage, 17.41%, followed by ethyl acetate (3.19%) and n-hexane (1.45%), respectively. For phytochemicals screening, all extracts exhibited the presence of alkaloids, flavonoids, saponins, terpenoids, and tannins, except the n-hexane extract, where flavonoids are not detected. The antioxidant assay was done by DPPH (2,2 diphenyl-1-picrylhydrazyl) assay at 1000 µg/mL concentration. It was found that the methanol extract gave the highest DPPH radical scavenging activity of 99.11 ± 0.50%, which is higher than the ascorbic acid standard used. The results justify its use as a medicinal plant. This local species has a higher potential as an antioxidant agent as well.

Keywords: *Quisqualis indica* L., phytochemical, antioxidant, DPPH

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Introduction

Plants are among several natural sources used long ago to discover potential bioactive compounds. It is a promising source because many plants still need to be explored. Medicinal plants always get attention from many researchers due to their historical benefits transferred from ancestors. In addition, their ability to cure illnesses without giving harmful side effects is reported in many publications and documented in television shows. Today, the use of herbal medicinal supplements and products has increased tremendously. Ekor (2014) reported that 80% of people worldwide rely on them for primary healthcare.

A medicinal plant that is still worth exploring is the Rangoon creeper. It is a ligneous creeper grown as an ornamental plant in many countries. This herbal has been used as a traditional medicine for a long time to treat many diseases due to its activity against common illnesses such as fever, boil, ulcer, headache, and diarrhoea. One of the reasons for using this plant in making many herbal products is its availability almost every season in Malaysia, and it grows faster. Each part of the plant has different uses. However, the scientific study to prove the claim must be more extensive. Therefore, exploring more about this plant's bioactive compounds, specifically from the local species is interesting.

Flower extract of *Q. indica* L. has many benefits due to the presence of active compounds that are responsible for specific bioactivity functions. This species' flower (Figure 1) was reported to relieve headaches (Sahu et al., 2012). In addition, it has also been proven to be rich in flavonoids and phenols that play an essential role in free radical scavenging activity (Singh et al., 2017). Therefore, this study aims to screen the phytochemical constituents of the *Q. indica* L. of flower extract from the local species and investigate the presence of bioactive compounds that can act as potent antioxidant properties. The findings of this study provide information about the local species of *indica* and, at the same time, prove their importance in medicinal potential.

Figure 1. Flowers of local *Q. indica* L.

Methods

Plant material and preparation of extracts

The wildly growing flowers of *Q. indica* L. were randomly collected along the village Darul Hidayah roadside in Hulu Langat, Selangor, Malaysia, from August 2022 to September 2022. Exactly 1200 g of fresh petals were washed with tap water and air-dried at room temperature for two weeks. The dried petals were weighed (420 g) and ground into powder form using a mixer grinder.

For the extract preparation, 400 g of powdered sample was soaked into 1.2 L of n-hexane (Bendosen, Malaysia) for 72 hours and occasionally stirred. Then, the mixture was filtered using Whatman No.1 filter paper. The same extraction process was repeated with ethyl acetate (1.2 L) (R&M, United Kingdom) and methanol (1.2 L) (HmbG Germany). The filtrate was concentrated to dryness using a rotary evaporator, and all crude extracts were preserved at 4°C in a refrigerator until further use.

Phytochemical screening

Phytochemical tests of crude flower extract were done to determine the presence of alkaloids, flavonoids, saponins, terpenoids, and tannins as follows:

Test for alkaloids (Wagner's Test)

About 50 mg of extract was dissolved in 5 mL distilled water and 2 mL hydrochloric acid (R&M, United Kingdom). Then, the mixture was filtered. Two drops of Wagner's reagent were added to the test solution. The presence of alkaloids was confirmed by a reddish-brown precipitate formed. (Anusha & Bai, 2017).

Test for flavonoids (Lead acetate test)
Approximately 50 mg of each extract was placed in a fresh test tube. Several drops of 10% lead acetate (R&M, United Kingdom) were then added. The presence of flavonoids was confirmed by the appearance of yellow precipitates (Shaikh & Patil, 2020).

Test for saponins (Foam test)
Precisely 50 mg of each extract was shaken with 5 mL of distilled water. The test tube was vigorously shaken by hand for 15 minutes. The presence of saponins was confirmed by the formation of foam on the top of the test tube (Kibria & Kar, 2019).

Test for terpenoids (Salkowski test)
About 50 mg of each extract was shaken with 2 mL chloroform (R&M, United Kingdom) and 2 mL concentrated H_2SO_4 (Merck, United States of America). The presence of terpenoids was confirmed by the formation of reddish-brown colouration of the interface (Roghini & Vijayalakshmi, 2018).

