Journal of Bioresource Management

Volume 2 | Issue 3 Article 6

A Study of DNA Protective Ability of Peels of Different Citrus Species

Nehrish Nisar
Institute of Natural and Management Sciences, Rawalpindi, Pakistan

Kainaat William
Bioresource Research Centre, Islamabad, Pakistan

Azra Khanum
Institute of Natural and Management Sciences, Rawalpindi, Pakistan

Recommended Citation

Nisar, N., William, K., & Khanum, A. (2015). A Study of DNA Protective Ability of Peels of Different Citrus Species, *Journal of Bioresource Management, 2* (3).
DOI: 10.35691/JBM.5102.0031
ISSN: 2309-3854 online

A Study of DNA Protective Ability of Peels of Different Citrus Species

J. Bioresource Manage. (2015) 2(3): 48-52.

A STUDY ON DNA PROTECTIVE ABILITY OF PEELS OF DIFFERENT CITRUS SPECIES

Nehrish Nisar[1], Kainaat William[2], Azra Khanum[1]

[1]Institute of Natural and Management Sciences (INAM), Rawalapindi, Pakistan.
[2]Bioresource Research Centre (BRC), Islamabad, Pakistan.
Email:

ABSTRACT

The aim of the present study was to assess the DNA protection ability, free radicals scavenging activity, and phytochemical constituents of peel extracts of various species of citrus. Results showed the presence of carbohydrates, amino acids, glycosides, tannins, steroids and alkaloids. DNA protection assay showed maximum protection with methanolic extracts of peels of *C. limon*, *C. reticulata*, *C. aurantium* and *C. sinensis* as compared to *C. limetta* which showed less protection. DPPH assay used to assess the antioxidants showed the highest activity in the methanolic extract of peels of *C. reticulata* and *C. aurantium* with 75% and 86% respectively at 10 mg/ml. It is therefore concluded that *C. limon*, *C. reticulata*, *C. aurantium* and *C. sinensis* in terms of DNA protection and *C. reticulata* and *C. aurantium* in terms of free radicals scavenging activity are the best ones and these are the potential candidates to be used for further studies.

Keywords: DNA protection assay, phytochemicals, DPPH assay, Free radicals, ROS.

INTRODUCTION

Nature has provided each cell with protective mechanisms against all kinds of effects caused by free radicals, which help in curing many degenerative and chronic diseases such as heart disease, diabetes mellitus, cancer, Alzheimer and aging (Lele *et al*., 2004). Intake of antioxidants by eating fruits and vegetables in the diet should protect cells against the reactive oxygen species and also help in decreasing the DNA damage caused by oxidation (Moller *et al.,* 2003). Various plant phytochemicals such as tannins, phenolic compounds and flavonoids are reported to have important antioxidant activity against the wide variation of free radicals (Koleckar *et al*., 2008; Kirmizibekmez *et al*., 2009; Choudhary & Swarnkar, 2011).

Oranges are a member of Rutaceae, which is commonly known as the rue or citrus family. In recent research studies, it has been proved that the phytonutrients compound of citrus has healing properties. Perez *et al*. (2010) studies showed *Citrus limetta* has healing effects in antihypertensive treatment. Kefford *et al*. (1960) found that citrus fruit consists of soluble solids content, ascorbic contents and acidity. The screening activity of citrus fruits identified the composition as carbohydrates in citrus juice, in citrus peels and pulps, ascorbic acid, vitamins, etc. Citrus is used for curing various illnesses, and reducing vomiting or diarrhea. So, the main objective of the current study is to determine the phytochemical constituents, DNA protective ability and scavenging activity of peel extracts of various citrus species.

MATERIAL AND METHODS

The fresh oranges of different varieties (*Citrus limetta, Citrus aurantium, Citrus sinensis, Citrus reticulata* and *Citrus limon)* were bought from a local market and their peels were air dried under shade. The peels were ground into fine powdered. Crude methanolic extracts (CME) of each plant were prepared by dissolving 5g of powdered sample into 50ml of methanol. Then, it was kept at room temperature by shaking it after every 2 hours for one day. Thereafter, it was filtered using Whattmann filter paper No. 1. The crude methanolic extracts were used for the phytochemical analysis by using procedures described by Harborne, 1973 and Sofowora, 1993. DNA protection assay was conducted by following the protocol described by Tian and Hua (2005). The antioxidant activity of all six samples was done by following the protocol described by Kulisic *et al*. (2004).

RESULTS AND DISCUSSION

For the estimation of antioxidant and DNA protection ability of different species of citrus: phytochemical screening, plasmid DNA protection assay and DPPH free radicals scavenging assay was performed in this study. Qualitative analysis of phytochemicals present in peels extract of citrus species is summarized in Table 1.

