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

Aziza Saif Rashid Alkhawalidy, Mohammad Amzad Hossain*

School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, P. O. Box 33, Postal Code 616, Nizwa, Sultanate of Oman

**ARTICLE INFO**

*Corresponding author: Mohammad Amzad Hossain, School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, P. O. Box 33, Postal Code 616, Nizwa, Sultanate of Oman.

**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 (topotecan, irinotecan), 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...
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 then made 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 dry powder leaves were used for the preparation of crude extracts using different polarities solvents and evaluate their total phenolics content and antioxidant activity of the selected species collected from Jabal Al Akhdar.

2.5. 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 all the test tubes were kept in 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:

\[
\text{% Inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

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 leaf 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.

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

| 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.

### Acknowledgments

The authors are grateful to University of Nizwa, Nizwa, Sultanate of Oman for providing all chemicals and other expenses from their internal fund to carry out this graduation project. The authors are also grateful to Prof. Dr. Nafsiah Binti Shamsudin, Dean, College of Pharmacy and Nursing, University of Nizwa, Sultanate of Oman for her continuous encouragement during the work. The authors wish to express sincere gratitude to the Central Instrument Laboratory, College of Agriculture and Marine Sciences, Sultan Qaboos University, Sultanate of Oman where the tests were confirmed (Grant No. 507/SOP/OB/1/2013).

### References

[1] Mariod AA, Abdewlahib S, Elikheir S, Ahmed YM, Fauzi PN, Chuen CS. Antioxidant activity of different parts from *Annona squamosa*, and *Catunaregam nilotica* methanolic extract. *Acta Sci Pol Technol Aliment* 2012; 11(3): 249-58.

[2] Deshmukh AB, Patel JK. Aqueous extract of *Annona squamosa* (L.)ameliorates renal failure induced by 5/6 nephrectomy in rat. *Indian J Pharmocol* 2011; 43(6): 718-21.

[3] Pandey N, Barve D. Phytochemical and pharmacological review on *Annona squamosa* Linn. *Int J Res Pharm Biomed Sci* 2011; 2(4): 1404-12.

[4] Damasceno DC, Volpato GT, Sartori TC, Rodrigues PF, Perin EA, Calderon IM, et al. Effects of *Annona squamosa* extract on early pregnancy in rats. *Phytomedicine* 2002; 9: 667-72.

[5] Saha R. Pharmacognosy and pharmacology of *Annona squamosa*: a review. *Int J Pharm Life Sci* 2011; 2: 1183-9.

[6] Hatware K, Annapurna A, Sravani K. Evaluation of in vitro antioxidant activity & effect of aqueous extract of *Annona squamosa* leaves on indomethacin induced peptic ulcer in rats. *Int J Res Pharm Chem* 2014; 4(3): 636-42.

[7] Chandrababu NR, Niranjana KA, Komuriah B, Satya SKV, Kotesh KJ, Ramakrishna KVS, et al. Fatty acid derivatives from seeds of *Annona squamosa* Linn. *J Chem Pharm Res* 2012; 4(10): 4558-61.

[8] Johns T, Windust A, Jurgens T, Mansor SM. Antimarial alkaloids isolated from *Annona squamosa*. *Phytopharmal* 2011; 1: 49-53.

[9] Manvi FV, Nanjawade BK, Shing S. Pharmacological screening of combined extract of *Annona squamosa* and *Nigella sativa*. *Int J Pharm Bio Sci* 2011; 2: 520-9.

[10] Mohamed Saleem TS, Christina AJM, Chidambaramathan N, Ravi V, Gauthaman K. Hepatoprotective activity of *Annona squamosa* Linn. on experimental animal model. *Int J Appl Res Nat Prod* 2008; 1(3): 1-7.

[11] Chitra S, Patil MB, Ravi K. Antibacterial and wound healing activity of the leaves of *Annona squamosa* Linn. *Res J Pharmacogn Phytochem* 2009; 1: 44-50.

[12] Saha R. Pharmacognosy and pharmacology of *Annona Squamosa*: a review. *Int J Pharm Life Sci* 2011; 2(10): 1183-9.

[13] Ponrasu T, Subamekala MK, Ganeshkuma M, Suguna L. Role of *Annona squamosa* on antioxidants during wound healing in streptozotocin-nicotinamide induced diabetic rats. *J Pharmacogn Phytochem* 2013; 2(4): 77-84.

[14] Suresh K, Mamoharan S, Panjamurthy K, Kavitha K. Chemopreventive and antilipidperoxidative efficiency of *Annona squamosa* bark extract. *Pak J Biol Sci* 2006; 9: 2600-5.

[15] Vanitha V, Umadevi KJ, Vijayalakshmi K. Determination of bioactive components of *Annona squamosa* L leaf by GC-MS analysis. *Int J Pharm Sci Drug Res* 2011; 3(4): 309-12.

[16] Hashmi LS, Hossain MA, Weli AM, Al-Riyami Q, Al-Sabahi JN. Gas chromatography-mass spectrometry analysis of different organic crude extracts from the local medicinal plant of *Thymus vulgaris* L. *Asian Pac J Trop Biomed* 2013; 3(1): 69-73.

[17] Hossain MA, Ali Al Kaltani MS, Al Farsi SAJ, Weli AM, Al-Riyami Q. Comparative study of total phenolics, flavonoids contents and evaluation of antioxidant and antimicrobial activities of different polarities fruits crude extracts of *Datura metel* L. *Asian Pac J Trop Dis* 2014; 4(5): 378-83.