Test for tannins (Ferric Chloride test)
Exactly 50 mg of each extract was treated with 5% ferric chloride (Bendosen, Malaysia) drops. The bluish-black formation confirmed the presence of tannins (Anusha & Bai, 2017).

Antioxidant assay
The method was adopted by Jani et al. (2020) with few changes. The DPPH (Merck, United States of America) free radical scavenging assay determined how good the extracts are at being antioxidants. About 1.0 mg of each sample was dissolved in 1 mL methanol to obtain a stock concentration of 1000 µg/mL. The stock solution was diluted in methanol to a final concentration of 500, 250, 125, and 62.5 µg/mL. Fresh DPPH solution was diluted in methanol to a final concentration of 50 µM. Then, 3.8 mL of DPPH solution was added to 0.2 mL of varied concentrations of sample solution. The solutions were well mixed using a vortex and left in a dark room for 30 minutes. The absorbance of the reaction was measured at 517 nm using a UV-vis spectrophotometer after 30 minutes.

Ascorbic acid (R&M, United Kingdom) was used as a standard reference (positive control), while the DPPH solution (3.8 mL DPPH and 0.2 mL methanol) served as the DPPH blank. The blank sample was prepared by mixing 0.2 mL of sample extract and 3.8 mL of methanol. The standard and extract were both tested in triplicate. The following formula below was used to calculate the percentage inhibition (%):

$$\text{Percentage Inhibition (\%)} = [A_{\text{DPPH blank}} - (A_{\text{Sample}} - A_{\text{blank sample}})] / A_{\text{DPPH blank}} \times 100$$

Where A is absorbance.

Result and Discussion

The percentage yield of extracts
Through the maceration extraction method, the extraction of *Q. indica* L. flowers was done successively using three (3) different solvents, n-hexane, ethyl acetate, and methanol. The extraction process began with non-polar to polar solvents to fully retain the compounds based on their polarity. Table 1 summarises the percentage of extractive yield of each extract obtained. For n-hexane, the extract obtained was 5.81 g, representing a 1.45% yield. For ethyl acetate, 12.77 g (3.19%) extract was recorded, and 69.66 g of extract was obtained from methanol, signifying a 17.41% extractive yield. It shows that methanol has the highest yield extract, followed by ethyl acetate and n-hexane. From the experiment, it can be concluded that the percentage of extractive yield of crude extracts is higher as the polarity of the solvent increases.

Table 1. The percentage of extractive yield of each *Q. indica* L. flower extract

Extracts	Weight of dried extract (g)	Extractive yield (%)
n-hexane	5.81	1.45
Ethyl Acetate	12.77	3.19
Methanol	69.66	17.41

Preliminary phytochemical screening

The qualitative identification of flower constituents was accomplished by phytochemical screening. Previous studies by Barik et al. (2020), Shah et al. (2019), Mukherjee & Chandra (2017), and Afify & Hassan (2016) reported that flower extracts of *Q. indica* L. possess alkaloids, flavonoids, saponins, glycosides, phenols, and terpenoids. In this study, the phytochemical screening test reveals the presence of alkaloids, flavonoids, saponins, terpenoids, and tannins, as displayed in Table 2. It was found that methanol and ethyl acetate extracts exhibited the presence of all tested phytochemical constituents. However, flavonoid was not detected in the n-hexane extract, which may be due to the polarity of the compounds. In general, flavonoids are polar compounds and cannot be extracted by a non-polar solvent (n-hexane). These results are also visually demonstrated, as shown in Figure 2 below.

Table 2. Preliminary investigation of phytochemical constituents in various extracts

Phytochemical	Extracts		
	n-hexane	Ethyl acetate	Methanol
Alkaloids (Wagner's Test)	+	+	+++
Flavonoids (Lead acetate test)	-	+	+++
Saponins (Foam test)	+	+	+++
Terpenoids (Salkowski test)	+	+++	++
Tannins (Ferric Chloride test)	+	++	+++

(+) Presence: low; (++) Presence: moderate; (+++) Presence: high; (-) Absence

Based on Figure 2, alkaloids were identified at a high level in the methanol extract compared to the other two extracts. The detection of flavonoids, saponins, and tannins was also more intense in methanol extract than n-hexane and ethyl acetate extracts. However, terpenoids were found abundant in ethyl acetate extract. The different levels of phytochemicals present in different extracts may be due to factors such as salinity, soil water, soil fertility, temperature, and light that could affect plants' production of bioactive substances (Yang et al., 2018).

