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Research Article

PHYTOCHEMICAL SCREENING OF ARTABOTRYS CRASSIFOLIUS HOOK.F. & THOMSON (ANNONACEAE JUSS.)

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ABSTRACT

Objective: The aim of the present study was to determine the phytochemical constituents of *Artabotrys crassifolius*.
Methods: The leaves and barks of *Artabotrys crassifolius* were extracted sequentially with hexane, chloroform and ethanol. The corresponding crude extracts obtained were then subjected to phytochemical screening.
Results: The phytochemical analysis of crude extracts of *Artabotrys crassifolius* demonstrated the presence of alkaloids, cardiac glycosides, flavonoids, phenolic compounds, saponins and terpenoids.
Conclusion: The chemical profile of these crude extracts can serve as a useful reference for further investigations in pharmacological activities of *Artabotrys crassifolius*.

Keywords: Phytochemical analysis, Artabotrys crassifolius, Annonaceae

INTRODUCTION

Since time immemorial, plants have been used extensively as a source of medicines for the treatment of various human ailments [1]. According to the World Health Organisation, approximately 80% of the people in developing countries still rely on traditional medicines for their primary health care needs [2], and a major part of the traditional therapy involves the use of plant extracts or their active constituents [3]. Furthermore, about 25% to 50% of current pharmaceuticals are plant-derived natural products, indicating the significance and efficacy of plants as an indispensable pharmacological tool [4].

Over the past few years, there has been a tremendous resurgence of interest in medicinal plants [5]. This revival might be attributed to several driving factors such as rise in population, insufficient supply of drugs in certain parts of the world, prohibitive cost of treatments for common ailments, side effects of several allopathic drugs in current usage as well as development of resistance to currently used drugs for diseases [6,7]. Consequently, exploitation of medicinal plants for bioactive compounds is of great potential and could be an imperative source of providing new vistas for novel drug discovery and development [8,9].

The tropical rainforest of Malaysia is regarded as one of the most evolved and complex ecosystems in the world that serves a vast untapped biodiversity of natural resources [10]. This unique natural heritage has brought renewed interest in the screening of indigenous medicinal plants for bioactive compounds. *Artabotrys* R.Br. is one of the largest genera of the custard-apple family, Annonaceae [11]. The genus *Artabotrys* comprises over 100 species of woody climbers and scandent shrubs distributed mainly in tropical and subtropical regions of the world, especially tropical Africa and Eastern Asia [12–14]. Moreover, *Artabotrys* species have a long history of traditional use for a wide range of medical conditions, particularly malaria [15], scrofula [16] and cholera [17].

To the best of our knowledge, no detailed studies have been reported on the phytochemical screening of *Artabotrys crassifolius*. Therefore, the objective in this research was to determine the phytochemical constituents of *Artabotrys crassifolius*.

MATERIALS AND METHODS

Collection and identification of plant material

The leaves and barks of *Artabotrys crassifolius*, with the local name of *akar mempisang*, were collected from Kuala Kangsar, Perak, Malaysia (4°46′N, 100°56′E) in March 2011. The plant was identified and authenticated by Mr. Kamarudin Saleh, Forest Biodiversity Division, Forest Research Institute Malaysia (FRIM). Voucher specimens were prepared and deposited in the Kepong Herbarium (KEP) of FRIM (PID 080311–05), and the School of Pharmacy, Faculty of Science, The University of Nottingham Malaysia Campus (UNMC 65) for future reference.

Preparation of plant material

After removal of extraneous matter, the freshly collected leaves and barks were air-dried in the shade at room temperature for at least 2 weeks. The dried leaves and barks were then finely pulverized by grinding prior to extraction. The pulverized leaves (1.30 kg) and barks (4.79 kg) were extracted sequentially with solvents of increasing polarity starting from hexane, chloroform and 95% (v/v) of ethanol. Each extraction was performed in triplicate at a solid-to-solvent ratio of 1:5 (w/v) in a 40°C water bath for three days. The respective extract was subsequently filtered through Whatman filter papers No. 1 and the collected filtrate was concentrated to dryness under reduced pressure at 40°C using rotary evaporator. Eventually, the dried extract obtained was weighed and stored at −20°C until further use. For stock solutions, each crude extract was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL and stored at 4°C.

