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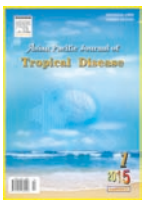

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Study on total phenolics and antioxidant activity of leaves crude extracts of *Annona squamosa* traditionally used for the treatment of cancerous tumours

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ABSTRACT

Objective: To use the leaves of *Annona squamosa* (*A. squamosa*) for the preparation of crude extracts and all crude extracts were used to evaluate their antioxidant activity and total phenolics content collected from Jabal Al Akhdar.
Methods: The leaves powder samples of *A. squamosa* were used for the preparation of crude extracts with methanol by Soxhlet extractor method. Defatted methanol crude extract by water was extracted by different polarities of solvents with increasing polarities. All crude extracts were used for the determination of total phenolics content and antioxidant activity by Folin-Ciocalteu reagent and 2,2-diphenyl-1-picrylhydrazyl methods.
Results: The high amount of total phenolics content found in the leaves crude extracts was in the range of 54.75 to 352.0 mg/100 g of dry powder samples. The highest antioxidant activity was found in methanol and the lowest was found in ethyl acetate crude extract.
Conclusions: The crude extracts from *A. squamosa* leaves showed strong antioxidant activity. Therefore, it could be used as a medicine for the treatment food borne diseases.

1. Introduction

Cancer is considered as one of the major causes of death worldwide. Only limited progress of this disease has been made to reduce the morbidity and mortality[1]. Incorrect diet, genetic predisposition, and via the environment are the main causes of cancers. About 95% cancers are caused by life style. It may take long time about 20–30 years to develop. American Cancer Society and International Union reported that 12 million of cancer patients were diagnosed last year. Among them, about 7 million die worldwide. These numbers are expected to be double by 2030[2].

More than 80% of world's population depend on traditional medicine for their primary health care systems reported by World Health Organization[3,4]. Plants have been used for the treatment of cancer and other incurable diseases. More than 60% anti-cancer agents available in the market come from natural sources[5]. Plant and plants drugs that are used for the treatment of cancer include vinca alkaloids (vincristine, vinblastine, vindesine, vinorelbine), taxanes (paclitaxel, docetaxel), podophyllotoxin and its derivative (etoposide, teniposide), camptothecin, its derivatives (topothecan, irinothecan), anthracyclines (doxorubicin, daunorubicin, epirubicin, idarubicin) and others[6,7]. All anti-cancer drugs used by the patients internationally were either natural products or their derivatives[6,7].

Annona squamosa (*A. squamosa*) belongs to Annonaceae family. In Oman, it is called Almostafal. The English name is sugar apple. America and West Indies are the suitable places for growing this plant. However, due to its medicinal importance now, it has been cultivated commercially all over the world, such as Indonesia, Thailand, Taiwan and India[8,9]. It is mainly grown in gardens for its fruits and ornamental value[10]. The plant height is about 3-7 m long. Its fruits are round to conical shape. The leaves occur singly and the edges are without teeth. Traditionally, all parts of *A. squamosa* are used by different ethnic communities for the treatment of different chronic diseases such as cancerous tumours, insect bites and other skin complaints[11]. It is either used as food and flavouring agent or some countries used it to treat toothache[12,13]. However, the seeds powder are toxic and used to kill head lice and fleas[7]. The leaves are used for long time to treat the diabetics, anti-depressants, epilepsy and spinal cord disorders[8-10]. It has also used as hepatoprotective powers[14]. Its roots are powerful purgatives and are also used in dysentery[15]. Steroid, terpeniod, glycoside, alkaloid, flavonoids, saponin and polyphenolic compounds are the main bioactive compounds presented in the selected plant[14]. However, there is no scientific data

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available on Omani *A. squamosa*. Therefore, the work deals to prepare different leaves crude extracts using different polarities solvents and evaluate their total phenolics content and antioxidant activity of the selected species collected from Jabal Al Akhdar.

2. Materials and methods

2.1. Materials

Hexane, chloroform, ethyl acetate and acetic anhydride were purchased from Scharlau, European Union, UK. Butanol and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich, Germany. Methanol was obtained from Emsure, Germany. Grinder (Japan, Super Deluxe, and India) and rotary evaporator (Yamato, Rotary Evaporator, and Model–RE 801) were used in this experiment for the preparation of samples. Shimadzu UV-1800 spectrophotometer, Japan was used to measure the absorbance. Ammonia was obtained from Appli Chem, Germany.

2.2. Plant samples

The leaves of *A. squamosa* were collected on December 13, 2013 at 4 pm from Al Jabal Al Akhdar, Oman. The samples were collected in a polyethylene bags and brought to the house and kept at room temperature for processing. The morphological identification was done by a botanist and specimen number (056) was deposited in the laboratory.

2.3. Preparation of samples

The leaves samples were washed and cut into small pieces by knife then dried at room temperature for 7 days. The dried samples were brought to the laboratory for powder. The dried samples were ground to make powder by heavy duty blender machine. The powder leaves samples were used for extraction by using Soxhlet extractor.

2.4. Extraction procedure from leaves samples

The dry powder leaves samples (140 g) were extracted with methanol (300 mL, 72 h) by using Soxhlet extractor[16]. The methanol solvent was filtered and evaporated at low temperature and pressure by rotary evaporator to give crude methanol extract (11.69 g). The methanol crude extract (10 g) was suspended in water. The water dissolve part was transferred to a separatory funnel for fractionated by hexane, chloroform, ethyl acetate and butanol solvents to give hexane (0.76 g), chloroform (6.17 g), ethyl acetate (0.62 g) and butanol (1.01 g) crude extracts. The water part in the separatory funnel was evaporated by rotary evaporator dissolved to give the water crude extract (1.10 g).