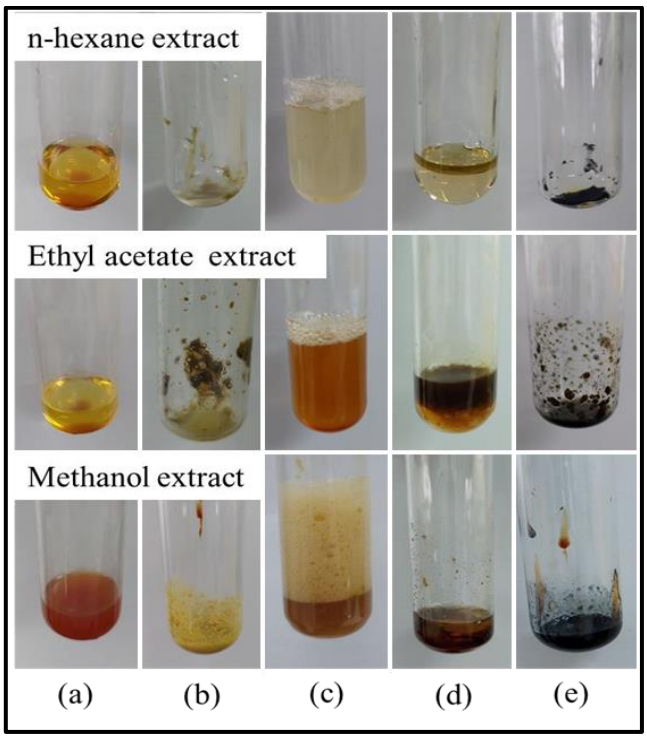

Figure 2. Phytochemical constituents screening on n-hexane, ethyl acetate, and methanol extracts for (a) alkaloids, (b) flavonoids, (c) saponins, (d) terpenoids, and (e) tannins

DPPH radical scavenging activity

Free radicals can be neutralised with a substance that contains antioxidant properties. The antioxidant will shield cell components from oxidative damage caused by unstable molecules. Also, they will act as a reducing agent, e.g., intermediate free radicals, and stop oxidation from happening by oxidising themselves. Usually, plants such as fruits, vegetables, herbs, and spices produce many antioxidant substances.

In this study, the DPPH radical scavenging activity of the flower extract of *Q. indica* L. was evaluated, as shown in Table 3. It was observed that as the polarity of solvent extraction increased, the scavenging activity increased as well. The result obtained was compared with a standard known as ascorbic acid. All extracts showed DPPH activity more significantly than 50%, and the methanol extract displayed the highest DPPH radical scavenging activity with 99.11 ± 0.50% inhibition compared to the other two extracts. The DPPH scavenging activity of methanol extract also demonstrated higher inhibition than a standard. This result was the same as a previous study by Dutta et al. (2019). The crude extract sample from the polar solvent used in their study exhibited potent antioxidant activity by inhibiting hydrogen peroxide radicals compared to standard ascorbic acid.

The present study also agrees with previous research reported by Sutar et al. (2020) in which the flower extract of *Q. indica* L. showed high DPPH radical scavenging activity from methanol extract. Flowers naturally contain polyphenols and flavonoids, essential in regulating the oxidation reaction. This statement can be supported by preliminary phytochemical constituents screening that showed the methanol extract contains flavonoids. An article written by Rashid et al. (2023) mentioned that flavonoids have hydroxyl groups. They can eliminate singlet and triplet oxygen or break down peroxides by giving up an electron or a hydrogen atom. Furthermore, flavonoids can neutralise free radicals, which are natural antioxidants that help prevent and fix damage caused by oxidation.

Table 3. Per cent inhibition of *Q. indica* L. flowers with three different extracts at 1000 µg/mL

Extracts	Concentration (µg/mL)	DPPH Scavenging Activity (%)
Methanol	1000	99.11 ± 0.50
Ethyl acetate		92.38 ± 0.47
n-hexane		78.18 ± 18.06
Ascorbic acid (standard)		94.81 ± 4.93

Conclusion

Extraction of flower *Q. indica* L. using the maceration technique was successfully achieved. The preliminary phytochemical constituents screening showed that methanol and ethyl acetate extracts exhibited the presence of alkaloids, flavonoids, saponins, terpenoids, and tannins, except for the n-hexane extract, which showed the absence of flavonoids. Methanol extract also showed antioxidant properties (99.11 ± 0.50%) is comparable to ascorbic acid (94.81 ± 4.93%). Thus, the findings of this work proved that local *Q. indica* L. flower extract has the potential as an antioxidant and may embark on further ideas to facilitate the standardisation of herbal formulations containing *Q. indica* L. flowers.

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Author Contribution

Nurul Izzah – collecting data, data processing and analysis, manuscript writing; Suhaidi Ariffin – analysis of experimental design, conceptualisation, supervision, manuscript writing, review, and editing.

Conflict of Interest

The authors declare no conflict of interest.

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