Haryana Veterinarian (Dec., 2025) **64(2),** 83-87 ISSN 0033-4359 (P)

Research Article

PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY ANALYSIS OF METHANOLIC EXTRACT OF *ANNONA SQUAMOSA* (CUSTARD APPLE) SEED EXTRACT

B. BEHERA, N. PAZHANIVEL, S. VAIRAMUTHU[1]*, S. SURESHKANNAN[2], T.M.A. SENTHIL KUMAR[3], P. JALANTHA and GANNE VENKATA SUDHAKAR RAO
Department of Veterinary Pathology, [1]Centralised Clinical Laboratory,
[2]Department of Veterinary Public Health and Epidemiology, Madras Veterinary College, Chennai-600007
[3]Zoonosis Research Laboratory, TANUVAS, Madhavaram Milk Colony, Chennai-600051

Received: 27.07.23; Accepted: 14.10.23

ABSTRACT

The plant *Annona squamosa* is also known as custard apple which possesses potent bioactive principles in all its parts. Acetogenins, a class of natural compounds, isolated from members of Annonaceae have potent antineoplastic, parasiticidal, pesticidal, antioxidant, free radical scavenging, antimicrobial activity, etc. Methanolic extract of *Annona squamosa* seed was prepared through the maceration method and undergoes through GC-MS analysis, both qualitative and quantitative phytochemical analysis, and antioxidant assay. GC-MS analysis revealed a total of 19 bioactive compounds in the methanolic extract and n-Hexadecanoic acid showed more probability percentage whereas cis-13-Octadecenoic acid, methyl ester, and trans-13-Octadecenoic acid, methyl ester having the least probability percentage. Quantitative analysis of methanolic extract showed phenol content is more than other phytochemicals whereas saponin content is lower than others. The methanolic extract also revealed very good antioxidant capacity which may be used as a remedy against oxidative stress-related diseases.

Keywords: *Annona squamosa*, Antioxidant, Custard apple, Methanolic seed extract

How to cite: Behera, B., Pazhanivel, N., Vairamuthu, S., Sureshkannan, S., Senthil Kumar, T.M.A., Jalantha, P. and Rao, G.V.S. (2025). Phytochemical and antioxidant activity analysis of methanolic extract of *Annona squamosa* (Custard apple) seed extract. *Haryana Veterinarian.* **64(2)**: 83-87.

Unhealthy dietary habits and lifestyle patterns (smoking and alcohol consumption), exposure to physical (ultraviolet [UV] light, ionizing radiations) or chemical agents (drugs, pollutants, pesticides) along with deficiencies in the physiological antioxidant defences may result in pathological stress to the cells and tissues, which can have multiple effects. The practice of traditional medicine using medicinal plants is as old as the origin of man (Trease and Evans, 2002). Substances found in medicinal plants are known as active principles. These compounds have been extracted and used in different forms such as infusions, syrups, decoctions, infused oils, essential oils, and creams. Plant-derived natural products such as flavonoids, terpenes, alkaloids, anthraquinones, saponins, tannins, steroids, lactones and volatile oils received considerable attention in recent years due to their diverse pharmacological properties, including cytotoxic and chemo-preventive effects.

The plant *Annona squamosa* is also known as custard apple which possesses potent bioactive principles in all its parts. Acetogenins are a class of natural compounds, isolated from members of Annonaceae having potent antineoplastic, parasiticidal, pesticidal, antioxidant, free radical scavenging, antimicrobial activity, etc. (Alali *et al.*, 1999; Ma *et al.*, 2017). Unfortunately, seeds are not to be eaten directly because of hardness though they have medicinal derivatives they can be processed in such a way to use for the cytotoxicity effect on cancerous cells by using scientific methods (Extraction, GC-MS analysis, both qualitative and quantitative test, Free radical scavenging assay, Total antioxidant capacity assay) by bring a person from sickness to healthy. (Saad *et al.*, 2006). The *in-silico* study between *Annona squamosa* seed methanolic extract and the genes responsible for mammary tumor also revealed about the anticancerous property of this extracts (Behera *et al.*, 2023).

