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Antioxidant Potentialities of Black Nightshade and Sweet Potatoes Leaves Consumed in Côte d'Ivoire

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Abstract Leafy vegetables contain nutrients and have also antioxidant potentialities. Two of them, black nightshade and sweet potatoes leaves, were analyzed for their antioxidant capacities. Total phenolic and flavonoids were determined. The antioxidant activities determinations were on radical scavenging activities, lipid peroxidation inhibition and reducing power. The results showed that total phenolic and flavonoids content were higher in sweet potatoes leaves than in black nightshade. Indeed, sweet potatoes total phenolic and flavonoids content were respectively 933.30 ± 15.30 mg Gallic Acid Equivalent (GAE)/g of dry matter (DM) and 149.00 ± 4.00 mg Quercetin Equivalent (QE)/g DM, while that of black nightshade were 300.00 ± 5.7 mg GAE/g DM and 54.60 ± 8.30 mg QE/g DM. Consequently, sweet potatoes leaves had best antiradical activities which were characterised by their IC_{50} value (3.12 ± 1.42µg/ml). That of black nightshade was 163.00 ± 0.82 µg/ml. The two leafy vegetables had a lipid peroxidation inhibitory capacities upper than that of gallic acid and these inhibitory capacities were more important in sweet potatoes leaves than in black nightshade leaves. Sweet potatoes leaves IC_{50} was about 206.33 ± 14.05 µg/ml and that of black nightshade was 272.33 ± 21.39 µg/ml. However, reducing power in leaves was lower than that of vitamin C (the molecular reference). Antioxidant activities were high in sweet potatoes leaves. But, it could decrease after cooking process as nutrients losses are occurred during cooking.

Keywords: *antioxidant activities, total phenolic, flavonoids, sweet potatoes leaves, black nightshade*

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1. Introduction

Human organism is exposed to free radicals which effects promote many diseases such as certain cancers, cardiovascular and metabolic diseases [1]. To escape the serious consequences of oxidative stress, fruits and vegetables, especially leafy vegetables, consumption is useful as they provide micronutrients (vitamins and minerals) and have antioxidant potentialities to fight against free radicals [2,3].

In Côte d'Ivoire, leafy vegetables take an important place in population eating habits [4]. Their consumption is cultural with for example, jute mallow and black nightshade in the Center, roselle and sweet potatoes leaves in the North, okra leaves in the West and spinach in the South. Among them, sweet potatoes leaves are the most consumed [5]. Populations really appreciate their taste, their relatively low cost and their availability several months in the year [6]. They are also consumed and recommended for their capacities to overcome anemia and other nutrients deficiencies [7,8], but, not for their antioxidant properties which could enhance such capacities.

Several studies have been conducted on antioxidant activity of sweet potatoes leaves and black nightshade, two leafy vegetables of interest for ivorian population [9,10]. They all revealed important antioxidant activity. But, these antioxidant activities were expressed only by DPPH radical scavenging activities. However, antioxidant activities could be also expressed with total reducing power determination, lipid peroxidation inhibition determination, superoxide radical scavenging activity and metal chelating activity. Making several determination could help to better appreciate leafy vegetables' antioxidant activities.

In this study the phenolic and flavonoid compounds of sweet potatoes leaves and black nightshade have been determined. Antioxidant activities have been appreciated through DPPH radical scavenging, total reducing power and lipid peroxidation inhibition determination.

2. Material and Methods

2.1. Material

Sweet potatoes leaves and black nightshade leaves were collected in « Gouro Market » a leafy vegetables wholesale trade in Abidjan, Côte d'Ivoire. For this study, there were chosen because sweet potatoes leaves were the most consumed leafy vegetables by Ivorian population and black nightshade were consumed as salad and sometime in raw form.

2.2. Sampling

Each leafy vegetable was collected with 3 sellers chosen randomly, then, mixed and transported to the laboratory for the analysis. The leafy vegetables were destalked, cleaned, washed at running water and 400g of each sample were dried at 16°C during 72h.

