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The antioxidant and antimicrobial properties of the methanolic extract from *Cocos nucifera* mesocarp

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Abstract

The antioxidant activity of the methanolic extract prepared from different stages of *Cocos nucifera L.* mesocarp was demonstrated, by DPPH, FRAP and deoxyribose assays, and suggests the potential of the mesocarp extract to be used for therapeutic purposes. Antimicrobial activity of the crude mesocarp extract was tested against *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 441, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* MTCC 7925. The extract exhibits a potent anti-staphylococcal activity. Probable compounds responsible for the bioactivity were identified by means of HPLC and UV/ESI–MS spectroscopic analyses. Their structures were deduced as 5-*O*-caffeoylquinic acid (chlorogenic acid), dicaffeoylquinic acid and three tentative isomers of caffeoylshikimic acid.

Keywords: Mesocarp; ESI/MS spectroscopy; Dicaffeoylquinic acid; HPLC; Chlorogenic acid

1. Introduction

Cocos nucifera L. (family Arecaceae), commonly known as coconut, is considered as an important fruit crop in tropical countries. Coconuts are unique in terms of their fruit (a drupe) morphology. The most interesting feature of the fruit is its wall. The fruit wall comprises of three layers exocarp, mesocarp and endocarp. Due to extensive cross linking between phenolics, lignin and polysaccharides, the mesocarp becomes hard and fibrous. Fibrous coconut fruit is not only edible but also suitable for multipurpose uses. As a traditional medicine in northeastern Brazil, coconut husks have been used for the treatment of diarrhoea and arthritis. Antimicrobial activity of the water extract of coconut husk has already been demonstrated (Esquenazi et al., 2002). However, studies regarding the polyphenol content of the coconut fruit wall are limited. In our previous work, we have discovered the major wall bound C_6–C_3 and C_6–C_1 phenolic compounds (Dey, Chakraborty, & Mitra, 2005; Dey, Sachan, Ghosh, & Mitra, 2003) present in the coconut mesocarp but the picture remains unclear regarding the accumulation of soluble phenolics.

Plant phenols are of interest because they an important group of natural antioxidants and some of them are potent antimicrobial compounds.

Among the polyphenols, caffeoyl derivatives draw special attention because of their ability as anti-HIV and anti-viral agents (Kwon et al., 2000; Robinson et al., 1996) and to increase hepatic glucose utilization (Hemmerle et al., 1997; Johnston, Clifford, & Morgan, 2003). Caffeoyl derivatives like chlorogenic acid (CGA) are strong antioxidants, which inhibit the in vitro oxidation of LDL (Frankel, Teissedre, & Waterhouse, 1995; Meyer, Yi, Pearson, Waterhouse, & Frankel, 1997). Caffeoylshikimates were reported to act as anti-thiamine factors.

Therefore, we have identified the different caffeic acid derivatives present in the methanolic extract of the young, mature and old coconut mesocarp and estimated the antioxidant property of the major polyphenols. In the recent past, research on antioxidants has increased considerably (Frankel & Meyer, 2000; Kusano et al., 2003). Due to

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the involvement of different complex mechanisms, accurate and quantitative measurement of antioxidant properties by a simple and universal method is not possible. The mechanism of antioxidant activity in vitro may involve direct inhibition of reactive oxygen species or the scavenging of free radicals (Dini, Tenore, & Dini, 2006). Thus we have used DPPH, FRAP and deoxyribose assays to evaluate the antioxidant property of the methanolic extract from the mesocarp. Apart from this, we also report on the antimicrobial activity of the methanolic coconut mesocarp extract.

2. Material and methods

2.1. Plant material

Young (3–4 months), mature (7–8 months) and old (12–14 months) coconuts were collected from the agricultural farm located at Indian Institute of Technology, Kharagpur, West Bengal, India.

2.2. Chemicals

Analytical grade chemicals were used in sample preparation and all the solvents for chromatographic purpose were HPLC grade, purchased from Merck. Muller Hilton Broth and Muller Hilton Agar were bought from Hi-Media, India (Mumbai). Standard Chlorogenic acid, Caffeic acid and Shikimic acid were bought from Aldrich chemicals (USA). Deionised water for all procedures was obtained from Barnstead/Thermolyne (Iowa, USA) Diamond-Nanopure™ water.