*Corresponding author: vairamuthupath@gmail.com

MATERIALS AND METHODS

Extraction Procedure

Around 4 kg of Custard apple seeds were collected manually from fruits and washed with distilled water. Then seeds were dried under shade conditions for 1 month without any contamination. Dried seeds were electrically ground and powders were collected in an air-tight container. Then powders were taken in a sterilized beaker, absolute methanol was added, and allowed for magnetic homogenization by stirring at 60-65º C for 15 minutes under 100 rpm. The supernatant was filtered using Whatmann #1 filter paper and condensation of the filtrate was done in a rotary evaporator (Heidolph, India) under reduced pressure and vacuum at 40º C and 30 rpm. Then the condensed filtrates were undergone for lyophilization and extract powder was prepared.

GC-MS Estimation

The analyses were performed using a GC-MS system (7890A-5975C, Agilent Technologies Inc., Santa Rosa,

CA, USA) equipped with a DB5 capillary column (30 m × 0.25 mm, 0.25 mm, Agilent Technologies Inc., Santa Rosa, CA, USA). The injection volume of each sample was 1 µL. Helium (99.999%) was used as the carrier gas at a flow rate of 1 mL/min. The temperature of the injection port was 250º C, in splitless mode and the column temperature program was as follows: 50º C for 2 min, followed by an increase to 180º C at a rate of 5º C/min, an increase to 270º C at a rate of 20º C/min, and maintenance at 270º C for 5 min. The MS conditions included an EI ion source temperature of 230º C, an ionization energy of 70 eV, and a mass scan range of 40-500 amu. The run time was 25 minutes and the post run was 1 min. The separated constituents were tentatively identified by comparing their mass spectra with those in the NIST08 MS library (National Institute of Standards and Technology, Gaithersburg, MD, USA). The analysis was performed at Pharmacovigilance Laboratory for Animal Feed and Food Safety, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai 600 051.

Qualitative test

The methanolic extract and Custard apple seed extract were screened for their phytochemical constituents according to the standard methods as discussed by Mini (2012) and Salau *et al.* (2013).

Test for alkaloids

To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. The appearance of green color or white precipitate indicates the presence of alkaloids.

Test for tannins

To 1 ml of freshly prepared 10% potassium hydroxide, 1 ml of the extract was added. The appearance of a dirty white precipitate indicates the presence of tannins.

Test for phenols

To 2 drops of 5% ferric chloride, 1 ml of the extract was added. The appearance of a greenish precipitate indicates the presence of phenols.

Test for glycosides

To 10 ml of 50% sulphuric acid, 1 ml of the extract was added, heated in boiling water for 15 min, and 10 ml of Fehling's solution was added and boiled again. The appearance of a brick-red precipitate indicates the presence of glycosides.

Test for saponins

To 2 ml extract, 2 ml of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise. The formation of a 1 cm layer of foam indicates the presence of saponins.

Test for flavonoids

To 1 ml of 10% sodium hydroxide, 3 ml of the extract was added. The formation of yellow color indicates the presence of flavonoids.

Test for steroids

To 5 drops of concentrated sulphuric acid, 1 ml of the extract was added. The appearance of red coloration indicates the presence of steroids.

Test for terpenoids

To 0.5 ml of extract, 2 ml of chloroform was added. The concentrated sulphuric acid was added carefully. The formation of red-brown color at the interface indicates the presence of terpenoids.

Test for quinones

To 1 ml of extract, 1 ml of concentrated sulphuric acid was added. The formation of red color indicates the presence of quinones.

Test for anthocyanin and betacyanin

To 2 ml of extract, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100º C. The formation of bluish-green color indicates the presence of anthocyanin and the formation of yellow color indicates the presence of betacyanin.

Test for proteins

To 2 ml of extract, a few drops of 0.2% ninhydrin was added and heated for 5 min. The appearance of blue color indicates the presence of proteins.

Quantitative Phytochemical Analysis

Quantitative tests were carried out for the quantification of active phytochemicals respectively.

Estimation of total phenolic content

The number of total phenolics in the extracts was determined by Folin- Ciocalteau method using gallic acid as a standard (Dhananjay and Maurya, 2010).

Concentrations of 0.5, 1.0, 5.0, 10.0, 20, 30, and 50 µg/ml of gallic acid standard were prepared in methanol, and the concentration of 1 mg/mL of *A. squamosa* seed extract was also prepared in methanol. 0.5 mL of standard and extract were taken in a test tube. To this, 2.5 mL of 10 folds dilute Folin-Ciocalteau reagent and 2.0 mL of 7.5% sodium carbonate were added, mixed thoroughly, and kept at room temperature for 30 minutes. The absorbance was measured at 760 nm in a UV-visible spectrophotometer. The concentration of total phenolics was calculated using the gallic acid standard curve and the total phenolics were expressed as mg of Gallic acid equivalent (GAE) / gram of extract.

