The current issue and full text archive of this journal is available at
www.emeraldinsight.com/0034-6659.htm



# Antioxidant activity of seed extracts of *Annona squamosa* and *Carica papaya*

Vijay Kothari and Sriram Seshadri
*Institute of Science, Nirma University, Ahmedabad, India*

## Abstract

**Purpose** – The purpose of this paper is to investigate various extracts of *Annona squamosa* L (Annonanceae) and *Carica papaya* L (Caricaceae) seeds for their antioxidant activity, free radical scavenging ability, total phenolic and flavonoid contents.

**Design/methodology/approach** – Samples from both the seeds were prepared by subjecting them to microwave-assisted extraction. After determining their antioxidant properties and polyphenolic contents, correlation between them was also investigated.

**Findings** – Highest antioxidant activity (3,179.66 g gallic acid equivalent/g of dry extract) and phenol content were registered by chloroform:methanol extract of *C. papaya* seeds. Maximum radical scavenging activity (3,201.63 ascorbic acid equivalent antioxidant capacity g/100 g of dry extract) was exerted by water extract of *A. squamosa* seeds, whereas acetone extract of *C. papaya* registered highest flavonoid content among all extracts. Polar extracts were found to be better free radical scavengers compared with those less polar. Hexane extracts showed least DPPH radical scavenging activity. Acetone proved efficient in extracting flavonoids, whereas phenols were best extracted in a mixture of chloroform and methanol. Phenolic metabolites seem to be contributing significantly towards antioxidant activity of the *C. papaya* extracts, but less so in the case of *A. squamosa*.

**Originality/value** – There have been few reports on antioxidant activity of non-edible parts of commonly consumed fruits. The research indicates that seeds may be a promising source of antioxidants, which may have therapeutic implications.

**Keywords** Plants, Fruits

**Paper type** Research paper

## Introduction

Reactive oxygen species (ROS) are $O_2$ free radicals and play dual role of being both deleterious and beneficial to biological systems (Rudolf, 2001). Apart from their role in the diseased conditions in the body, ROS are also known to have a role in the spoilage of food by the autoxidation of lipids, the enzymatic oxidation, during storage and processing in fats, oils and fat-containing foods (Matthaus, 2002). Antioxidants are defined as substances, present at low concentration relative to the oxidizable substrate, which significantly delay or prevent oxidation of substrate (Godfraind, 2005). Human body does not synthesize overwhelming amount of antioxidants to compensate with the damaging effects of ROS (Halliwell, 1996). Although synthetic antioxidants such as butylated hydroxy toluene, butylated hydroxy anisole, gallic acid esters and tertiary butylated hydroquinone have potential to neutralize free radicals, they have been criticized due to possible toxic effects, low solubility along with moderate antioxidant activity. Hence there arises a need to discover new potential natural sources of antioxidants.

Several studies have successfully correlated the phenolic content with antioxidant activity. For example, natural phytochemicals present in berry crops, tea, oilseeds, beans, fruits and vegetables, herbs and spices such as rosemary, thyme, sage, nutmeg, turmeric, white pepper, chili pepper ginger and several medicinal plants are good

The authors thank Nirma Education and Research Foundation (NERF) for financial assistance, and Prof. Y.T. Jasrai (Botany Dept, Gujarat University) for seed authentication.

Nutrition & Food Science
Vol. 40 No. 4, 2010
pp. 403-408

0034-6659
DOI 10.1108/00346651011062050

source of antioxidant activity (Emami, 2007) and this antioxidant activity have been correlated to the plant secondary metabolites such as flavonoids, carotenoids, alkaloids, tannins and phenolic compounds.

Many fruits have inedible seeds and are not part of human diet. However, such seeds are a part of ayurvedic preparations against many diseases. Further, since seeds are the primary stage of plant life cycle, they have strong defence mechanism possibly due to the presence of phytoconstituents contributing to antioxidant activity. In the present study, the seeds of *Annona squamosa* L (Annonaceae; commonly known as custard apple) and that of *Carica papaya* L (Caricaceae) were evaluated for their antioxidant activity. The relationship of antioxidant activity with total phenolic and flavonoid content was also studied. Pulp of *C. papaya* is already known to have antioxidant properties (Fischer, 1998).