2.3. Spectroscopic apparatus

Mass spectrometric results were analyzed using Micromass^{R} LCT™ mass spectrometer. Spectrometric data was obtained at a capillary voltage of 3800 V, sample cone of 30 V and extraction cone of 3 V. Acetonitrile and water (50:50 v/v) was used as solvent. UV-spectral scanning was carried out using a SPECORD S100 UV–VIS scanning diode-array dual beam spectrophotometer. For analytes one pair of quartz cuvettes were used and for antioxidant assay 4 ml glass cuvettes were used.

2.4. Chromatographic apparatus

HPLC analysis was carried out using a Waters (waters, Milford, CA, USA) BREEZE™ HPLC system consisting of a binary pump (Waters 1525) and a UV detector (Water 2487). Data were collected and analyzed in BREEZE™ software (version 3.20) and elutes were monitored at 280 and 310 nm. A *Phenomenex*™ Synergy 4 μ Hydro-RP 80 C_{18} column (250 × 4.6 mm^2), coupled to a guard column Phenomenex™ Security Guard™ C_{18} ODS (4 × 3.0 mm^2) was used. ÄKTAprime™ Low Pressure Chromatography system (Amersham Pharmacia Biotech Ltd, Hong Kong) was equipped with a Sephadex LH-20 column and fraction collector of 65 tubes. Data were monitored using Prime-View™ software.

2.5. Extraction of methanol-soluble phenolic compounds

Ten grams of mesocarp tissues were separated from the young coconut mesocarp. Separated pieces were soaked in 25 ml of 50% methanol (containing 2.5 mM of sodium fluoride to reduce the browning reaction) overnight at 4 °C. Methanol extract was centrifuged at 5000 rpm for 20 min to settle the debris. The supernatant was passed through a Sephadex LH-20 column (40 cm × 2.5 mm, calibrated with 70% methanol for 15 min), with 70% methanol as solvent and at a flow rate of 2.5 ml/min for 30 min followed by collection of eluent in the fraction collector. Three different peaks were detected ($frac_1$, $frac_2$, $frac_3$). The fractions related to each peak were pooled together separately and evaporated to dryness under reduced pressure in a rotary evaporator. The residue was dissolved in 5 ml of 50% (v/v water:methanol) methanol. 20 μl of the extract was injected into the HPLC to identify the compounds present in the three different LPC fractions.

2.6. DPPH· radical scavenging or hydrogen donating method

The effect of the $frac_3$extract from three different stages of coconut mesocarp on DPPH· radical was estimated according to the procedure described in literature (Brand-Williams, Cuvelier, & Berset, 1995). For each concentration tested, the percentage of DPPH· left at the steady state was calculated as follows: %DPPH· REM = $[DPPH\cdot]_T/[DPPH\cdot]_{T=0}$, where T is the time require to reach the steady state. The antioxidant property was measured in terms of amount of methanolic extract necessary to reduce the initial DPPH· concentration by 50% (EC50). An aliquot of methanol (0.1 ml) solution containing different concentrations of methanolic extract was added to 2.9 ml of 60 μM DPPH (dissolved in methanol). The decrease in the absorbance was determined at 515 nm when the reaction reached a plateau. Methanol was used as blank. Ascorbic acid was taken as a standard antioxidant. The absorbance of the DPPH· radical without any antioxidant was measured as control.

2.7. Ferric reducing antioxidant power (FRAP assay)

FRAP assay was performed following the procedure described in the literature (Benzie & Strain, 1996). FRAP reagent was freshly prepared by mixing together 10 mM 2,4,6-tripyridyl triazine (TPTZ) and 12 mM ferric chloride and sodium acetate buffer (pH 3.6) at a ratio of 1:1:10. Aliquots (20 μl–1 mM) of the methanolic mesocarp extract were added separately to 3 ml of FRAP solution and kept in the incubator at 37 °C. The absorbance at 593 nm was determined at 37 °C for 90 min at an interval of 10 min. Reducing potential was expressed in units of mmol Fe(II)/g dry weight of mesocarp.

