World Journal of Science and Research. 7 (4): 01-06 (2022)

ISSN: 2455 2208

Available online at http://www.harmanpublications.com

World Journal of Science and Research

Research Article Biochemistry

IN VITRO ANTIOXIDANT PROPERTIES OF *Annona muricata* FRUIT PEELS EXTRACT

JJ. Vimala Suji and S. Velavan

Department of Biochemistry, Marudupandiyar College (Affiliated to Bharathidasan University), Thanjavur Tamil Nadu, India

Received on 20th Oct. 2022; Revised on 30th Nov. 2022 Online 17th Dec. 2022

ABSTRACT

In this study, the antioxidant activity of ethanol extracts of *Annona muricata* peel was evaluated by various antioxidant assays, including DPPH, superoxide anion scavenging, Fe2+ chelating and hydroxyl radical activities. The maximum inhibitory concentration (IC_{50}) in all methods viz., DPPH, superoxide, Fe2+ chelating and hydroxyl radical activity of *A. muricata*peel peels were found to be 55.72, 59.01, 60.46 and 61.27µg/ml respectively at different concentrations. The various antioxidant activities were compared to standard antioxidants such as ascorbic acid. This study indicates significant free radical scavenging potential of the *A. muricata*peel peels which can be exploited for the treatment of various free radical mediated ailments.

Keywords: *Annona muricata,* antioxidant activity, ascorbic acid

INTRODUCTION

Plants are sources of a wide range of therapeutic molecules and hence hold a great value for new drugs. Medicinal plants typically contain several different pharmacologically active compounds that may act individually, additively or in synergy to improve health (Azaizeh *et al.,* 2003; Gurib-Fakim, 2006). Plants are rich sources of important metabolites, which are potential sources of antioxidant activity (Daffodil *et al.,* 2012). The utilization of herbal medicine in treating infectious diseases have been practiced for 1000s of years and will continue to provide mankind with new remedies (Cragg and Newman, 2007). Earlier reports have demonstrated a distinct correlation between the higher intake of plant foods and lower risk of mortality from diseases (Chidambaram *et al.,* 2013). Recent interest has escalated in the finding antioxidant properties of natural origin plants due to their superior safety and consumer acceptability (Gorinstein *et al.*, 2003). Numerous *in vitro* assays are used to determine the antioxidant activity of biological samples. Comparing one assay with another is hard, and evaluating the antioxidant activity using a single antioxidant test method only is not possible because different methods measure antioxidant activity from different angles (Alam *et al.,* 2012; Pisoschi and Negulescu, 2011).

The oxidation by free radicals give rise to the disintegration of the cell membrane, DNA mutation and damage of membranous protein, also a number of diseases can initiate or propagate, like diabetes, liver injury, rheumatism, cardiovascular disorders and cancer (Liao and Yin, 2000). An antioxidant biomolecule can detain or interrupt the oxidation of molecules by oxidising themselves, so these are often called as reducing agents such as thiol, ascorbic acid, polyphenols etc. (Alam *et al.,* 2013). Thus, antioxidants may improve the quality of life by preventing the origin of degenerative diseases. Due to their beneficial effects, the investigations are focused on naturally occurring antioxidant molecules, especially

plant phytochemicals viz., phenolic compounds, flavonoids, carotenoids, benzoic acid derivatives, proanthocyanidins, coumarins, stilbenes and ligninsto replace the synthetic antioxidants compounds, which have various side effects. The medicinal plants contain several phytochemical constituents, which attributed to the antioxidant potential and prevent chronic disease progression (Sylvie *et al.,* 2014; Tamuly *et al.,* 2014). Hence the present study was carried out to investigate the antioxidant activity of the *Annona muricata* peel as a direction for further research.

MATERIALS AND METHODS

Collection of plant materials

The peel of *Annona muricata* were collected from Thanjavu, Tamil Nadu, India.

Preparation of hydro-alcoholic extract

10grams of *Annona muricata* peel powder were used for extraction. Extraction was performed with cold extraction using the maceration method into ethanol for 24 hours using the “intermittent shaking” method to obtain an extract. The extract was filtered using Whatman filter No 1 paper and filtrate was used for *in vitro* antioxidant assay.