2.3. Analysis

2.3.1. Extraction and Total Phenolic and Flavonoid Determination

Before extraction, samples were ground in a grinder (IKAMAG) and 10 g of each powder were soaked in 100 ml of methanol/water solution (90:10, v/v). The mixture was shaked with an orbital shaker (Biobase) during 24 hours. After that, it was filtered with Whatman paper n°1 and the filtrat was stored in an oven (Biobase) at 40°C during 24 hours for solvent evaporation. The final paste was the total extract [11]. Total phenolic compounds were determined by Folin-Ciocalteu method at 765nm and expressed as gallic acid equivalents (GAE) in milligrams per gram DM using a standard curve generated with gallic acid [12]. Total flavonoids were determined at 415nm and espressed at quercetin equivalents (QE) in microgram per gram DM using the standard curve of quercetin [13].

2.3.2. Free radical Scavenging Activities and Anti-radical Power Determination

The free radical scavenging activity of the extracts was measured with the DPPH method [14]. This test consists to evaluate the capacity of extract to fixed DPPH free radical by the measurement of color diminution at 517 nm. Vitamin C (100µg/ml) was used as standard and the percent inhibitory activity was calculated as follow in equation 1:

$$\text{Inhibition DPPH}\ (\%) = \frac{\text{Abs}_c - \text{Abs}_e}{\text{Abs}_c} \text{x}100 \qquad (1)$$

where Abs_c was the absorbance of the control, and Abs_e the absorbance of the extract/standard.

The efficient concentration (EC_{50}), which is the sample concentration which can reduce 1 µmol of DPPH was also determined (Equation 2).

$$EC_{50} = \frac{IC_{50}}{\text{DPPH solution concentration}} \qquad (2)$$
$$(\text{mg of sample} / \mu\text{mol of reduced DPPH})$$

IC_{50}: Sample concentration which inhibe 50% of DPPH
EC_{50}: Efficient concentration for 50% of DPPH

The efficient concentration allows calculating the anti-radical power (Equation 3) [15].

$$\text{ARP} = \frac{1}{EC_{50}} \qquad (3)$$

(µmol of reduced DPPH /mg of sample)
ARP: anti-radical power.

2.3.3. Lipid Peroxidation Inhibitory Activity Determination

The lipid peroxidation inhibitory activity was determined according to ammonium thiocyanate test with some slight modifications [16]. A quantity 0.5ml of samples extracts at graduate concentrations (0.2 to 6 mg/ml) has been mixed to 0.2 ml of diluted linoleic acid (20 mg/ml in ethanol 99%) and 0.4 ml of phosphate buffer (50 mM; pH 7.4). The mixture was heated in a water bath at 40 °C for 15 min. Then, 0.1ml of mixture was add to the reaction mixture which was composed of 3ml of ethanol (70%), 0.1 ml of ammonium thiocyanate (30 mg/ml) and 0.05 ml of $FeSO_4$ (2.45 mg/ml in HCl 3.5% (v/v)). After agitation and incubation at ambient temperature during 3 min, the absorbance was determined at 500 nm. Gallic acid (100µg/ml) was used as standard.

The inhibitory percentage of lipid peroxidation was calculated in equation 4:

$$\text{Lipid peroxidation inhibitory}(\%) = 1 - \frac{\text{Abs}_e}{\text{Abs}_c} \text{x}100 \qquad (4)$$

Abs_c: control absorbance
Abs_e: absorbance of extract/standard.

2.3.4. Reducing Power Determination

The determination of reducing power was conducted according to Oyaizu method [17] using potassium ferricyanide. The solution of plant extracts (1 ml, 0 - 300 mg/ml) was spiked with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide (1% (p/v)). The mixture was then placed in water bath at 50°C for 20 minutes. After cooling rapidly, 1 ml of trichloroacetic acid (TCA) (10%) was added and the mixture was centrifuged at 3000 rpm during 10 minutes. The supernatant (1 ml) was then mixed with 0.1 mL of ferric chloride (1%) and 2 ml of distilled water. After incubation at ambient temperature during 10 minutes, the absorbance at 593 nm was recorded. Vitamin C (100µg/ml) was used as standard.

2.4. Statistical Analysis

Graphic representations were made with Graph Pad Prism 5.0 (Microsoft U.S.A) and data were compared with the same software. Results made in triplicate were expressed as means with standard deviation. A one-way ANOVA was performed and means were separated using Tukey test ($p \leq 0.05$) or Dunnett test ($p \leq 0.05$).