Table 1: Phytochemicals present in crude methanolic extract of different Citrus species.

	C. limon	***C. limetta***	***C. aurantium***	***C. reticulata***	***C. sinensis***
Carbohydrates	+	+	+	+	+
Amino acids	+	+	+	+	+
Tannins	+	+	+	+	+
Glycosides	+	+	+	+	+
Steroids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Phlobatannins	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Saponins	-	-	-	-	-

Positive sign (+) indicated presence of compound and negative sign (-) indicated absence of compound.

Lalitha *et al*. (2009) also evaluated preliminary phytochemical screening of *Citrus limon* and confirmed the presence of cardiac glycosides and steroids, which is similar to our results. Further, a study of phytochemical screening conducted by Kumar *et al*. (2011) showed the presences of flavonoids, saponins, steroids, terpenoids, tannins and alkaloids. This study partially supports our study with the presence of steroids, tannins and alkaloids while saponins were absent in the peel extracts. This difference may be due to the difference of fruits used.

DNA protective ability of the methanolic extract of Citrus species was determined by using a DNA protection assay, which is based on photolysing H_2O_2 and $FeSO_4$. In this assay, plasmid pBR322 was used with four dilutions, i.e. 1000 ppm, 100 ppm,

10 ppm and 1 ppm of different samples. After one hour of incubation, samples were loaded, along with the positive and negative controls, on agarose gel electrophoresis. Normal pBR322 of the control series showed 2 bands in agarose gel electrophoresis. The faster moving band represented the native form of supercoiled circular DNA (scDNA) and the slower moving band corresponded to the open circular form (ocDNA). The obtained results are given in Figure 1.

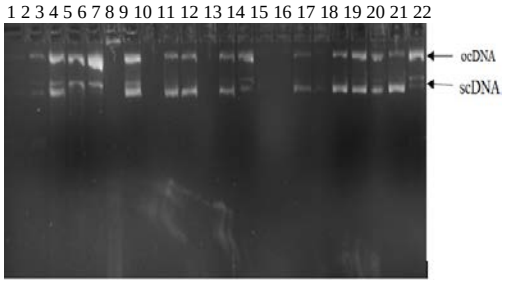

Figure 1: Plasmid DNA Protection Assay. Protection assay mixture contained 3 µL of 2mMFeSo$_4$, 4 µl of H_2O_2, 5 µl of PBS, 3µl of Plasmid with different concentrations of methanolic extract of various citrus species. Methanolic extract of *C. limon* having 1000 ppm, 100 ppm, 10 ppm and 1 ppm in Lanes 1-4, Methanolic extract of *C. reticulata* having 1000 ppm, 100 ppm, 10 ppm and 1 ppm in Lanes 5-8, Methanolic extract of *C. aurantium* having 1000 ppm, 100 ppm, 10 ppm and 1 ppm in Lanes 9-12,Methanolic extract of *C. limetta* having 1000 ppm, 100 ppm, 10 ppm and 1 ppm in Lanes 13-16, Methanolic extract of *C. sinensis* having 1000 ppm, 100 ppm, 10 ppm and 1 ppm in Lanes 17-20, Negative control (3 µl of 2mMFeSo$_4$, 4 µl of H_2O_2, 5 µl of PBS, 3µl of Plasmid) in Lane 21, Positive control (3 µl of Plasmid, 5 µl of PBS) in Lane 22.

DPPH assay performed for the evaluation of the antioxidant activity of the peel extracts of different citrus fruit samples showed *C. limon* and *C. sinensis* have 50% and *C. limetta* have 17% antioxidant activity, while *C. reticulata* and *C. aurantium* showed more than 75% activity at 10 mg/ml. Since *C. reticulata* and *C. aurantium* showed more than 75% antioxidant activity further dilutions were made as 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml. Barreca *et al.* (2011), in their study, carried out DPPH radical quenching and FRAP assays which demonstrated that *C. limetta* juice showed remarkable antioxidant activity. Their results do not support our study. This may be due to their use of *C. limetta* juice, while in the current study the peels extract was used. Four citrus species were characterized in relation to their contents of minerals, ascorbic acid, total polyphenols and the antioxidant capacity of pulps and peels by Barros *et al*. (2012). They concluded that aside from citrus pulps, the peels are also good sources of bioactive compounds and minerals, and can play a considerable role in food products. This study supports our study in terms of the use of peels. The results of antioxidant activity of citrus species are mentioned in Table 2.