2.8. Deoxyribose assay

Hydroxyl radical scavenging potential of the extract was determined using the deoxyribose assay (Aruoma, 1994a, 1994b; Halliwell, Gutteridge, & Aruoma, 1987). The assay mixture contained in a final volume of 1 ml the following reagents: 200 μl KH_2PO4–KOH (100 mM), 200 μl deoxyribose (15 mM), 200 μl $FeCl_3$ (500 mM), 100 μl EDTA (1 mM), 100 μl isolated compound, 100 μl H_2O_2 (10 mM) and 100 μl ascorbic acid (1 mM). Reaction mixtures were incubated at 37 °C for 1 h in the dry bath incubator. At the end of the incubation period, 1 ml 1% (w/v) thiobarbituric acid (TBA) was added to each mixture, followed by the addition of 1 ml 2.8% (w/v) trichloroacetic acid (TCA). The solutions were heated in a water bath at 80 °C for 20 min to develop the pink colored malondialdehyde MDA-$(TBA)_2$adduct. As turbidity was encountered, the MDA-$(TBA)_2$ chromogen was extracted into 2 ml butan-1-ol and its absorbance measured at 532 nm. Results are expressed as % inhibition of deoxyribose oxidation/g dry wt mesocarp.

2.9. Antimicrobial activity and preparation of inoculum

Stock cultures were maintained at 4 °C on slopes of nutrient agar. Stock cultures are the type culture (kindly donated by Dr Ujjal Mukherjee, West Bengal Pollution Control Board, India). Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller–Hinton broth (MHB) and incubated without agitation for 24 h at 37 °C and 25 °C, respectively. The cultures were diluted with fresh MHB to achieve optical densities corresponding to $2.0 \cdot 10^6$colony forming units (CFU/ml).

2.10. Antimicrobial susceptibility test

The disc diffusion method was used to screen the antimicrobial activity (Bauer, Kirby, Sherris, & Turck, 1966). The MHA plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 min, 0.1% inoculum suspension was spread uniformly, and the inoculums were allowed to dry for 5 min. Twenty microliters of crude plant extract were loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium, the compound was allowed to diffuse for 5 min, and the plates were kept for incubation at 37 °C for 24 h. Streptomycin and ampicillin discs were taken as positive controls. At the end of incubation, inhibition zones formed around the disc were measured with a transparent ruler in millimeters.

2.11. Statistical analysis

All the experiments were done in triplicate. The triplicate data were subjected to an analysis of variance for a completely random design using statistical analysis software, SPSS 10.0. The significance level was fixed at 0.05 for all statistical analysis. Standard deviations were expressed in each table.

3. Result and discussion

3.1. Extraction and isolation

Crude methanolic extract may contain thousands of compounds including flavones, proanthocyanidin and even soluble polysaccharides. To establish a better characterization of the methanolic extract and to get a clear HPLC profile, fractionation through a Sephadex LH-20 column was carried out. As per UV behavior three fractions were obtained, $frac_1$, $frac_2$ and $frac_3$. HPLC elution of $frac_3$ exhibits five major compounds in high concentration. In $frac_1$ and $frac_2$ these five compounds were present (low concentration) along with some other compounds (not identified). In the present study we only focused on the identification of the five compounds of the $frac_3$. An HPLC profile of the $frac_3$ recorded at 310 nm is illustrated in Fig. 1. The identification of the compounds was tentatively made on the basis of UV and mass spectral characteristics. UV absorption of spectra at 330 and 352 nm and pseudo molecular ion peak at 353 $(M–H)^-$ and 515 $(M–H)^-$ in the negative ESI–MS spectra of compound 1 and 5, respectively, were used to identify the compounds as caffeoylquinic and dicaffeoylquinic acid (DCQA), respectively (Carnini, Aldini, Furlanetto, Stefani, & Facino, 2001; Clifford, Jhonston, Knight, & Kunnert, 2003). Further the retention time and mass spectra of compound 1 was compared with the authentic standards to confirm that it is 5-caffeoylquinc acid (CGA). With the UV absorbance maxima of compound 2, 3, 4 at 326 nm (Veit et al., 1992) and the negative ion mass spectral peak at m/z 335 (MW.336) it can be assumed that these compounds are isomers of caffeoylshikimic acid. Ester hydrolysis of the HPLC eluents of peak 2, 3, 4, produces caffeic acid and shikimic acids. Products were confirmed by HPLC comparing with

