Research Article
ISSN: 0974-6943

C. Chandrashekar et al. / Journal of Pharmacy Research 2011,4(3),610-611

Available online through
www.jpronline.info

Isolation,Characterizations and Free radical scavenging activity of *Annona squamosa* leaf

C. Chandrashekar, V. R. Kulkarni*

Central Research Lab, Department of Chemistry, Gulbarga University, Gulbarga, Karnataka. India.

Received on: 05-10-2010; Revised on: 14-12-2010; Accepted on:09-02-2011

ABSTRACT

The present study was to isolation and determines the chemical constituent of leaves of *Annona Squamosa* from ethanolic extract. The chemical compound isolated was analyzed by IR, LC-MS and the compound was confirmed flavones type compound on the basis of spectral data. The *in vitro* antioxidant activity of isolated compound (AS-1) was evaluated by free radical scavenging activity of different concentrations (10µg, 50 µg, and 100 µg) using 1, 1-diphenyl-2 picryl hydrazil method (DPPH). The results of assay were then compared with synthetic antioxidant Butylated hydroxyl anisole (BHA).The isolated compound exhibit (9.62, 24.28, and 45.62%) significant free radical scavenging activity.

Key words: *Annona squamosa*, custard apple, flavonoids, antioxidant activity, DPPH method.

INTRODUCTION

The family (Annonaceae), is a large family which comprising about 130 genera over 2000 species; the most important genera having a largest number of species are Annona, with 120 species, from genera, the species of Annona Squamosa commonly known as custard apple is cultivated throughout India, mainly edible fruit. The plant is tradionally used for the treatment of epilepsy, dysentery, cardiac problem, worm infection, constipation, hemorrhage, antibacterial infection, dysuria, fever, and ulcer. It also has anti fertility, anti tumor and abortifacient properties. [1-4] Phytochemical pharmacological, antibacterial and antiovulatory have been studied with seed extract.[5] The aqueous leaf extract has also been reported to ameliorate hyper thyroidism.[6] Ethanolic extract of leaves and stem are reported to have an anti cancerous activity[7]. It is used as an insecticidal agent and has been investigated by several workers.[8] Free radical scavenging activity[9], Hypoglycemic anti diabetic effect [10-11] and Hepato protective activity of Annona squamosa was reported in the leaf extract. [12] Aporphine alkaloids, [13-14] flavonoids, [15] glycoside, [16]terpine derivatives[17] and Novel diazepine, squamoline[18] were isolated from this plant.

With the importance of above literature survey, in our present investigation, we here in report the isolation and evaluation of compound from the leaf extract of Annona squamosa for free radical scavenging activity.

MATERIALS AND METHODS:

Sample collection and preparation:
The fully matured *Annona squamosa* leaf was collected in the month of august-2008 from Ananthagiri hills, Rangareddy dist, Andhra Pradesh, India. The leaf was identified and authenticated voucher specimen no.HGUG19 at herbarium, Department of Botany, Gulbarga University, Gulbarga Karnataka India.

Preparation of extracts:
The leaves of *Annona squamosa* was washed, air-dried and grounded into a fine powder (200g), was successively extracted with in order non polar to polar solvents like pet ether (40-60 ^{0}C), chloroform, ethanol and water using a sox let extractor. The extracts were filtered through a watman No-1 filter paper and evaporated to dryness under reduced pressure. The yield of pet ether, chloroform, ethanol and water, extracts were 1.10%, 2.86%, 3.92% and 4.82% respectively. All extracts were stored in the refrigerator for future use.

Phytochemical screening:
All the extracts were subjected to phytochemical screening using the standard procedure[19] and their results are tabulated in table-1.

Table -1: Preliminary Phytochemical screening of leaf extract of *Annona squamosa.*

Constituents	Pet ether ext	chloroform ext	methanol ext	water ext
Steroids	+	+	-	-
Triterpine	-	+	-	-
Glycoside	+	+	+	+
Alkaloids	-	-	+	+
Flavonoids	-	-	+	+
Saponins	-	-	+	+
Phinolic compounds	+	+	+	+

***Corresponding author.**
Dr. V. R. Kulkarni,
Department of chemistry,
Gulbarga University,
Gulbarga, Karnataka, India.
Tel: +91 9448585914
Email: vrk_chem@yahoo.com

Isolation of flavonoids.
Ethanol part of extract (5gm) was successively extracted with EtOAc, and then the EtOAc extract was concentrated to dryness. The EtOAc extract was subjected to a column of silica gel (60-120 mesh; 3cm dia, × 60 cm length) being eluted a gradient of Pet ether /EtOAc with increasing polarity.10 main fractions were collected and individual fractions were tested for presence of the active bioflavonoid compounds. Thus, from fraction of Pet ether /EtOAc (10:90), compound 1 was separated by using preparative TLC using Hexane/ EtOAc in ratio of 8:2 system as eluent.