Determination of extraction yield

For each extraction, the extraction yields of crude extracts were calculated. The extraction yield was expressed as the weight percentage of the dried plant extract obtained with respect to the dried plant material used [18,19], which was given as follows:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dried plant extract (g)}}{\text{Weight of dried plant material (g)}} \times 100\%$$

Phytochemical screening

The phytochemical screenings of crude extracts were carried out using standard procedures. Each crude extract (final concentration of 1 mg/mL) was assayed for the presence of phytochemical constituents such as alkaloids, cardiac glycosides, flavonoids, phenolic compounds, saponins, tannins and terpenoids.

Test for alkaloids (Dragendorff's test)

Prior to detection of alkaloids, solution A was prepared by dissolving 1.7 g of bismuth subnitrate in 100 mL of 4:1 (v/v) of distilled water and acetic acid, whereas 40 g of potassium iodide was dissolved in 100 mL of distilled water as solution B. To prepare Dragendorff's reagent, 5 mL of solution A and B was added in 20 mL of acetic acid and topped up with distilled water to 100 mL [20].

Approximately 1 mL of each crude extract was mixed with 4 mL of methanol. The mixture was filtered and the filtrate was divided into two test tubes with one portion as the control. Another portion of the filtrate was treated with 1 mL of 1% (v/v) of hydrochloric acid (HCl) and warmed on steam bath, followed by addition of a few drops of Dragendorff's reagent. Reddish orange precipitation indicated the presence of alkaloids [21].

Test for cardiac glycosides (Keller-Kiliani test)

Approximately 1 mL of each crude extract was mixed with 4 mL of distilled water. The mixture was filtered and the filtrate was divided into two test tubes with one portion as the control. Another portion of the filtrate was treated with 2 mL of acetic acid containing one drop of 5% (w/v) of ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$). This was underlayed with 1 mL of concentrated sulfuric acid (H_2SO_4, 98%). A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring might appear below the brown ring, while in the acetic acid layer, a greenish ring might form just above the brown ring and gradually spread throughout this layer [22].

Test for flavonoids (Shinoda test)

Approximately 1 mL of each crude extract was mixed with 4 mL of ethanol. The mixture was filtered and the filtrate was divided into two test tubes with one portion as the control. Another portion of the filtrate was treated with few fragments of magnesium ribbon, followed by dropwise addition of concentrated HCl (fuming 37%). Pink scarlet, crimson red or occasionally green to blue color appeared after few minutes indicated the presence of flavonoids [23].

Test for phenolic compounds (Ferric chloride test)

Approximately 1 mL of each crude extract was mixed with 4 mL of distilled water. The mixture was filtered and the filtrate was divided into two test tubes with one portion as the control. Another portion of the filtrate was treated with a few drops of 5% (w/v) of $FeCl_3 \cdot 6H_2O$. Bluish-green or bluish-black color indicated the presence of phenolic compounds [24].

Test for saponins (Frothing test)

Approximately 1 mL of each crude extract was mixed with 4 mL of distilled water. The mixture was filtered and the filtrate was divided into two test tubes with one portion as the control. Another portion of the filtrate was shaken vigorously for 1 min and allowed to stand for 15 min. Frothing persistence indicated the presence of saponins [25].