## Materials and methods

### *Materials*

Solvents (Merck): methanol, hexane, acetone, chloroform and ethanol. Molybdate assay: sodium phosphate (SRL, Mumbai, India), ammonium molybdate (S-d fine chemicals, Mumbai), $H_2SO_4$ (Merck). DPPH free radical scavenging assay: 2,2-diphenyl-1-picryl hydrazyl (DPPH, Himedia). Total phenolic content: sodium carbonate (S-d fine chemicals), Folin-Ciocalteu reagent (SRL). Total flavonoid content: ammonium chloride (Merck), potassium acetate (Merck), quercetin (S-d fine chemicals).

### *Extract preparation*

Seeds of both the plants were procured from local market of Ahmedabad city. They were authenticated for their unambiguous identity by Prof. Y.T. Jasrai, Head of Botany Department, Gujarat University, Ahmedabad. Seed extracts were prepared by microwave-assisted extraction technique (Kothari *et al.*, 2009). One gram of seed powder was added to 50 ml of respective solvent and pre-leached for 1 min. Five different solvents used were hexane, acetone, chloroform:methanol (2:1), ethanol (50 percent) and water. The extracts obtained after microwave heating (at 720 W, for 300, 120, 50, 70 and 180 s in hexane, acetone, chloroform:methanol, ethanol and water, respectively, with intermittent cooling for avoiding overheating) were cooled, centrifuged at 10,000 rpm for 15 min and filtered through Whatman #1 filter paper (Whatman International Ltd., England). The supernatants were evaporated at the respective boiling points of the solvents. Dried extracts were then reconstituted in respective solvents, and stored in a refrigerator.

## Antioxidant assays

### *Total antioxidant activity by molybdate assay*

The assay is based on the reduction of Mo (VI) to Mo (V) by the sample and subsequent formation of a green phosphate/Mo (V) complex at acid pH (Pilar *et al.*, 1999). The tubes containing extract and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of each tube was measured at 695 nm. The standard curve was prepared by using the known concentrations of gallic acid. The antioxidant capacity of extracts was expressed in terms of g of gallic acid equivalent (GAE)/g of dry extract.

### *DPPH radical-scavenging activity*

The capacity of the extracts to scavenge the stable DPPH free radical was measured (Duan *et al.*, 2007). A volume of 0.1 ml of extract was mixed with 2.9 ml of 0.1 mM DPPH

solution. The blank contained 0.1 ml of extract and 2.9 ml of DPPH solution. Negative control was prepared by mixing 0.1 ml of methanol with 2.9 ml of DPPH solution. The radical scavenging activity was calculated in terms of ascorbic acid equivalent antioxidant capacity (AEAC) by using the following formula (Lim *et al.*, 2007):

$$\text{AEAC} = A_{\text{control}} - A_{\text{sample}}/A_{\text{control}} - A_{\text{ascorbic acid}} \times \text{concentration of ascorbic acid} \times (\text{mg/ml}) \times \text{volume} \times 100/\text{g of sample}$$

where $A$ is Absorbance.

*Estimation of total phenolic content*

Folin-Ciocalteu method was used to determine the total phenolic content of the sample as described by Singleton and Rossi (1965). In all, 0.2 ml 10 percent v/v Folin-Ciocalteu reagent was added to the 0.1 ml of the sample, and was vortexed for 5 min, followed by addition of 0.8 ml of sodium carbonate. This reaction mixture was incubated for 2 h at room temperature. The absorbance was measured at 765 nm. The calibration curve was prepared by employing gallic acid at concentrations of 0.4 to 1.6 mM.

*Estimation of total flavonoid content*

Aluminum chloride colorimetric method was used for flavonoids determination (Chang *et al.*, 2002). 0.5 ml of each plant extract was separately mixed with 1.5 ml of methanol, 0.1 ml of 10 percent aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The reaction mixture was allowed to stand at room temperature for 30 min and the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by using quercetin at concentrations of 12.5 to 100 μg/ml in methanol.

*Statistical analysis*

Statistical significance between experimental results was evaluated with a Mann-Whitney $U$ test. $P$ values less than 0.05 were considered to be statistically significant.

## Results and discussion

Results of different assays performed for all the extracts are presented in Table I.

*Total antioxidant activity*

Different extracts showed the total antioxidant activity in the wide range of 107 to 3179.66 g GAE/g of dry extract. Highest total antioxidant activity was exerted by the chloroform-methanol extract of *C. papaya* seeds, and lowest by its acetone extract. Higher activity in polar extracts suggests that polar phytochemicals are largely contributing to the total antioxidant activity of the seeds. Ascorbic acid (0.1 mM) used as a positive control registered an activity of 53 g GAE/g.