There were two different spots on the TLC plate, when illuminated with UV light with Rf value of compound 0.28 respectively from point of origin of sample. The compound with Rf value of 0.28, showed relatively higher concentration of flavonoids tests, (Shinoda and NaOH). Compound labeled as AS-1 (Rf 0.28). The isolated compound was identified after analyzing spectra obtained from IR and LCMS spectrophotometer

Compound AS-1: Brownish yellow crystal, mp: 225^o C, R_f value: 0.28, LC-MS (SHIMADZU)- 328 $(M)^+$. FT-IR (Jasco-5300) (KBR) V max/cm: 3414 (-OH), 1651 (Unsaturated -C=O), 2928 (C-Me), 1072 cm^{-1} glycosidic (C-O) groups that are found in flavonoids.[20-21]

Free radical scavenging activity by DPPH method:
The free radical scavenging activity was followed by the DPPH method.[22] Initially different concentrations (10µg, 50 µg, and 100 µg) of sample and Butylated hydroxyl anisole (BHA) were taken in different test tubes. The volume was adjusted to 100 µl by adding methanol. Five milliliter of 0.1mM methonolic solution of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was added to these tubes and shaken vigorously. The tubes were allowed to stand at Room temperature for 30 min. The control was prepared as above without any exreact.The absorbances of the sample were measured at 517 nm. Radical scavenging activity was calculated using the following formula.
% of radical scavenging activity = [(Control OD – Sample OD) / (Control OD)] ×100
Where, control OD is the absorbance of the control reaction (Containing all reagents except the test sample), and sample OD is the absorbance of the test sample. Synthetic antioxidant butylated hydroxyl anisole (BHA) was used as positive control and all test were carried out in triplicate.

RESULTS AND DISCUSSION:

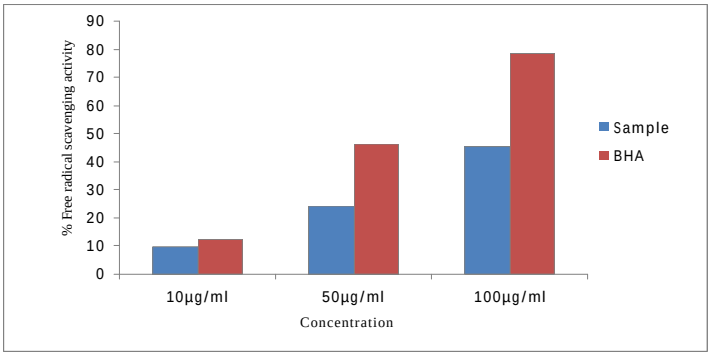
Compound AS-1 was brownish yellow crystal. Mp: 225^{o}C .The mass spectrum showed molecular ion peak at m/z 328 [$(M)^+$, 100%] which corresponds to the molecular weight of the compound. The IR spectrum exhibited characteristic strong absorption band at 3414 cm^{-1} for hydroxyl group, 1651 cm^{-1} for carbonyl group, 2926cm^{-1} for methoxy group and 1072 cm^{-1} for glycosidic group. The above spectral data supports and suggest that the isolated compound belongs to flavones (Leitao *et al.*,) type of compound.

Several concentrations ranging from 10-100µg/ml of the AS-1 was tested their antioxidant activity *in vitro* model. It was observed that free radicals were scavenged by the test compound in a concentration dependent manner in the model. The compound AS-1 showed 9.62, 24.28, 45.62% inhibition respectively. The results given in table-2.

Table -2: Results of DPPH radical scavenger activity

Test Material	Conc. (µg/ml)	% DPPH radical scavenging
BHA	10	12.12
	50	46.21
	100	78.54
Test sample	10	09.62
	50	24.28
	100	45.62

Fig-1: Free radical scavenger activity



Free radicals are chemical entities that can exit separately with one or more unpaired electrons. The generation of free radical can bring about thousands of reactions and thus cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals.[23] Antioxidants may offer resistance against oxidative stress by scavenging the free radicals.

1,1-diphenyl-2-picryl hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds such as phinolic and anthocyanins or crude mixtures of plant extracts. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH.

The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wave length 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. DPPH is a relatively stable radical. The assay is based on the measurement of the scavenging ability of antioxidants toward the stable radical DPPH which reacts with suitable reducing agent. The electrons become paired off and solution loses color stochiometrically depending on the number of electrons taken up.[24] DPPH was used to determine the proton radical scavenging action of isolated compound AS-1, because it possesses a proton free radical and shown a characteristic absorbance at 517 nm. It showed excellent anti radical activity with comparable to that observed with synthetic BHA showed figure-1.