3. Results

3.1. Total Phenolic and Flavonoid Content

Total phenolic and flavonoids content of leafy vegetables were presented in Table 1. Leafy vegetables total phenolic

content was about 933.30 ± 15.30 and 300.00 ± 5.70 mg GAE/g DM respectively in sweet potatoes leaves and black nightshade. Flavonoids content were about 149.00 ± 4.00 and 54.60 ± 8.30 mg QE/g DM respectively in sweet potatoes and black nightshade leaves. There was a significant statistical difference between the 2 leafy vegetables for total phenolic and flavonoid content.

Table 1. Total phenolic and flavonoid content of leafy vegetables

Leafy vegetables	Total phenolic (mg GAE/g DM)	Flavonoid (mg QE/g DM)
Sweet potatoes leaves	933.30 ± 15.30[a]	149.00 ± 4.00[a]
Black nightshade	300.00 ± 5.70[b]	54.60 ± 8.30[b]

In row, means with different superscript differ significantly (Tukey test, $p \leq 0.05$).

3.2. Antioxidant Activities in Leafy Vegetables

3.2.1. Free Radical Scavenging Activities and Anti-radical Power of Leafy Vegetables

DPPH free radical scavenging activities of vitamin C and leafy vegetables were presented in Figure 1. These activities increased with leafy vegetables concentrations. The graphic determination of IC_{50} indicated that sweet potatoes leaves had the best antiradical activities. This is shown by is IC_{50} value (3.12 ± 1.42 µg/ml) which is closed to that of the standard (vitamin C) value (1.74 ± 0.33 µg/ml). IC_{50} of black nightshade was 163.00 ± 0.82 µg/ml. The efficient concentration (EC_{50}) which can reduce 1 µmol of DPPH and the anti-radical power capacities of leafy vegetables was expressed in Table 2. Sweet potatoes leaves had the best anti-radical power. For black nightshade, anti-radical power differed significantly to that of vitamin C.

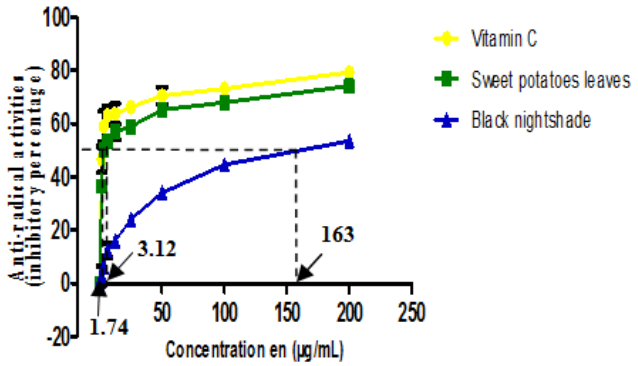

Figure 1. Evolution of anti-radical activities of vitamin C and leafy vegetables

Table 2. Inhibitory concentration, efficient concentration and anti-radical power of vitamin C and leafy vegetables

Leafy vegetables	IC_{50}	EC_{50}	ARP
Sweet potatoes leaves	3.12 ± 1.42[a]	0.03 ± 0.01[b]	37.63 ± 19.02[b]
Black nightshade***	163.00 ± 0.82[b]	0.01 ± 0.01[a]	0.61 ± 0.00[a]
Vitamin C	1.74 ± 0.33[a]	0.02 ± 0.00[b]	58.90 ± 11.37[b]

In row, means with different superscript differ significantly.
*** Significant difference between vitamin C (reference) and leaves. Dunnett test ($p \leq 0.05$).

3.2.2. Lipid Peroxidation Inhibitory Activities of Leafy Vegetables

In the different leafy vegetables, lipid peroxidation inhibitory activity increased with extract concentration (125, 250 and 500 µl/ml) (Figure 2). In sweet potatoes leaves and black nightshade, the lipid peroxidation inhibitory activity varied respectively from 34.50 ± 3.24% to 96.94 ± 2.16% and from 26.17 ± 2.58% to 80.90 ± 2.41%. All these inhibitory activities were upper than that of standard (gallic acid). Leafy vegetables lipid peroxidation inhibitory capacities, according to IC_{50}, could be range as follow: sweet potatoes leaves (206.33 ± 14.05 µg/ml) first and then black nightshade (272.33 ± 21.39 µg/ml) (Table 3).