2.5. Antioxidant assay

The antioxidant activity of each isolated crude extract from the leaves of *A. squamosa* was determined by DPPH[17]. All the test tubes were labeled according to the prepared concentration. Each crude extract (2 mg) was taken in a 10 mL volumetric flask and diluted with methanol solvent. Serial dilution technique was followed for the preparation of 200, 100, 50, 25 and 12.5 μg/mL. Each concentration solution of each crude extract (1 mL) was transferred into a separate test tube and were added 3 mL of DPPH solutions and all the test tubes were kept in the dark for an hour and half and recorded the absorbance at 517 nm wavelength by UV-visible spectrophotometer.

The activity was measured by the following formula:

$$\% \text{ Inhibition} = \text{A control} - \frac{\text{A extract}}{\text{A control}} \times 100$$

2.6. Determination of total phenolics content

The concentration of total phenolics of all crude isolated extracts from the leaves was determined by using Folin-Ciocalteu reagent and external calibration with gallic acid. The crude sample was taken in a test tube and added 1.5 mL of Folin-Ciocalteu reagent[17]. The whole mixture was mixed thoroughly by hand. Then the test tube was kept in dark place for 5 min. About 1.5 mL of sodium carbonate solution was added to it and the mixture was allowed to stand for 2 h at room temperature. The absorbance was measured at 760 nm using Shimadzu UV-1800 spectrophotometer. The concentration of the total phenolics was determined as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve. The estimation of phenolics compounds in the fractions was carried out in triplicate and the results were averaged.

3. Results

The leaves powder samples were used for the preparation of crude extracts through Soxhlet method[16]. The crude extract was dissolved by water and fractionated with hexane, ethyl acetate, chloroform, butanol and methanol to give hexane, ethyl acetate, chloroform, butanol and methanol crude extracts.

The antioxidant activity of different crude extracts such as hexane, ethyl acetate, chloroform, butanol and methanol obtained from the leave of *A. squamosa* was determined through DPPH method[17]. The results of antioxidant activity of the crude extracts from the leaves of *A. squamosa* were presented in Table 1.

Table 1
Antioxidant activity of different leaves crude extracts of *A. squamosa*.

Extract	Concentration (μg/mL)	Absorbance standard	Absorbance sample	Inhibition (%)
Methanol	200.0	1.467	0.020	98.63
	100.0		0.021	98.56
	50.0		0.024	98.36
	25.0		0.029	98.02
	12.5		0.208	85.82
Hexane	200.0	1.467	0.018	98.77
	100.0		0.018	98.77
	50.0		0.027	98.15
	25.0		0.028	98.09
	12.5		0.143	90.25
Chloroform	200.0	1.467	0.017	98.84
	100.0		0.021	98.56
	50.0		0.027	98.15
	25.0		0.029	98.02
	12.5		0.143	90.25
Ethyl acetate	200.0	1.467	0.020	98.63
	100.0		0.023	98.43
	50.0		0.027	98.15
	25.0		0.029	98.02
	12.5		0.188	87.18
Butanol	200.0	1.467	0.017	98.84
	100.0		0.023	98.43
	50.0		0.025	98.29
	25.0		0.025	98.29
	12.5		0.157	89.29
Water	200.0	1.467	0.020	98.63
	100.0		0.020	98.63
	50.0		0.022	98.50
	25.0		0.022	98.50
	12.5		0.133	90.93

The total phenolics content of all crude extracts from the leaves of *A. squamosa* was determined through Folin–Ciocalteu reagent method. The results of total phenolics contents in our present study from different crude extracts were present in Table 2[17].

Table 2
Total phenolics content from the leaves crude extracts of *A. squamosa.*

Different crude extracts	Total phenol mg/100 g dry leaves powder
Water	100.50
Chloroform	200.25
Hexane	105.50
Butanol	352.00
Ethyl acetate	54.75
Methanol extract	105.25

4. Discussion

The leaves powder samples were used for the preparation of crude extracts through Soxhlet method. The isolated crude extract was dissolved by water and fractionated with hexane, ethyl acetate, chloroform, butanol and methanol to give hexane, ethyl acetate, chloroform, butanol and methanol crude extracts. The highest yield of extraction from the leaves was chloroform and the lowest was ethyl acetate.

The antioxidant activity in different crude extracts such as hexane, ethyl acetate, chloroform, water, butanol and methanol crude extracts from the leaves samples of *A. squamosa* at different concentrations (12.5, 25.0, 50.0, 100.0 and 200.0 μg/mL) was determined through DPPH using spectrophotometer method. The results showed that the absorbance was increased with increasing concentration of the crude extracts from leaves samples[17]. The highest antioxidant activity was hexane crude extract and the lowest was ethyl acetate crude extract among the six crude extracts prepared from the leaves and the order was found hexane > methanol > butanol > water > chloroform > ethyl acetate crude extract.

The total phenolics content of all crude extracts from the leaves of *A. squamosa* was determined through Folin–Ciocalteu reagent method. The results of total phenolics content in our present study from different crude extracts are present[17]. The highest total phenolics content in leaves crude extracts of *A. squamosa* was in butanol crude extract and the lowest was in ethyl acetate crude extract. Almost similar results were reported by the other authors on total phenolics contents and antioxidant activity of the crude extracts of *A. squamosa*[5,7,9].

All the defatted crude extracts from the leaves showed very high contents of total phenolics and antioxidant activity. Therefore, according to our study, all the crude extracts from *A. squamosa* could be used as medicine for the treatment of different chronic diseases. Further extensive study will be needed for the isolation and characterization of bioactive compounds from leaves crude extracts of *A. squamosa*.

Conflict of interest statement

We declare that we have no conflict of interest.

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