In vitro antioxidant studies

DPPH radical-scavenging activity was determined by the method of Shimada *et al*. (1992). The superoxide anion radicals scavenging activity was measured by the method of Liu *et al.,* (1997). The chelating activity of the plant extract for ferrous ions Fe^{2+} was measured according to the method of Dinis *et al.,* (1994). The scavenging activity for hydroxyl radicals was measured with Fenton reaction by the method of Yu *et al.* (2004).

Statistical analysis

Tests were carried out in triplicate for 3 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50% (IC_{50}), was graphically determined by a linear regression method using Ms-Windows based graphpad Instat (version 3) software. Results were expressed as graphically/mean±standard deviation.

RESULTS AND DISCUSSION

Antioxidant compounds are known for their capabilities to prevent or delay the oxidation of other molecules. They inhibit the free radical chain reactions by donating an electron without becoming themselves free radicals, and nowadays, it becomes a vital part of our body. In the present study, antioxidant activity of the *Annona muricata* peel has been assessing. The results revealed the antioxidant potential of the ethanol extract of the peel.

DPPH radical scavenging activity

The antioxidant principle in their interaction depends on oxidative free radicals. The main mechanism of DPPH method is that the bioactive compounds react with the stable free radical i.e., a, a-diphenyl-β-picrylhydrazyl. It is converted to a,a-diphenyl-β-picrylhydrazine with color change. The gradually color change indicates the scavenging activities of the plant crude sample due to bioactive compounds such as phenolic compounds, flavonoids, terpenoids and derivatives (Daffodil *et al.,* 2012; Nishanthini *et al.,* 2012). DPPH radical scavenging activity of *Annona muricata* and compared with ascorbic acid. The *Annona muricata* and ascorbic acid showed the minimum DPPH inhibitory activity in 20µg/ml concentration range (19.78% and 22.61%) while maximum inhibitory activity in100µg/ml concentration range (88.69and 93.63%). The half inhibition concentration (IC_{50}) of *Annona muricata* and ascorbic acid were 55.72µg/ml and 49.29µg/ml respectively. *Annona muricata* has potential DPPH activity and near to standard. Their antioxidant activity is affected by phenolic compound on DPPH which able to donate hydrogen atoms to form stable (Sathishkumar *et al.,* 2009).

Table 1: DPPH radical scavenging activity

Concentrations (µg/mL)	% of inhibitions	
	Annona muricata	Std. (Ascorbic acid)
20	19.78±0.13	22.61±0.18
40	34.27±0.27	41.69±0.33
60	55.83±0.35	61.48±0.47
80	69.96±0.59	78.44±0.68
100	88.69±0.66	93.63±0.81
IC_{50} (µg/mL)	55.72	49.29

Values were expressed as mean ± Standard deviation for triplicates; IC: Inhibitions concentration

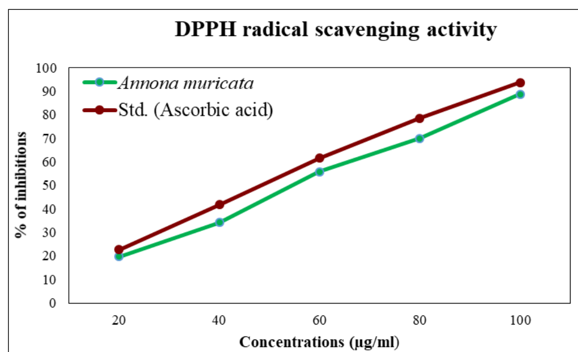

Figure 1: DPPH radical scavenging activity

Superoxide anion scavenging activity

Superoxide anion is also another harmful reactive oxygen species as it damages cellular components in biological systems. This species is produced by a number of enzyme systems in auto-oxidation reactions and by non-enzymatic electron transfers that univalently reduce molecular oxygen. It can also reduce certain iron complexes such as cytochromes. Superoxide anion radical scavenging activity of *Annona muricata* and compared with ascorbic acid. The *Annona muricata* and ascorbic acid showed the minimum superoxide anion scavenging inhibitory activity in 20µg/ml concentration range (17.39% and 21.73%) while maximum inhibitory activity in100µg/ml concentration range (84.05% and 91.30%). The half inhibition concentration (IC_{50}) of *Annona muricata* and ascorbic acid were 59.01µg/ml and 51.84µg/ml respectively. *Annona muricata* has potential superoxide anion scavenging activity and near to standard.