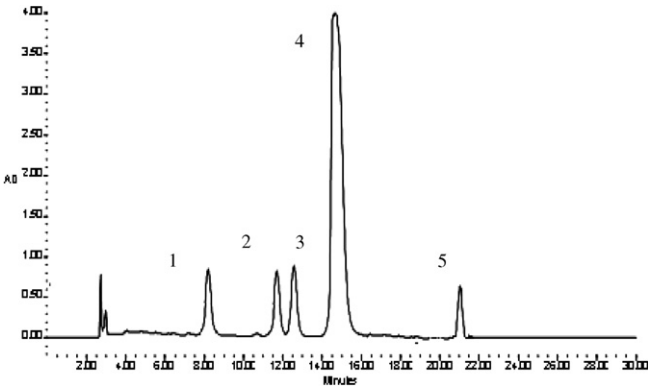

Fig. 1. A HPLC profile of the $frac_3$ (eluted from the Sephadex LH-20 column) of methanolic extract of coconut mesocarp recorded at 310 nm in an isocratic mode (aqueous TFA: methanol 68:32). 1 = chlorogenic acid, 2, 3 and 4 = isomers of caffeoylshikimic acid, 5 = dicaffeoylquinic acid.

authentic standards (data not shown). Shikimate and quinate esters were quantified by HPLC considering chlorogenic acid as standard (Table 1).It had been observed that with age of the mesocarp its antioxidant potential gradually diminishes. With age soluble phenolics gradually decreases because most of them become associated with the wall or are utilised as intermediates of the lignin pathway. This fact was also supported by the quantification data of the caffeic acid derivatives. Thus, suggesting that the antioxidant potential of the mesocarp was primarily comprised of these caffeic acid derivatives.

3.2. Antioxidant activity of the methanolic extract

The antioxidant property of the phenolics mainly depends on the number and position of the hydroxyl group (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). In general, caffeic acid derivatives are considered as well-known antioxidants because the catechol structure donates the phenolic hydrogens or electron to the acceptors such as lipid peroxyl groups or reactive oxygen species (Herrmann, 1989; Ho, 1992). Orthodihydroxy configuration of some caffeic acid derivatives like caffeoylshikimates makes them potent antioxidant compounds. The antioxidant property of the extract was verified by the DPPH scavenging method (Table 2, Fig. 2). Antioxidant capacity was evaluated from 0 to 30 min at an interval of 10 min. Near equilibrium state was achieved at 20 min, showing that the reaction rate is quite high. DPPH test confirms the radical scavenging potentiality of the mesocarp extract by measuring the inactivation potential of the radical in an aqueous medium. Free radical scavenging property of the mesocarp extract was further verified by the deoxyribose assay (Table 3). In the deoxyribose assay, free radicals were generated by Fentons reaction (Nordberg & Arne'r, 2001), which react with deoxyribose and produce MDA. MDA on being incubated with thiobarbituric acid (TBA) produces a pink chromogen (Paya, Halliwell, & Hoult, 1992). Free radical scavenging compounds scavenge the free hydroxyl radical generated by the Fenton reaction and limits its availability to react with the deoxyribose, thus reducing the formation of pink chromogen. The change in the color of the reaction mixture was quantified to evaluate the scavenging property of the tested compound. Deoxyribose assay therefore further authenticated the free radical scavenging property of the extract.