*DPPH radical scavenging assay*

Extracts showed radical scavenging activity in the range of 61.19 to 3201.63 AEAC g/100 g of dry extract. Highest and lowest activity was exerted, respectively, in water and hexane extract of *A. squamosa* seeds (Table I). Extracts prepared in polar solvents are better free radical scavengers than those prepared in less polar solvents as evident from the fact that hexane extracts of both seeds exhibited least DPPH scavenging ability, whereas water extracts exhibited the highest. The potency of the extracts in scavenging the radicals is due to the number of hydrogens available for donation by the hydroxyl groups (Chen and Ho, 1995). Higher activity of the aqueous extracts may be due to the

**Table I.**
Results of different assays of *A. squamosa* and *C. papaya* seed extracts[a]

| Seed | Extract prepared in | Total antioxidant activity (g GAE/g of dry extract) | DPPH radical scavenging capacity (g AEAC/100 g of dry extract) | Total phenolic content (g GAE/g of extract) | Total flavonoid content (mg QE/g of dry extract) |
|---|---|---|---|---|---|
| *A. squamosa* | Hexane | 268.75 ± 2.32 | 61.19 ± 0.19 | 24.45 ± 1.32 | 9.86 ± 0.22 |
| | Acetone | 229.29 ± 2.21 | 582.82 ± 4.27 | 29.95 ± 3.11 | 32.66 ± 8.13 |
| | Chlorofom-methanol | 203.81 ± 9.31 | 647.42 ± 12.02 | 242.82 ± 5.08 | 23.15 ± 0.33 |
| | Ethanol | 427.14 ± 2.87 | 1,925.91 ± 23.02 | 171.58 ± 7.31 | 42.44 ± 1.13 |
| | Water | 777.64 ± 15.05 | 3,201.63 ± 27.31 | 208.70 ± 2.09 | 5.72 ± 0.38 |
| *C. papaya* | Hexane | 470.35 ± 28.2 | 123.40 ± 9.02 | 156.53 ± 13.09 | 39.37 ± 0.2 |
| | Acetone | 107 ± 9.07 | 782.49 ± 22 | 23.51 ± 0.15 | 77 ± 2.4 |
| | Chloroform-methanol | 3,179.66 ± 18.8 | 827.43 ± 13.4 | 528.29 ± 8 | 22.51 ± 3.5 |
| | Ethanol | 912.77 ± 67.5 | 156.31 ± 15.6 | 307.32 ± 3 | 10.20 ± 0.3 |
| | Water | 1,371.54 ± 11 | 1,681.99 ± 26.2 | 211.31 ± 11.3 | 4.42 ± 0.4 |

**Notes:** [a]Each value in the table was obtained by calculating the average of three experiments ± standard deviation; GAE: gallic acid equivalent; AEAC: ascorbic acid equivalent antioxidant capacity; QE: quercetin equivalent

presence of high hydroxyl groups, which is proportionate to their phenolic content. Free radicals act as a trigger to a number of degenerative diseases, therefore samples having free radical scavenging activity can be of potent medicinal importance.

*Total phenolic content*

Plant phenolics present in fruits and vegetables have received considerable attention because of their potential antioxidant activity. Total phenolic content of different extracts was recorded in the range of 23.51 to 528.29 g GAE/g of dry extract. The highest total phenolic content appeared to be present in chloroform-methanol extracts. Acetone appears to be poor in extracting phenolics.

*Total flavonoid content*

The flavonoid content as analyzed by the aluminum chloride colourimetric method was found to fall in the range of 5.72 to 77 mg quercetin equivalent/g of dry extract. Acetone extract of *C. papaya* seeds showed maximum flavonoid content, followed by ethanol extract of *A. squamosa*. Water extracted least amount of flavonoids, as opposed to notable amount of phenols extracted in it. Acetone appeared best for flavonoid extraction. Suitability of acetone for extraction of flavonols has already been reported by Afolayan and Meyer (1997).

*Correlation of the total phenolic and total flavonoid content with the antioxidant capacity*

Neither flavonoid nor phenolic content of the seed extracts of *A. squamosa* was found to have linear correlation with the total antioxidant capacity. In case of *C. papaya* total phenolic content of the seed extracts was found to have a positive linear correlation with the total antioxidant activity, the correlation coefficient being 0.88 (Figure 1). Chloroform-methanol extract of *C. papaya* seeds was found to be the most active antioxidant as well as with highest phenolic content indicating contribution of phenolic phytoconstituents towards antioxidant activity. In neither of the seeds, flavonoid content of the seed extracts was found to have a significant linear correlation with the total antioxidant activity. Thus majority of the phytochemicals responsible for antioxidant activity of the *C. papaya* extracts seem to belong to phenol group (possibly other than flavonoids) of plant metabolites.