CONCLUSION

Citrus species used in the current study consists of various phytochemicals such as carbohydrates, amino acids, glycosides, tannins, steroids and alkaloids. They also have the capability for DNA protection, which was shown by the methanolic extracts of peels of *C. limon*, *C. reticulata*, *C. aurantium* and *C. sinensis*. *C. limetta* showed less protection as compared to other species. DPPH assay used to assess the

Table 2: The percent scavenging activity of different dilutions of plant extracts using DPPH assay.

Samples	Dilutions			
	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL
C. limon	51%	-----	------	------
C. sinensis	50%|	-----	------	------
C. limetta	17%	-----	-------	-----
C. reticulate	75%	80%	81%	17%
C. aurantium	86%	84%	82%	-----
Ascorbic acid	87%	-----	------	------

antioxidants showed the highest activity in the methanolic extract of peels of *C. reticulata* and *C. aurantium* with 75% and 86% respectively at 10 mg/ml. It is therefore concluded that *C. limon, C. reticulata, C. aurantium* and *C. sinensis,* in terms of DNA protection, and *C. reticulata* and *C. aurantium,* in terms of free radicals scavenging activity, are the best ones and these are the potential candidates which can be used for further studies.

REFERENCES

Barreca D, Bellocco E, Caristi C, Leuzzi U, Gattuso G (2011). Distribution of *C*- and *O*-glycosyl flavonoids, (3-hydroxy-3-methylglutaryl) glycosyl flavanones and furocoumarins in *Citrus aurantium* L. juice. J Food Chem, 124, 576–582.

Barreca D, Bellocco E, Caristi C, Leuzzi U, Gattuso G (2011). Flavonoid profile and radical-scavenging activity of Mediterranean sweet lemon (*Citrus limmeta* Risso) juice. J Food Chem, 129 (2), 417-422.

Barros F, Varela SA, Pereira HM, Vicente L (2012). Subtidal benthic macroinfaunal assemblages in tropical estuaries Generality amongst highly variable gradients. J Ethnobiol Ethnomed, 8, 37.

Choudhary RK, Swarnkaar PL (2011). Antioxidant activity of phenolic and flavonoids compounds in some medicinal plants of India. J Nat Prod Res, 25, 1101-1109.

Harbone JB (1973). Phytochemical Methods. Chapman and Hall Ltd., Koche, D.K and R. P Shirsat (eds), London, 49-188.

Keffort JF (1960). The chemical constituents of citrus fruits. J Adv Food Res, 9, 285-372.

Kirimizibekmez H, Bassarello C, Piacente S, Celep E, Atay I, Mercanoglu G (2009). Phenolic compounds from Hypericum calycinum and their antioxidant activity. J Nat Prod Commun, 4, 531-534.

Koleckar V, Kubikova K, Rehakova Z, Kuca K, Jun D, Jahodar L (2008). Condensed and hydrolysable tannins as antioxidants influencing the health. J Mini Res Med Chem, 8, 436-447.

Kulisic T, Radonic A, Katalinic V (2004). Use of different

methods for testing antioxidant activity of oregano essential oil. J Food Chem, 85, 633-640.

Kumar A, Purabi R, Sarika A, Vinod S (2011). Preliminary study of the antioxidant properties of flowers and roots of Pyrostegia venusta (Ker Gawl) Miers. BMC Complement. Altern, Med, 11, 69.

Lalitha G, Sivaraj R, Maheswari SU, Nirmala A (2009). Antimicrobial Activity and Preliminary Phytochemical screening of *Allium cepa*, *Allium sativum* and *Citrus lemon*. J Medicinal Food Plants, 1, 11-15.

Lele RD, Devasagayam TPA, Tilak JC, Boloor KK, Ketaki SS, Saroj SG (2004). Free radicals and antioxidants in human health: Current status and future prospects. J Assoc Physicians India, 52, 794-804.

Møller P, Vogel U, Pedersen A, Lars OD, Brittmarie S, Steffen L (2003). No Effect of 600 Grams Fruit and Vegetables Per Day on Oxidative DNA Damage and Repair in Healthy Nonsmokers. Cancer Epidemiol, 12, 1016–1022.

Pandey A, Kaushik A, Tiwari SK (2001). Evaluation of antimicrobial activity and phytochemical analysis of *C.limon*. JPBMS, 13(13).

Perez Y, Jimene-ferrer YE, Alonso D, Botello-Amaro CA, Zamilpa A (2010). *Citrus limetta* leaves extract antagonizes the hypertensive effect of angiotensin II. J Ethnopharmacol, 128 (3), 611-614.

Sofowora A (1993). Medicinal plants and traditional in Africa. Spectrum Books Ltd. Ibadan, Nigeria, 191-289.

Tian B, Hua Y (2005). Concentration-dependence of prooxidant and antioxidant effects of aloin and aloe-emodin on DNA. J Food Chem, 91, 413-418.