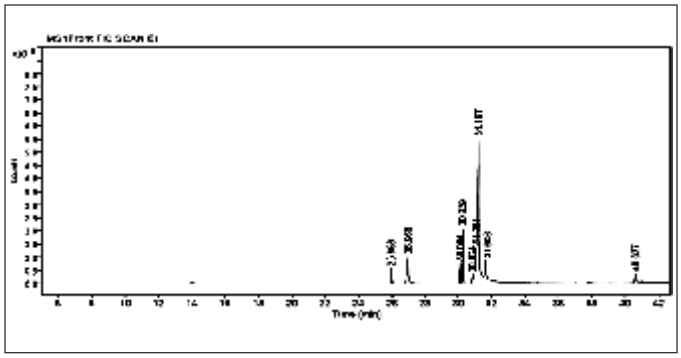

Fig. 1. GC-MS Analysis of methanolic extract of Custard apple (*Annona squamosa*) seed

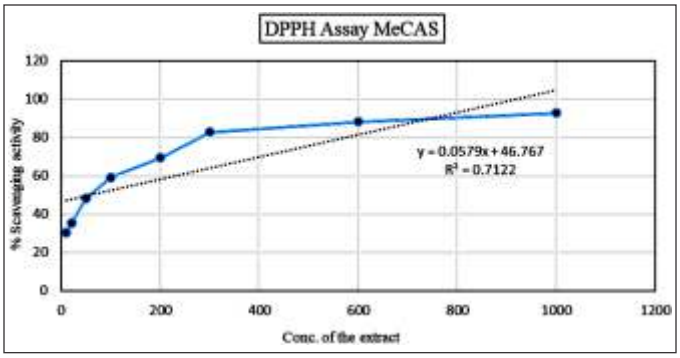

Fig. 2. DPPH Assay of methanolic extract of Custard apple (*Annona squamosa*) seed

Estimation of total flavonoids

The total flavonoid content of the extracts was determined by an aluminium chloride colorimetric assay (Kamtekar *et al.*, 2014).

Standard

Catechin was used as a standard. Stock standard of catechin (1000 µg/ml) was prepared by dissolving 10 mg catechin in 10 ml of absolute alcohol. Working concentrations of 20, 40, 80, 100, 200 and 400 µg/ml were prepared in distilled water.

0.5 ml of extract and different concentrations of the standard were taken in a test tube. To this, 2.0 ml of distilled water and 0.15 ml of 5% sodium nitrite were added and incubated at room temperature for 5 minutes. To each tube, 0.15 ml of 10% aluminium chloride was added and after 6 minutes, 1 ml of 1 M sodium hydroxide was added. Finally, the volume was made up to 5 ml using distilled water. The contents were mixed well and incubated at room temperature for 15 minutes. The development of orange yellowish color was measured using a UV-Visible spectrophotometer at 510 nm. The concentration of total flavonoid content was calculated using the catechin standard curve and expressed as mg of catechin/100 grams of extract.

Estimation of alkaloids

5 grams of powdered *Annona squamosa* seed was taken into 20ml of n-butanol and stirred vigorously. The content was transferred into a reagent bottle and kept overnight at room temperature. The slurry was centrifuged at 6000 rpm for 10 minutes and the supernatant made up to 50 ml with n-butanol. The obtained sample was used for the estimation of total alkaloids by the titrimetric method.

10 ml of supernatant solution was taken in a 100 ml separating funnel and 0.1 N HCl of 10 ml was added and the funnel was shaken thoroughly for 2-3 minutes. This results in the solubility of alkaloids. The lower layer contains alkaloids neutralized with 0.1 NHCl and the top layer consists of n-butanol. 10 ml HCl portion was collected in a beaker and 2-3 drops of methyl red was added as an indicator, which turns the solution into a slightly reddish color. The content in the beaker is titrated against 0.1 N NaOH, till the color changes to pale yellow color. The neutralization point was determined. The same procedure was repeated in triplicate (Bikash *et al.*, 2015).