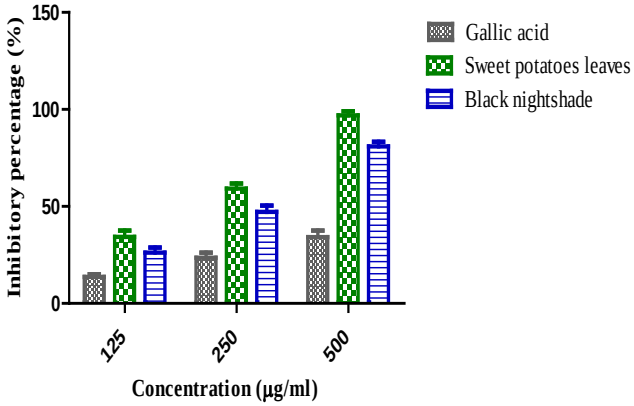

Figure 2. Evolution of lipid peroxidation inhibitory activities of gallic acid and leafy vegetables

Table 3. Lipid peroxidation inhibitory concentration

	Sweet potatoes leaves	Black nightshade
IC_{50} (µg/ml)	206.33 ± 14.05[a]	272.33 ± 21.39[b]

In line, means with different superscript differ significantly (Tukey test, $p \leq 0.05$).

3.2.3. Reducing Power Activities of Leafy Vegetables

Vitamin C and leafy vegetables reducing power capacity increased with concentration. However, in comparison to the reference, leafy vegetables reducing power activities is low and the statistical difference is significative (Figure 3). Sweet potatoes reducing power activities were higher than that of black nightshade.

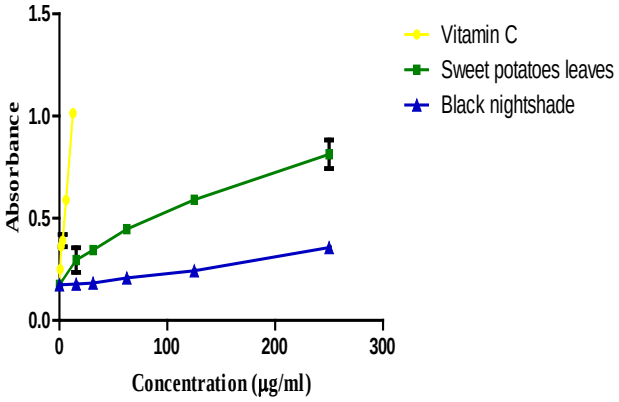

Figure 3. Evolution of reducing power activities of vitamin C and leafy vegetables

4. Discussion

The studied leafy vegetables content total phenolic, flavonoids and had antioxidant activities. Sweet potatoes leaves total phenolic and flavonoids content were higher than that of black nightshade. Total phenolic content of sweet potatoes leaves were also higher than 163.20 mg GAE/g DM as indicated by [13] in their study and that of black nightshade were also higher than the result of [18] (13.17 mg GAE/g DM). Sweet potatoes leaves had high antiradical power. This is characterized by their IC_{50} value which was closed to the reference (vitamin C). This result was in concordance with [19] study who indicated a better IC_{50} value (0.92 µg/ml) in sweet potatoes leaves. For black nightshade, our results which revealed a low antioxidant capacity were in contradiction to that of [9] which showed an IC50 value of 0.19 µg/ ml and consequently, a high antiradical power. All these antioxidant capacities were correlated with their phenolic compounds rates which were implicated in oxidative stress inhibition by donating a single electron or hydrogen atom for reduction [20,21].

The studied leafy vegetables had a lipid peroxidation inhibitory capacity which is higher than that of the reference (gallic acid) and which increased with concentration. This inhibitory activity was due to the extracts total flavonoids content [22]. According to [23], there is a positive linear correlation between flavonoids and the linoleic acid lipid peroxidation inhibition. The inhibitory effect of linoleic acid lipid peroxidation in sweet potatoes black nightshade leaves is higher than that of *Celosioides gomphrena* which varied from 16.18 at 125µg/ml to 48.50 at 500 µg/ml as indicated by [24] in their study. According to [25], the flavonoids had the capacity of reducing peroxyl radicals by electrons transfer thanks to their low redox potential.