Test for tannins (Gelatin-salt test)

Approximately 1 mL of each crude extract was mixed with 4 mL of hot distilled water. The mixture was filtered and the filtrate was divided into three test tubes. To the first portion of the filtrate, 1 mL of 1% (w/v) of sodium chloride (NaCl) was added as the control. Second portion of the filtrate was treated with 1 mL of 1% (w/v) of NaCl and 1 mL of 5% (w/v) of gelatin, whereas a few drops of 5% (w/v) of $FeCl_3 \cdot 6H_2O$ were added to the third portion of the filtrate. Formation of a precipitate in the second treatment suggested the presence of tannins, and a positive response after addition of $FeCl_3 \cdot 6H_2O$ to the third portion supported this inference [26].

Test for terpenoids (Salkowski test)

Approximately 1 mL of each crude extract was mixed with 4 mL of chloroform. The mixture was filtered and the filtrate was divided into two test tubes with one portion as the control. Another portion of the filtrate was carefully treated with 3 mL of concentrated H_2SO_4 to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids [27].

RESULTS AND DISCUSSION

Extraction yield is a measure of the solvent efficiency to extract specific components from the original material [28,29]. The extraction yield in percentage for each crude extract is shown in Table 1. Among the different solvents used for extraction, ethanol provided the highest yield of crude extracts from both leaves and barks with extraction yields of 5.00% and 4.02% respectively. In contrast, the lowest extraction yield was recorded for hexane extract of barks with 0.53%. This indicates that ethanol is a superior extraction solvent to hexane or chloroform in terms of providing a better yield due to its high polarity.

Prior to pharmacological evaluation of plant extracts, phytochemical screening is the initial and essential step towards understanding the nature of active principles in medicinal plants [30]. Based on the phytochemical analysis of crude extracts presented in Table 2, barks extracts were found to have more secondary metabolites than leaves extracts. Both chloroform and ethanol extracted the widest range of phytochemical constituents from barks including cardiac glycosides, flavonoids, phenolic compounds and terpenoids. The only difference detected between these extracts was the presence of alkaloids and saponins in chloroform and ethanol extracts of barks respectively. Additionally, hexane extract of barks showed positive results for alkaloids, cardiac glycosides and terpenoids.

On the other hand, ethanol extract of leaves possessed similar phytochemical constituents to ethanol extract of barks. However, hexane and chloroform extracts of leaves exhibited positive reaction only to Keller-Kiliani test for cardiac glycosides. Among the phytochemical constituents analyzed, tannins were absent in all of the tested extracts. This implies that the extracts from leaves and barks may constitute a different source of secondary metabolites that can serve as a constructive reference for further detailed studies on the pharmacological activities of *Artabotrys crassifolius*.

Table 1: Extraction yields of crude extracts of *Artabotrys crassifolius*

Extraction yield	Crude extract					
	Leaves hexane	Leaves chloroform	Leaves ethanol	Barks hexane	Barks chloroform	Barks ethanol
Extraction yield (%)	1.99	1.24	5.00	0.53	1.13	4.02

Table 2: Phytochemical screenings of crude extracts of *Artabotrys crassifolius*.

Phytochemical constituent	Test used	Crude extract					
		Leaves hexane	Leaves chloroform	Leaves ethanol	Barks hexane	Barks chloroform	Barks ethanol
Alkaloids	Dragendorff's test	–	–	–	+	+	–
Cardiac glycosides	Keller-Kiliani test	+	+	+	+	+	+
Flavonoids	Shinoda test	–	–	+	–	+	+
Phenolic compounds	Ferric chloride test	–	–	+	–	+	+
Saponins	Frothing test	–	–	+	–	–	+
Tannins	Gelatin-salt test	–	–	–	–	–	–
Terpenoids	Salkowski test	–	–	+	+	+	+

Note: (+) indicates the presence of phytochemical constituents, (–) indicates the absence of phytochemical constituents.

CONCLUSION

The phytochemical analysis of crude extracts of *Artabotrys crassifolius* revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenolic compounds, saponins and terpenoids. Consequently, the chemical profile of these crude extracts can help to provide guidance for further investigations in pharmacological properties of *Artabotrys crassifolius*.

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