The total amount of alkaloids was calculated by: 1 ml 0.1 N HCl = 0.0162 g alkaloid

Estimation of terpenoids

Annona squamosa seed extract of 100 mg (Wi) was taken and soaked in 9 ml of ethanol for 24 hours. The extract after filtration was extracted in 10 ml of petroleum ether in a separating funnel. The ether extract was separated into pre-weighed glass vials and waited for complete drying (Wf). Ether was evaporated and the yield (%) of total terpenoid contents was measured by using the formula (Wi - Wf/Wi x 100) (Malik *et al.*, 2017).

Quantitative determination of saponins

5 grams of *Annona squamosa* seed extract was taken in a 250 ml conical flask and 100 ml of 20% ethanol was added and kept in the water bath at 55º C for 4 hours with continuous stirring. The residue mixture was re-extracted with 20% ethanol for 4 hours at 55º C with continuous stirring. The combined extract was evaporated to 40 ml over a water bath at 90º C. 20 ml diethyl ether was added to the concentrate in a 250 ml separator funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. 60 ml of n- butanol was added and extracted twice with 10 ml of 5% sodium chloride. After discarding the sodium chloride layer remaining solution was heated in a water bath for 30 minutes. The solution was then transferred into a crucible and was dried in an oven to a constant weight (Chukwuma *et al.*, 2016). The saponin content was calculated in percentage:

$$\% \text{ Saponin} = \frac{\text{Weight of Saponin}}{\text{Weight of sample}} \times 100$$

Quantitative determination of anti-oxidants

1. DPPH (2, 2-diphenyl-1-picryl hydrazyl) Assay

The free radical scavenging activity of the methanolic extract of *Annona squamosa seed* was measured using DPPH (2, 2-diphenyl-1-picryl hydrazyl) as per the method of Zahin *et al.* (2009). One mL of various concentrations of the extract was added to one mL of 0.1 mM solution DPPH in methanol. The solutions were mixed and incubated in the dark for 30 minutes at room temperature. After 30 min, absorbance was measured at 517 nm using a UV-visible spectrophotometer. The free radical scavenging activity was calculated using the formula

$$\% \text{ scavenging activity} = \frac{\text{Control absorbance - Test absorbance}}{\text{Control absorbance}} \times 100$$

The effective concentration required for a 50% reduction of the DPPH radical (IC_{50}) was calculated by regression analysis.

2. Total antioxidant capacity assay

The total antioxidant capacity assay of the methanolic extract of *Annona squamosa* was determined by a standard method (Wan *et al.*, 2011). Different concentrations of *Annona squamosa* seed extracts (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml) were added to 1.0 ml of the reagent solution (0.6 M sulphuric acid, 28 mmol sodium phosphate, and 4 mmol ammonium molybdate. The reaction mixture was incubated at 95° C for 90 min. After cooling, the absorbance was measured at 695 nm against a blank using a UV-visible spectrophotometer (UV 1650 Pc, Schimadzu). Ascorbic acid was used as a standard. Experiments were performed in triplicates.

$$\% \text{ Inhibition} = A0 - A1 / A0 \times 100$$

Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract.

RESULTS AND DISCUSSION

Nowadays different plant extracts have revealed the presence of various phytochemicals which may be used in different disease conditions (Maphosa and Masika, 2010). Methanolic extracts of *A. squamosa* (Custard apple) seed were obtained through the maceration method and then Lyophilized powder was made. The qualitative phytochemical analysis of a methanolic extract of *A. squamosa* (Custard apple) seed showed the presence of various bioactive compounds like tannins, saponins, flavonoids, alkaloids, quinones, terpenoids, phenol, Coumarins (Table No. 1). Presence of different bioactive secondary metabolites may be responsible for the medicinal value of plant extract. Our findings are almost in accordance with the findings of Sachan *et al.* (2015).

Gas chromatography and mass spectrometry (GC-MS)

The GC-MS results showed the presence of different peaks for *A. squamosa* (Custard apple) seed extract (Fig 1.) and total 19 bioactive compounds were found. All the compounds along with their retention time and probability percentage of *A. squamosa* (Custard apple) seed extract represented in Table no. 2. In Custard apple seed extract, n-Hexadecanoic acid showed more probability percentage whereas cis-13-Octadecenoic acid, methyl ester and trans-13-Octadecenoicacid, methyl ester having less probability percentage. The analysis of GC-MS revealed the presence of several compounds like different cyclopeptides and acetogenins which are also reported by others (Zahid *et al.*, 2018).