Finally, this study has identified chloroform:methanol extract of *C. papaya* seeds to possess high antioxidant activity (attributable to its high phenolic content), and water extract of *A. squamosa* seeds to possess high radical scavenging activity. The presence of antioxidant activity in extracts prepared in all the different solvents (which differ widely in their polarity) indicate that phytochemicals contributing to the antioxidant activity of the seeds tested belong to different groups of plant metabolites and vary

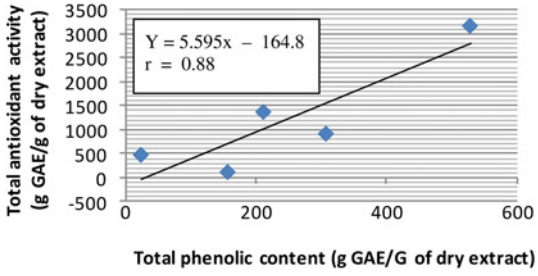


**Figure 1.**
Correlation of the total antioxidant activity with total phenolic content of *C. papaya* seed extracts

widely with respect to their chemical properties. Further investigation for isolation and identification of the phytoconstituents responsible for antioxidant activity is desirable. Such findings can contribute to the increasing database of the medicinal plants and may be of importance in varietal improvement, food preservatives, nutraceuticals, cosmetics and biopharmaceuticals in a race with the degenerative diseases like cancer, cardiovascular diseases and neurodegenerative diseases.

## References

Afolayan, A.J. and Meyer, J.J. (1997), "The antimicrobial activity of 3,5,7 trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*", *Journal of Ethnopharmacology*, Vol. 57, pp. 177-81.

Chang, C., Yang, M., Wen, H. and Chern, J. (2002), "Estimation of total flavonoid content in propolis by two complementary colorimetric methods", *Journal of Food and Drug Analysis*, Vol. 10, pp. 178-82.

Chen, C.W. and Ho, C.T. (1995), "Antioxidant properties of polyphenols extracted from green and black tea", *Journal of Food Lipids*, Vol. 2, pp. 35-46.

Duan, X., Wu, G. and Jiang, Y. (2007), "Evaluation of the antioxidant properties of litchi fruit phenolics in relation to pericarp browning prevention", *Molecules*, Vol. 12, pp. 759-71.

Emami, S. (2007), "Antioxidant activity of leaves and fruits of Iranian conifers", *eCAM*, Vol. 4 No. 3, pp. 313-19.

Fischer, N. (1998), "Flavour components in selected exotic fruits", *Food Australia*, Vol. 50 No. 4, pp. 165-8.

Godfraind, T.O. (2005), "Antioxidant effects and the therapeutic mode of action of calcium channel blockers in hypertension and atherosclerosis", *Philosophical Transactions of the Royal Society B*, Vol. 360, pp. 2259-72.

Halliwell, B. (1996), "Antioxidants in human health and disease", *Annual Reviews of Nutrition*, Vol. 16, pp. 33-50.

Kothari, V.O., Punjabi, A.B. and Gupta, S.R. (2009), "Optimization of microwave-assisted extraction of *Annona squamosa* seeds", *The ICFAI University Journal of Life Sciences*, Vol. 3 No. 1, pp. 55-60.

Lim, Y.Y., Lim, T.T. and Tee, J.J. (2007), "Antioxidant properties of several tropical fruits: a comparative study", *Food Chemistry*, Vol. 103, pp. 1003-8.

Matthaus, B. (2002), "Antioxidant activity of extracts obtained from residues of different oilseeds", *Journal of Agriculture and Food Chemistry*, Vol. 50, pp. 3444-52.

Pilar, P., Manuel, M. and Miguel, A. (1999), "Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E", *Analytical Biochemistry*, Vol. 269, pp. 337-41.

Rudolf, S. (2001), "The benefits and hazards of antioxidants: controlling apoptosis and other protective mechanisms in cancer patients and the human population", *Journal of American College of Nutrition*, Vol. 20, pp. 464-72.

Singleton, V. and Rossi, J. (1965), "Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents", *American Journal of Enology and Viticulture*, Vol. 16, pp. 144-58.

## Corresponding author

Sriram Seshadri can be contacted at: sriramsjpr@gmail.com; sriram.seshadri@nirmauni.ac.in