The FRAP assay was performed to estimate the capacity of the mesocarp extract to reduce Fe^{3+}/Fe^{2+} (Table 3)

Table 1
Retention time and soluble phenolic acids content of coconut mesocarp (at young mature and old stage)

Compounds	Stages of coconut	RT[a]	Quantification[b]
Chlorogenic acid	Young mesocarp	8.9	4.4 ± 0.4
	Mature mesocarp		2.1 ± 0.5
	Old mesocrp		0.9 ± 0.3
Caffeoylshikimic acid[c]	Young mesocarp	11.2	4.4 ± 0.3
	Mature mesocarp		3.1 ± 0.2
	Old mesocrp		2.2 ± 0.3
Caffeoylshikimic acid[c]	Young mesocarp	12.4	4.1 ± 0.1
	Mature mesocarp		3.6 ± 0.4
	Old mesocrp		1.2 ± 0.4
Caffeoylshikimic acid[c]	Young mesocarp	15.6	21.4 ± 0.1
	Mature mesocarp		20.4 ± 0.5
	Old mesocrp		19.5 ± 0.2
Dicaffeoylquinic acid	Young mesocarp	21.2	5.6 ± 0.3
	Mature mesocarp		4.4 ± 0.4
	Old mesocrp		0.4 ± 0.5

[a] Retention time.
[b] Quantified in terms of chlorogenic acid equivanlent mg/g dry wt; mean values ± = standard deviation ($n = 3$).
[c] The isomers of Caffeoylshikimic acid.

Table 2
Efficient concentrations 50 of the methanolic extract (μg/ml) ($frac_3$) prepared from the young, mature and old coconut mesocarp

Coconut mesocarp	EC 50[a] (μg/ml)	Kinetic[b] (min)
Young stage	32.3 ± 0.6	20
Mature stage	45.4 ± 0.4	20
Old stage	90.2 ± 0.3	20
Ascorbic acid	22.7 ± 0.3	5

[a] Concentration of the extract at which 50% was completed.
[b] Time require to reach the steady state.

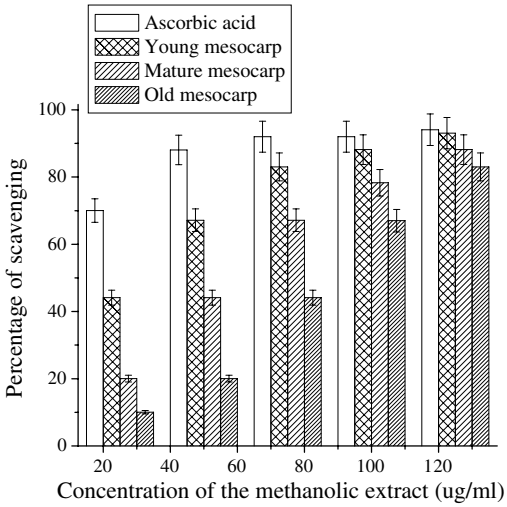

Fig. 2. DPPH radical scavenging activity (%) by the methanolic extract ($frac_3$) from young, mature and old mesocarp. Ascorbic acid is taken as standard antioxidant.

Table 3
Near equilibrium steady-state antioxidant capacity

Coconut mesocarp	Deoxyribose[a]	FRAP[b]
Young stage	40.6 ± 2.6	1292 ± 3
Mature stage	22.5 ± 3.1	1098 ± 1
Old stage	16.2 ± 2.9	768 ± 1

[a] Results are expressed as % inhibition of deoxyribose oxidation/g dry wt mesocarp.
[b] expressed in units of mmol Fe(II)/g dry wt weight of mesocarp.± = standard deviation ($n = 3$).

Table 4
Antimicrobial activity of methanolic extract ($frac_3$) of coconut mesocarp

Microbes		Zone of inhibition (mm)[a]				
		Methanolic extract (mg/ml dry wt)				
		0.5	1.00	1.50	Strep [b]	Amp[c]
S. aureus	Young	8.0 ± 0.2	10.0 ± 0.1	15 ± 0.2	16 ± 0.2	–
	Mature	8.0 ± 0.4	8.5 ± 0.3	12 ± 0.2		
	Old	–	–	8.0 ± 0.5		
E. coli	Young	–	–	–	13 ± 0.2	7 ± 0.3
	Mature					
	Old					
P. aeruginosa	Young	–	–	–	10 ± 0.1	8 ± 0.1
	Mature					
	Old					
B. subtilis	Young	7 ± 0.5	8.5 ± 0.3	10 ± 0.2	13 ± 0.2	10 ± 0.1
	Mature	7 ± 0.4	8.0 ± 0.1	8.0 ± 0.3		
	Old	–	–	–		

[a] Twenty microliter of extract was poured in each disc (6 mm).
[b] Streptomycin (50 μg/ml).
[c] Ampicillin (50 μg/ml) were taken as positive control.