Quantitative Phytochemical Analysis

Quantitative estimation of five phytochemicals, namely phenols, flavonoids, alkaloids, terpenoids, and saponin was carried out in methanolic extract of *A. squamosa* seed extracts and the results were given in Table No. 3. Phenol content is more as compared to other phytochemicals whereas saponin content is least as compared to others. There is a lot of research has been carried out since 1925 mainly for the isolation and characterization of total alkaloid contents in *A. squamosa* species (Berkov *et al.*, 2006). The presence of terpenoids in the extract exhibits antiviral and antibacterial properties (Chandraiah, 2022).

***In vitro* Antioxidant activity**

There is a continuous generation of reactive nitrogen species and reactive oxygen species due to the result of continual interaction with the environment, normal biochemical reactions, and consumption of xenobiotics. (Nimse *et al.*, 2015). Different disease conditions are mainly due to these free-radical species (Kim and Byzova, 2014). Various use of antioxidants can manage different pathological conditions. As synthetic antioxidants are mainly associated with various toxic and mutagenic effects (Kadhum *et al.*, 2011), so more importance has been given to natural antioxidants derived from different plants (Yao *et al.*, 2004).

1. DPPH (2, 2-diphenyl-1-picryl hydrazyl) Assay

There are a lot of *in-vitro* antioxidant assessing techniques in general practice. Also, antioxidant test models are not same always, so one method cannot be compared with other methods (Badarinath *et al.*, 2010). Mainly, *in-vitro*, antioxidant experiments by means of free radical scavengers are relatively direct to carry out (Alam *et al.*, 2013). In this study, DPPH scavenging activity and total

antioxidant capacity assay were done for the methanolic extract *A. squamosa* seed. DPPH Scavenging activity of methanolic extract of *A. squamosa* (Custard apple) seed is presented in Table no. 4 and Fig 2. The effective concentration required for a 50% reduction of the DPPH radical (IC_{50}) was calculated and the IC50 value of *A. squamosa* (Custard apple) seed extract was 0.558 mg/mL.

2. Total Antioxidant capacity assay

The total antioxidant capacity of the methanolic extract of *A. squamosa* seed as measured by the phosphomolybdate method was 117.81+13.91 (nmol ascorbic acid/g). The result is also in accordance with other reports (Badarinath *et al.*, 2010; Alam *et al.*, 2013).

CONCLUSION

This study observed the presence of different phytochemicals like tannins, saponins, flavonoids, alkaloids, quinones, terpenoids, phenol and coumarins. GC-MS analysis revealed a total of 19 bioactive compounds in the methanolic extract and n-Hexadecanoic acid showed more probability percentage whereas cis-13-Octadecenoic acid, methyl ester, and trans-13-Octadecenoicacid, methyl ester having least probability percentage. Quantitative analysis of methanolic extract showed phenol content is more than other phytochemicals whereas saponin content is lower than others. The methanolic extract also revealed very good antioxidant capacity which may be used as a remedy against oxidative stress-related diseases.

REFERENCES

Alali, F.Q., Liu, X.X. and McLaughlin, J.L. (1999). Annonaceous acetogenins: recent progress. *J. Nat. Prod.* **623**: 504-540.

Alam, Md., Nur, N., Jahan B. and Md Rafiquzzaman. (2013). Review on *in-vivo* and *in-vitro* methods evaluation of antioxidant activity. *Saudi Pharm. J.* **21(2)**: 143-152.

Badarinath, A.V., Rao, K.M., Chetty, C.M.S., Ramkanth, S.T.V.S.R., Ranjan, T.V.S. and Gnanaprakash, K. (2010). A review on *in vitro* antioxidant methods: comparisions, correlations and considerations. *Int. J. Pharma. Tech. Res.* **2(2)**: 1276-1285.

Behera, B., Pazhanivel, N., Vairamuthu, S., Sureshkannan, S., Senthil Kumar, T.M.A., Jalantha, P., Srinivasan, M.R. and Rao, G.V.S. (2023). Anticancerous effect of *Annona squamosa* (Custard apple) seed extract in the breast cancer- An *in-silico* approach. *Indian Vet. J.* **100(12)**: 37-43.

Bikash, D.C., Md. Jashim, U., Pataric, P., Dash, M., Maiti, D.C. and Mannab, K. (2015). Estimation of alkaloids and phenolics of five edible cucurbitaceous plants and their antibacterial activity. *Int. J. Pharmacy Pharm Sci.* **7**: 12.