Our results indicate that isolated compound of leaves of Annona squamosa possesses free radical scavenging activity. Further studies are needed to better characterize the important active constituents responsible for the free radical scavenging activity.

ACKNOWLEDGEMENT:

We thank Dr. M. G. Purohit (Retired professor) Gulbarga University, Gulbarga. For providing constant support throughout study.

REFERENCES:

1. Wealth of India: A Dictionary of India raw material and Industrial products. Vol V. Publication and information directorate, CSIR, New Delhi, 1959.
2. Watt, G., Periodic Expert; Dictionary of the Economic product of India, 1972, vol.1, P.260.
3. Asholkar LV, Kakkar KK, Chakre OJ. Glossary of Indian Medicinal plants with active Principle, Publication and information Directorate, New Delhi, 1992, 72-73.
4. Yoganarasiman SN. Medicinal plants of India-Tamilnadu. Vol II, International Book Publisher, print, Cyper media, Bagalore, 2000, 48-62.
5. Vohora SB, Kumar I, Naqvi S. Phytochemical, Pharmacological, anti bacterial and anti Ovulatory studies on *Annona squamosa*. Planta medica, 28, 1975, 97-100.
6. Sunanda P, Anand K, Possible amelioration of hyper thyroidism by the leaf extract of *Annona squamosa* L. Current Science. 84, 2003, 1402-1404
7. Bhakuni DS, Dhar ML, Dhar MM, Dhavan BN, Mehrotra BB. Screening of Indian Medicinal Plants for biological activity; part-II. Indian J.Exp.Biol. 7, 1969, 250-262
8. Cheema PS, Dixit RS, Koshi T, Petri SL. Insecticidal properties of the seed oil of *Annona squamosa* L. J.Sci.Ind Res, 1985, 19, 1251-1252
9. Shirwaikar A, Rajendran K, Dinesh Kumar C. In vitro Antioxidant studies of *Annona Squamosa* L. leaves. Indian J.Exp.Boil. 42, 2004,803
10. Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G. Hypoglycemic and Antidiabetic effect of Ethanolic extract of leaves of *Annona squamosa* L. in experimental animals. J.Ethano Pharmacol. 99(1), 2005, 75-81
11. Kalleem M, Asif M, Ahmed QU, Bano B. Antidiabetic and antioxidant activity of *Annona squamosa* L. extract in streptozotocin- Induced diabetic rats. Singapore Med.J. 47(8), 2006,670-675
12. Mohamed Saleem TS, Christina AJM, Chidambaranathan N, Ravi V, Gauthaman K. Hepatoprotective activity of *Annona squamosa* on experimental animal model. 1(3), 2008, 1-7
13. Bhakuni DS, Tewari S, Dhar MM. Aporphine alkaloids of *Annona squamosa* L. Phytochemistry. 11, 1972, 1819-1822
14. Bhaumic PK, Mukhargee B, Juneau JP, Bhacca NS, Mukharjee. Alkaloid from leaves of *Annona squamosa* L. Phytochemistry. 18, 1979, 1584-1586
15. Seetharaman TR. Flavonoids from the leaves of Annona squamosa L. and *Polyathia longifolia* L.Fitoterapia. 57, 1986, 189-198
16. Forgas P, Desconclois JF, Provost R, Tiberghien et touché A.UnNovel Heteroside nitre extrait D. *Annona squamosa*. Phytochemistry. 19, 1980, 1251-1252.
17. Bohlmann F, and Nagabhushan R. Naturally occurring terpene derivatives; XXI, on the constituents of *Annona squamosa* L. Chem. Ber. 106, 1973, 841-844
18. Yang T H and Chi-Ming C. Structure of Squamoline, a novel diazepine from *Annona squamosa* L. J.Chi.Chem.Soc.(Taipei) 19, 1972,149-151
19. Harbarne, JB. In: Phytochemical methods, Ist Edn; Chapman and Hall London; 1973, 70
20. Kalsi, P.S In: Spectroscopy of organic compounds, 5th Edn; Newage publisher, Mumbai. 2002, 88
21. Masbry TJ, and Markham KP, In: The systematic Identification of flavonoids, Springer verlag, Berlin. 1970, 41
22. Singh RP, Murthy KNC, and Jayaprakasha GK. Studies on the antioxidant activity of Pomegranate (*Punica granatum*) peel and seed extracts using in vitro model.J.Agric. Food.Chem. 50, 2002, 81-86
23. Cotran R S, Kumar V and Collins T, in Robbin's pathological basis of disease, 6 th ed .Thomson press (I) Ltd. Noida, India 1999, 1.
24. Blois M S, Antioxidant determination by the use of stable free radicals, Nature, 26, 1958, 1199

Source of support: Nil, Conflict of interest: None Declared