(Pulido, Bravo, & Saura-Calixto, 2000). This study was possible because the caffeic acid derivatives, which were identified in the extract, do not have any functional group whose reduction potential is less than that of Fe^{3+}/Fe^{2+} half reaction. FRAP therefore confirms the reducing potentiality of the mesocarp extract.

3.3. Antimicrobial activity of the plant extract

Antimicrobial activity of the methanolic extract shows strong activity against *Staphylococcus aureus* and an inhibition zone was formed against *Bacillus subtilis* (Table 4). However, it shows no response against *Escherichia coli* and *Pseudomonas aeruginosa*. A brown zone was developed around the diffusion disc in case of *E. coli*,, which was not found in case of other bacteria. Antimicrobial activity of DCQA and CGA has been reported (Zhu, Zang, & Lo, 2004), so it may be that the synergistic effect of these compounds is responsible for the antimicrobial activity of the crude extract. Some other potent antimicrobial compounds might present in the extract but were not isolated in the present method of isolation. In this respect, further investigation is ongoing.

4. Conclusion

To our knowledge, for the first time we have reported about the strong antioxidant and antimicrobial property of the methanolic extract from young coconut mesocarp. The probable compounds responsible for these activities were identified as three isomers of CSA, CGA, and DCQA, respectively.Though, there are literature reports (Mansouri et al., 2005) suggesting the presence of caffeoylshikimic acid in the palmae family, the presence of such high amount of caffeoylshikimic acid in the young coconut mesocarp was reported for the first time. These young coconut mesocarps are discarded as waste and it is considered as one of the major agro wastes of the tropical countries. Therefore, our study will definitely open up a scope for future utilization of these agro wastes for therapeutic purposes.

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References

Aruoma, O. I. (1994a). Nutrition and health aspects of free radicals and antioxidants. *Food Chemistry and Toxicology, 32*, 671–683.
Aruoma, O. I. (1994b). Deoxyribose assay for detecting hydroxyl radicals. *Methods in Enzymology, 233*, 57–66.
Bauer, R. W., Kirby, M. D. K., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by standard single disc diffusion method. *American Journal of Clinical Pathology, 45*, 493–496.
Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry, 239*, 70–76.
Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Technology, 28*, 25–30.
Carnini, M., Aldini, G., Furlanetto, S., Stefani, R., & Facino, M. R. (2001). LC coupled to ion trap MS for rapid screening and detection of polyphenol antioxidants from *Helichryum stoechas*. *Journal of Pharmaceuticals and Biomedical Analysis, 24*, 517–526.
Clifford, M., Jhonston, K. L., Knight, S., & Kunnert, N. (2003). Hierarchial scheme for LC–MS^n identification of chlorogenic acids. *Journal of Agricultural and Food Chemistry, 51*, 2900–2911.
Dey, G., Chakraborty, M., & Mitra, A. (2005). C_6–C_3 and C_6–C_1 phenolic metabolites in *Cocos nucifera*. *Journal of Plant Physiology, 162*, 375–381.
Dey, G., Sachan, A., Ghosh, S., & Mitra, A. (2003). Detection of major phenolic acids from the dried mesocarpic husk of mature coconut by

thin layer chromatography. *Industrial Crops and Products, 18*, 171–176.

Dini, I., Tenore, C. G., & Dini, A. (2006). New polyphenol derivative in *Ipomoea batatas* tubers and its antioxidant activity. *Journal of Agricultural and Food Chemistry, 54*, 8733–8737.