Table 2: Superoxide anion scavenging activity

Concentrations (µg/mL)	% of inhibitions	
	Annona muricata	**Std. (Ascorbic acid)**
20	17.39±0.15	21.73±0.20
40	35.26±0.27	40.09±0.34
60	49.27±0.38	57.48±0.52
80	68.11±0.55	74.87±0.71
100	84.05±0.76	91.30±0.95
IC_{50} (µg/mL)	59.01	51.84

Values were expressed as mean ± Standard deviation for triplicates;
IC: Inhibitions concentration

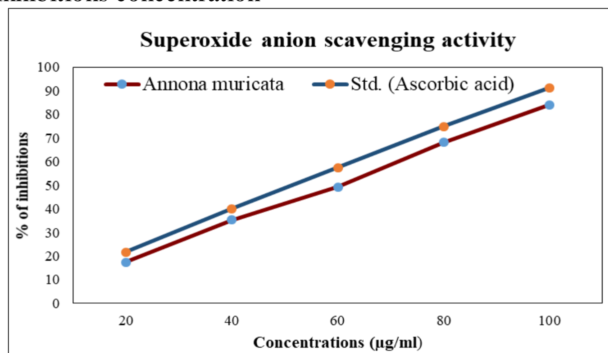

Figure 2: Superoxide anion scavenging activity

Fe2+ chelating activity

In the measurement of the reducing ability, it has been investigated from the Fe^{3+}-Fe^{2+} transformation. Fe^{3+} reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action and can be strongly correlated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating hydrogen atom (Oyedemi and Afolayan, 2012; Shajeesh *et al.,* 2011). Fe^{2+} chelating activity of *Annona muricata* and compared with ascorbic acid. The *Annona muricata* and ascorbic acid showed the minimum Fe^{2+} chelating inhibitory activity in 20µg/ml concentration range (16.83% and 20.61%) while maximum inhibitory activity in100µg/ml concentration range (82.47 and 92.09%). The half inhibition concentration

(IC_{50}) of *Annona muricata* and ascorbic acid were 60.46µg/ml and 52.39µg/ml respectively. *Annona muricata* has potential Fe^{2+} chelating activity and near to standard.

Table 3: Fe^{2+} chelating activity

Concentrations (µg/mL)	% of inhibitions	
	Annona muricata	**Std. (Ascorbic acid)**
20	16.83±0.18	20.61±0.22
40	30.92±0.28	38.48±0.31
60	51.54±0.42	57.73±0.56
80	66.32±0.71	75.25±0.84
100	82.47±0.93	92.09±1.02
IC_{50} (µg/mL)	60.46	52.39

Values were expressed as mean ± Standard deviation for triplicates;
IC: Inhibitions concentration

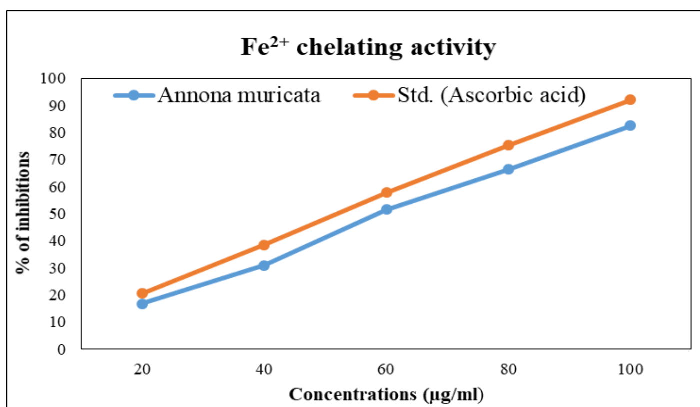

Figure 3: Fe^{2+} chelating activity

Hydroxyl radical scavenging activity

Hydroxyl radical is an extremely reactive species formed in biological systems. It is capable of damaging almost every molecule found in living cells. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition, hydroxyl radical is considered to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen

Table 4: Hydroxyl radical scavenging activity

Concentrations (µg/mL)	% of inhibitions	
	Annona muricata	**Std. (Ascorbic acid)**
20	18.70±0.15	23.74±0.21
40	32.37±0.34	39.56±0.33
60	45.68±0.54	55.75±0.67
80	68.34±0.72	73.02±0.79
100	79.85±0.88	90.28±0.96
IC_{50} (µg/mL)	61.27	52.23

Values were expressed as mean ± Standard deviation for triplicates;
IC: Inhibitions concentration

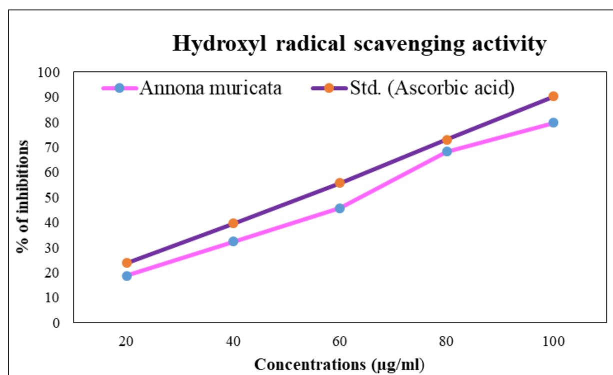

Figure 4: Hydroxyl radical scavenging activity

atoms from unsaturated fatty acids (Murugan and Mohan,2012). Hydroxyl radical scavenging activity of *Annona muricata* and compared with ascorbic acid. The *Annona muricata* and ascorbic acid showed the minimum hydroxyl radical scavenging inhibitory activity in 20µg/ml concentration range (18.70% and 23.74%) while maximum inhibitory activity in100µg/ml concentration range (79.85 and 90.28%). The half inhibition concentration (IC_{50}) of *Annona muricata* and ascorbic acid were 61.27µg/ml and 52.23µg/ml respectively. *Annona muricata* has potential hydroxyl radical scavenging activity and near to standard.

Some of the secondary metabolites from a single or combined with others in plant extracts are liable for the antioxidant activity (Molan *et al.,* 2009). Some phytochemicals have antioxidant activity where it provides protection against damage and the risk of developing chronic disease can be substantially reduced (Dai and Mumper, 2010; Velavan, 2011, 2015). The present study compared the antioxidant potential of *Annona muricata* and ascorbic acid of the different concentrations against different free radicals. The antioxidant activity estimated by inhibition concentration (IC_{50}); the *Annona muricata* displayed better antioxidant activity in the study. The values of the four different assays used to measure the antioxidant activity of *Annona muricata* peel. These differences are attributed to the varying reaction mechanisms of the assays. Moreover, the antioxidants from extracts have different abilities to mitigate peroxyl radicals and to reduce the DPPH free radical and ferric ion (Huang *et al.*, 2005; Thaipong *et al.,* 2006).

CONCLUSION

Over all it can be concluded that *Annona muricata* peel contains potential antioxidant activity confirmed through various *in vitro* method. The antioxidant activity was directly proportional to the concentration of peel extract. Therefore, further studies should be carried out to active principles having antioxidant property.

REFERENCES

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi pharmaceutical journal*, *21*(2), 143-152.

Alam, M. N., Wahed, T. B., Sultana, F., Ahmed, J., & Hasan, M. (2012). In vitro antioxidant potential of the methanolic extract of *Bacopa monnieri* L. *Turk J Pharm Sci*, *9*(3), 285-292.

Azaizeh, H., Fulder, S., Khalil, K., & Said, O. (2003). Ethnobotanical knowledge of local Arab practitioners in the Middle Eastern region. *Fitoterapia*, *74*(1-2), 98-108.

Chidambaram, U., Pachamuthu, V., Natarajan, S., Elango, B., & Ramkumar, K. M. (2013). *In vitro* evaluation of free radical scavenging activity of *Codariocalyx motorius* root extract. *Asian Pacific journal of tropical medicine*, *6*(3), 188-194.