Leafy vegetables reducing power were lower than the reference (vitamin C) but proportional to their total phenolic content. So, sweet potatoes reducing power was higher than that of black nightshade as their total phenolic content were elevated. These reducing power capacities were due to hydroxyl function in total phenolic compounds which were able to give electons [21] and revealed potential antioxidant activities for these leafy vegetables [26,27].

5. Conclusion

This study was on antioxidant activities in sweet potatoes and black nightshade leaves. It had revealed that sweet potatoes leaves content more total phenolic and flavonoids rate than black nightshade. Consequently, sweet potatoes leaves had a high antiradical and reducing power and a better lipid peroxidation inhibitory capacity than black nightshade. These leafy vegetables could be a natural source of antioxidant substances and help in metabolic diseases linked to oxidant stress.

However, as they were consumed after cooking, it should be great to evaluate the effect of high temperature in cooking time on their phenolic compounds availability and their antioxidant power.

Statement of Competing Interests

The authors have no competing interest in relation to their work.

List of Abbreviations

ARP: anti-radical power
DPPH: 2, 2-diphenyl-1-pycrylhydrazyl
EC_{50}: Efficient concentration for 50% of DPPH
IC_{50}: Inhibitory concentration for 50% of DPPH
GAE: gallic acid equivalent
QE: quercetin equivalent
DM: Dry matter

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References

[1] Pastre, J.O.C., “Intérêt de la supplémentation en antioxydants dans l’alimentation des carnivores domestiques, école nationale vétérinaire Toulouse”, Thèse de doctorat. Ecole Nationale Vétérinaire de Toulouse, 2005, 116.

[2] Rigaux, C., “Méthodes de monte carlo du second ordre et d’inférence bayésienne pour l’évaluation des risques microbiologiques et des bénéfices nutritionnels dans la transformation des legumes”, 2013, 207.

[3] Blanc, J-P., “Les Antioxydants naturels”, Seine, 9, 1996. Available: www.cabinet-de-nutrition-et-dietetique.eu, [Accessed January 2015].

[4] Agbo, E., Kouamé, C., Mahyao, A., N’Zi, J.C. and Fondio L., “Nutritional importance of indigenous leafy vegetables in Côte d’Ivoire”. Underutilized Plant species for food, nutrition, income and sustainable development Symposium. Mars 2008, Arusha, Tanzanie. Proc. IS on Underutilized Plants. Eds.: Jaenicke et al., Acta Hort. 806, ISHS, 1: 361-366. 2009.

[5] Agbo A. E., Kouamé C., Anin A. O. L., Soro L. C., N’zi J.-C., Fondio L. and Gnakri D., “Seasonal variation in nutritional compositions of spider plant (Cleome gynandra L.) in South Côte d’Ivoire”. IJAPR, 2 (11): 406-413. 2014.

[6] Kahane, R., Temple, L., Brat, P. and De B.H., “Les légumes feuilles des pays tropicaux: diversité, richesse économique et valeur santé dans un contexte très fragile”, Colloque Angers ; Les légumes : un patrimoine à transmettre et à valoriser, 2005, 9.

[7] Vyas, S., Collin, S., Bertin, E., Davys, G and Mathur B., “Leaf concentrate ace adolescent year alternate to iron and folic acid supplements for anemic girls: randomized controlled trial in India”, Public health Nutrition. 1-6. 2009.

[8] Grela, E. R. and Pietrzak, K., “Production Technology, Chemical Composition and Use of Alfalfa Protein-Xanthophyll Concentrate as Dietary Supplement”. J Food Process Technol, 5: 373. 2014.

[9] Mibei, E.K., Ojijo, N.K.O., Karanja, S.M. and Kinyua, J.K., “Phytochemical and antioxidant analysis of methanolic extracts of four african indigenous leafy vegetables”. Annals. Food Science and Technology. 13 (1): 37-42. 2012.

[10] Zoro, A.F., Zoué, L.T., Bédikou, M.E., Kra, S.A. and Niamké S.L., “Effect of cooking on nutritive and antioxidant characteristics of leafy vegetables consumed in Western Côte d’Ivoire”. Archives of Applied Science Research, 6 (4):114-123. 2014.