Esquenazi, D., Wagg, M. D., Miranda, M. M., Rodrigues, H. M., Tostes, J. B., Rozental, S., et al. (2002). Antimicrobial and antiviral activities of polyphenolics from *Cocos nucifera*Linn. (Palmae) husk fiber extract. *Research in Microbiology, 153*, 647–652.

Frankel, E. N., & Meyer, A. S. (2000). The problems of using one dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture, 80*, 1925–1941.

Frankel, E. N., Teissedre, P. L., & Waterhouse, A. L. (1995). Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of low-density lipoprotein. *Journal of Agricultural and Food Chemistry, 43*, 890–894.

Halliwell, B., Gutteridge, J. M. C., & Aruoma, O. I. (1987). The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry, 165*, 215–219.

Hemmerle, H., Burger, H., Bellow, P., Schubert, G., Rippel, R., Schindler, P. W., et al. (1997). Chlorogenic acid and synthetic chlorogenic acid derivatives: Novel inhibitors of hepatic glucose-6-phosphate translocase. *Journal of Medicinal Chemistry, 40*, 137–145.

Herrmann, K. (1989). Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. *Critical Reviews in Food Science and Nutrition, 28*, 315–347.

Ho, C. T. (1992). Phenolic compounds in food. In *Phenolic compounds in food and their effects on health*. In C. T. Ho, C. Y. Lee, & M. T. Hung (Eds.). *ACS symposium series* (vol. 506, pp. 2–7). Washington, DC: American Chemical Society.

Johnston, K. L., Clifford, M., & Morgan, L. M. (2003). Coffee acutely modifies gastrointestinal hormone secretion and glucose tolerance in humans: glycemic effects of chlorogenic acid and caffeine. *American Journal of Clinical Nutrition, 78*, 728–733.

Kusano, A., Seyama, Y., Usami, E., Katayose, T., Shibano, M., Tsukamoto, D., et al. (2003). Studies on the antioxidant active constituents of the dried powder from *Bidens pilosa* L. var. radiata Sch. *Nature Medicine, 57*, 100–104.

Kwon, H. C., Jung, C. M., Shin, C. G., Lee, J. K., Choi, S. U., Kim, S. Y., et al. (2000). A new caffeoyl quinic acid from Asterscaber and its inhibitory activity against human immunodeficiency virus-1 (HIV-1) integrase. *Chemical and Pharmacology Bulletin, 48*, 1796–1798.

Meyer, A. S., Yi, O., Pearson, D. A., Waterhouse, A. L., & Frankel, E. N. (1997). Inhibition of human low density lipoprotein oxidation in relation to composition of phenolic antioxidants in grapes (*Vitis vinifera*). *Journal of Agricultural and Food Chemistry, 45*, 1633–1643.

Nordberg, J., & Arne'r, E. S. J. (2001). Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radio Biology and Medicine, 31*, 1287–1312.

Paya, M., Halliwell, B., & Hoult, J. R. S. (1992). Interactions of a series of coumarins with reactive oxygen species. *Biochemical Pharmacology, 44*, 205–214.

Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry, 48*, 3396–3402.

Rice-Evans, C. A., Miller, N. J., Bolwell, P. G., Bramley, P. M., & Pridham, J. B. (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Research, 22*, 375–383.

Robinson, W. E., Cordeiro, M., Abdel-Malek, S., Jia, Q., Chow, S. A., & Reinecke, M. G. (1996). Dicaffeoylquinic acid inhibitors of human immunodeficiency virus integrase: inhibition of the core catalytic domain of human immunodeficiency virus integrase. *Molecular Pharmacology, 50*, 846–855.

Veit, M., Weidner, C., Strack, D., Wray, V., Witte, L., & Czygan, F. C. (1992). The distribution of caffeic acid conjugates in the Equisetaceae and some ferns. *Phytochemistry, 31*, 3483–3485.

Zhu, X., Zang, H., & Lo, R. (2004). Phenolic compounds from the leaf extract of Artichoke (*Cynara scolymus* L.) and their antimicrobial activities. *Journal of Agricultural and Food Chemistry, 52*, 7272–7278.