Cragg, G. M., & Newman, D. J. (2007). Drug from nature: past achievement, future prospect. *Adv. Phytomed.* 1, 23–37.

Daffodil, E. D., Rajalakshmi, K., & Mohan, V. R. (2012). Estimates total phenolic, flavonoid content and in vitro antioxidant activity of root of *Suaeda monaica* Forssk ex Gmel (Chenopodiaceae). *Elixir Applied Botany*, *53*, 11885-11889.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10), 7313-7352.

Dinis, T. C., Madeira, V. M., & Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of biochemistry and biophysics*, *315*(1), 161-169.

Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular aspects of Medicine*, *27*(1), 1-93.

Huang, D., Ou, B. & Prior, R. L. (2005) The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* 53, 1841–1856.

Liao, K. L., & Yin, M. C. (2000). Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems: importance of the partition coefficient. *Journal of agricultural and food chemistry*, *48*(6), 2266-2270.

Liu, F., Ooi, V. E. C., & Chang, S. T. (1997). Free radical scavenging activities of mushroom polysaccharide extracts. *Life sciences*, *60*(10), 763-771.

Molan, A. L., Flanagan, J., Wei, W., & Moughan, P. J. (2009). Selenium-containing green tea has higher antioxidant and prebiotic activities than regular green tea. *Food Chemistry*, *114*(3), 829-835.

Murugan, M., & Mohan, V. R. (2012). *In vitro* antioxidant studies of *Dioscorea esculenta* (Lour). Burkill. *Asian Pacific Journal of Tropical Biomedicine*, *2*(3), S1620-S1624.

Nishanthini, A., Ruba, A. A., & Mohan, V. R. (2012). Total phenolic, flavonoid contents and in vitro antioxidant activity of leaf of *Suaeda monoica Forssk* ex. Gmel (Chenopodiaceae). *International Journal of Advanced Life Sciences (IJALS)*, *1*(5), 34-43.

Oyedemi, S. O., & Afolayan, A. J. (2012). Antibacterial and antioxidant activities of hydroalcoholic whole plant bark extract of *Schotia latifolia* Jacq. *Asian Pac. J. Trop Biomed*, 4, 952-958 .

Pisoschi, A. M., & Negulescu, G. P. (2011). Methods for total antioxidant activity determination: a review. *Biochem Anal Biochem*, *1*(1), 106.

Sajeesh, T., Arunachalam, K., & Parimelazhagan, T. (2011). Antioxidant and antipyretic studies on *Pothos scandens* L. *Asian Pacific Journal of Tropical Medicine*, *4*(11), 889-899.

Sathishkumar, R., Lakshmi, P. T. V., Annamala, A (2009). *J. Med. Plants Res*, *3*(3), 93-101.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthum on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*, 40,945–948.

Sylvie, D. D., Anatole, P. C., Cabral, B. P., & Veronique, P. B. (2014). Comparison of in vitro antioxidant properties of extracts from three plants used for medical purpose in Cameroon: *Acalypha racemosa, Garcinia lucida* and *Hymenocardia lyrata*. *Asian Pacific Journal of Tropical Biomedicine*, *4*, S625-S632.

Tamuly, C., Hazarika, M., Bora, J., & Gajurel, P. R. (2014). Antioxidant Activities and Phenolic Content of *Piper wallichii* (Miq.) Hand.-Mazz. *International Journal of Food Properties*, *17*(2), 309-320.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. & Byrne, D. H. (2006) Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Compos. Anal.* 19, 669–675.

Yu, F., Yu, F., Li, R., & Wang, R. (2004). Inhibitory effects of the *Gentiana macrophylla* (Gentianaceae) extract on rheumatoid arthritis of rats. *Journal of ethnopharmacology*, *95*(1), 77-81.

Velavan S. (2015) Phytochemical Techniques - A Review. World Journal of Science and Research. 1(2): 80-91.

Velavan Sivanandham. (2011) Free radicals in Health and Diseases -A Mini Review. Pharmacologyonline Newsletter: 1062-1077.