[11] Bala, I. M., Manigundan, K., Usha J. and Shanmuga P. A., “Antioxidant and Anti-proliferative activity of methanolic leaf extract of Eupatorium glandulosum L.”, International Journal of Advanced Research, 2 (7): 717-723. 2014.

[12] Mc Donald, S., Prenzler, P.D., Autolovich, M. and Robards, K., "Phenolic content and antioxidant activity of olive extracts". Food Chemistry, 73: 73-84. 2001.

[13] Chang, C., Yang, M., Wen, H. and Chern, J., "Estimation of total flavonoid content in propolis by two complementary colorimetric methods". Journal of Food and Drug Analysis, 10: 178-182. 2002.

[14] Parejo, I., Codina, C., Petrakis, C. and Kefalas, P., "Evaluation of scavenging activity assessed by Co (II)/EDTA-induced luminol chemilunescence and DDPH (2, 2-diphenyl-1-pycryl-hydrazyl) free radical assay". *Journal of Pharmacological and Toxicological* Methods, 44: 507-512. 2000.

[15] Kroyer, G. T., "Red clover extract as antioxidant active and functional food ingredient". Innovative Food Science and Emerging Technologies, 5, 101-105. 2004.

[16] Lee, C. Y., Sharma, A., Cheong, J. E. and Nelson, J. L., "Synthesis and antioxidant properties of dendritic polyphenols". Bioorganic and Medicinal Chemistry Letters, 19: 6326-6330. 2009.

[17] Oyaizu, M., "Studies on products of browing reaction: antioxidative activity of products of browing reaction prepared from glucosamine". Journal of Nutrition, 44: 307-315. 1986.

[18] Akbugwo, I. E., Obasi, A. N., and Ginika, S. C., "Nutritional potential of the leaves and seeds of black nightshade- Solanum nigrum L. Var virginicum from Afikpo-Nigeria". Pakistan Jouranl of Nutrition, 6 (4): 323-326. 2007.

[19] Xu, W., Liu, L., Hu, B., Sun, Y., Ye, H., Ma, D. and Zeng, X., "TPC in the leaves of 116 sweet potato (Ipomea batatas L.) varieties and Pushu 53 leaf extracts". Journal of food composition an analysis. 23: 599-604. 2010.

[20] Gramza, A., Khokhar, S., Yoko, S., Gliszczynska-Swiglo, A., Hes, M. and Korczak J., "Antioxidant activity of tea extracts in lipids and correlation with polyphenol content". European Journal of lipid Science and technology, 108 (4): 351-362. 2006.

[21] Bidié, A. P., Banga, B., Yapo, A. F., N'guessan, J. D. and Djaman, A. J., 2011. "Activités antioxydantes de dix plantes médicinales de la pharmacopée ivoirienne". Science Natural. 1: 1-11. 2011.

[22] Oktay, M., Gulcin, I. and Kufrevioglu, O. I., "Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts". Lebensmittel-Wissenchaft und Technologie, 36: 263-271. 2003.

[23] Amiour, D. S., "Etude quantitative des composés phénoliques des extraits de trois variétés de dattes (phoenix dactylifera l.) et évaluation in vitro de leur activité biologique", Thèse de doctorat, Université El-Hadj Lakhdar – Batna (Algerie), 2009, 159.

[24] Méité, S., Gogahy, K., Yapi, H. F., Yapo, A. F., Djaman, A. J. and Nguessan, J. D., "Antioxidant in vivo, in vitro activity assessment and acute toxicity of aqueous extract of Gomphrena celosioides (amaranthaceae)". The Experiment, 23 (3): 1601-1610. 2014.

[25] N'khili, E. Z., "Polyphénols de l'alimentation: extraction, interaction avec les ions du fer et du cuivre, oxydation et pouvoir antioxidant". Thèse de doctorat, Montpellier, 2009, 306.

[26] Jeong, S. M., Kim, S. Y., Kim, D. R., Jo, S.C., Nam, K. C., Ahn, D. U. and Lee S. C., "Effects of heat treatment on the antioxidant activity of extracts from citrus peels". Journal of Agriculture and Food Chemistry, 52: 3389-3393. 2004.

[27] Kumaran, A. and Karunakaran, R. J., "In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India". Lebensmittel-Wissenschaft und Technologie, 40: 344-352. 2007.