Chandraiah, G. (2022). Studies on the phytochemical and pharmacological properties of *Annona squamosa* L. *Int. J. Creative Res. Thoughts.* **10(12)**: 2320-2882.

Chukwuma, S., Ezeonul, M. and Chigozie, E. (2016). Qualitative and quantitative determination of phytochemical contents of indigenous nigerian softwoods. Hindawi Publishing Corporation New *J. Sci.* **9**: 2-3.

Dhananjay, S. and Maurya, S. (2010). Quantitative analysis of total phenolic content in *Adhatoda vasica* nees extract. *Int. J. Pharma Tech. Res.* CODEN (USA): IJPRIF. **4**: 2403-2406.

Kadhum, A.A.H., Al-Amiery, A.A., Musa, A.Y. and Mohamad, A.B. (2011). The antioxidant activity of new coumarin derivatives. *Int. J. Mol. Sci.* **12(9)**: 5747-5761.

Kamtekar, S., Keer, V. and Patil, V. (2014). Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. *J. Applied Pharm. Sci.* **4**: 61-65.

Kim, Y.W. and Byzova, T.V. (2014). Oxidative stress in angiogenesis and vascular disease. *Blood.* **123(5)**: 625-631.

Ma, C., Chen, Y., Chen, J., Li, X. and Chen, Y. (2017). A review on *Annona squamosa* L.: phytochemicals and biological activities. *Am. J. Chin. Med.* **45(05)**: 933-964.

Malik, S.K., Ahmad, M. and Khan, F. (2017). Qualitative and quantitative estimation of Terpenoid contents in some important plants of Punjab, Pakistan. *Pakistan J. Sci.* **69**: 2.

Maphosa, V. and Masika, P.J. (2010). Ethnoveterinary uses of medicinal plants: A survey of plants used in the ethnoveterinary control of gastro-intestinal parasites of goats in the Eastern Cape Province, South Africa. *Pharm. Biol.* **48(6)**: 697-702.

Mini, K.P. (2012). *In-vitro* assessment of anthelmintic effect of aristolochia species plants against *Haemonchus contortus*. Ph. D thesis submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India.

Nimse, S., Satish, B. and Pal, D.K. (2015). Free radicals, natural antioxidants and their reaction mechanisms. *RSC Advances.* **5(35)**: 27986-28006.

Saad, B., Dakwar, S., Said, O., Abu-Hijleh, G., Al Battah, F., Kmeel, A. and Aziazeh, H. (2006). Evaluation of medicinal plant hepatotoxicity in co-culture of hepatocytes and monocytes. *Evid. Based Complementary and Altern. Med.* **3**: 93-98.

Sachan, A., Daya, S., Jaiswal, A.K. and Sudan, V. (2015). *In-vitro* ovicidal assessment of methanol, ethyl acetate and chloroform extracts of *Annona squamosa* and *Chenopodium album* against caprine gastrointestinal nematodiosis. *J. Parasit. Dis.* **39(1)**: 62-66.

Salau, A.K., Yakabu, M.T. and Oladiji, A.T. (2013). Cytotoxic activity of aqueous extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* root barks against ehrlich ascites carcinoma cells. *Indian J. Pharmacol.* **15(4)**: 381-385.

Trease, G.E. and Evans, W.C. (2002). Pharmacognosy. (15th Edn.) Edinburg: Saunders WB. p. 585.

Wan, C., Yu, Y., Zhou, S., Liu, W., Tian, S. and Cao, S. (2011). Antioxidant activity and free radical-scavenging capacity of *Gynura divaricate* leaf extracts at different temperatures. *Pharmacogn. Mag.* **7(25)**: 40.

Yao, L.H., Jiang, Y.M., Shi, J., Tomas-Barberan, F.A., Datta, N., Singanusong, R. and Chen, S.S. (2004). Flavonoids in food and their health benefits. *Plant Foods Hum. Nutr.* **59(3)**: 113-122.

Zahid, M., Mujahid, M., Singh, P.K., Farooqui, S., Singh, K., Praveen, S. and Arif M. (2018). *Annona squamosa* Linn. (Custard apple): An aromatic medicinal plant fruit with immense nutraceutical and therapeutic potentials. *Int. J. Pharm. Sci Res.* **9**: 1745-1759.

Zahin, M., Aqil, F. and Ahmad, I. (2009). The *in vitro* antioxidant activity and total phenolic content of four Indian medicinal plants. *Int. J. Pharm. Pharm. Sci.* **1**: 1.