MOL2NET, International Conference Series on Multidisciplinary Sciences
http://sciforum.net/conference/mol2net-03

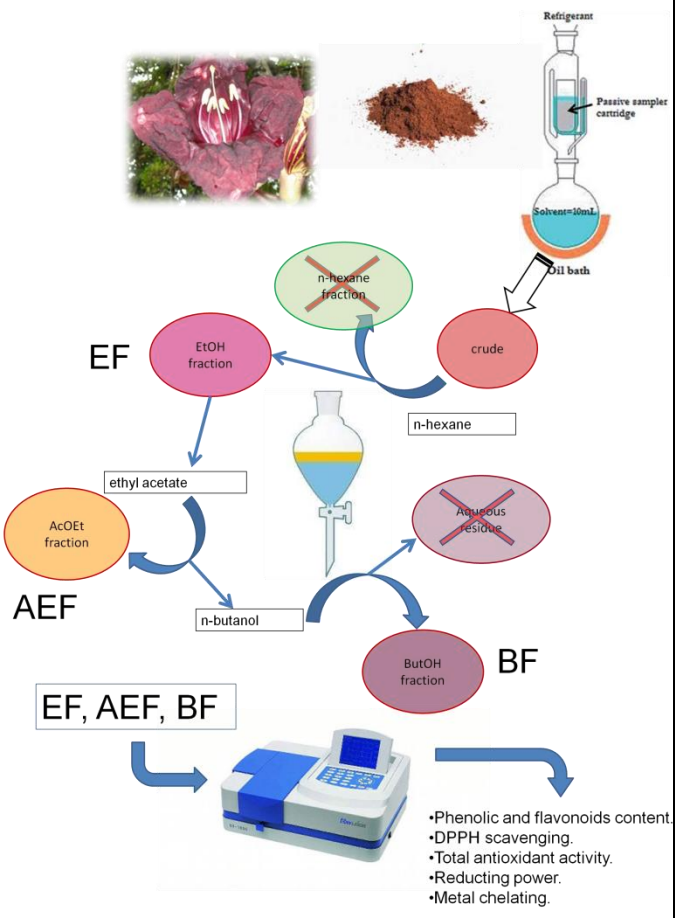
Potent antioxidant activity of *Kigelia africana* flower fractions on cell-free systems.

Enoel Hernández Barreto (enoelh@uclv.edu.cu)*[a], Luis A. Torres Gómez (luistg@ifal.uh.cu)[b], Venancio Ribalta Ribalta[a], Yuniesky Armas González[a], Vivian Ruz Sanjuan[a], Mirtha Mayra González Bedia[a].

[a] *Departamento de Farmacia. Facultad de Química y Farmacia. Universidad Central ´´Martha Abreu´´ de Las Villas. Santa Clara, Villa Clara. Cuba.*

[b] *Instituto de Farmacia y Alimentos. Universidad de la Habana. La Habana. Cuba.*

Graphical Abstract



Abstract

The aim of this work is to explore the antioxidant properties of three organic fractions obtained from *Kigelia africana* flowers on several cell-free systems. The vegetal material was subject to extraction with ethanol (90%) by soxhlet apparatus. Ethanolic **(EF)**, ethylacetate **(EAF)** and buthanolic **(BF)** fractions were obtained from crude ethanolic solution by liquid-liquid extraction procedures. Total Phenolic content (TPC) and Total Flavonoids content (TFC) were determined by Folin-Ciocalteu and $AlCl_3$ spectrophotometric methods respectively. The antioxidant and radical scavenging profile was assessed through 2, 2 – diphenyl – 1 – picrylhydrazyl (DPPH), Reducting power, Total antioxidant activity and Metal Chelating tests. Quercetin, rutin, gallic acid, hesperidin, ascorbic acid and Na_2EDTA were used as references. The antioxidant potency was strongly related with TPC and TFC values. This study reveals for the first time the antioxidant properties of *K. africana* flower fractions on cell-free systems. **Key Words:** Antioxidant, DPPH, *K. africana*.

Introduction

Many active compounds from medicinal plants, especially polyphenols and flavonoids, exhibit potential use as antioxidant agent against oxidative damage and cardiovascular disease (1), the first death cause in the world (2). The relation between oxidative stress and many human diseases as cancer, obesity, autism, arthritis, enteritis, hepatitis, diabetes mellitus, Parkinson disease, Alzheimer, cataracts, chronic renal disease, atherosclerosis and ageing are well documented (3-12).

Kigelia africana (Lam.) Benth. of Bignoniaceae family is an african medicinal tree from tropical zones that has been used as remedy in folkloric and natural medicines. The plant is used traditionally for numerous diseases such as psoriasis, eczema, wounds healing, fungal infections, rheumatism, diarrhea and stomach ailments. It is also use for skin care (13, 14). Some studies reported the antioxidants properties of *K. africana* aerial parts (15, 16). Nevertheless, the antioxidant potential of *K. africana* flowers not has been reported (13).

Materials and Methods

Fresh flowers of *K. africana* were collected in the Botanical Garden of the Central University of Las Villas. Plant sample was identified as *Kigelia africana* (Lam.) Benth. (Bignoniaceae) by a taxonomic expert of above Institution. The vegetal material was subject to extraction with ethanol (90%) by soxhlet apparatus. Ethanolic **(EF)**, ethylacetate **(EAF)** and buthanolic **(BF)** fractions were obtained from crude ethanolic solution by liquid-liquid extraction procedures.

The qualitative phytochemical analysis was carried out according to the ferric chloride, Shinoda, Baljet, *Bornträger*, Drangendorff, Kedde and Lieberman-Burchard tests as previous reported with slight modifications (17). For quantitative purposes, total phenolic content (TPC) was determined by Folin-Ciocalteu spectrophotometric method, reported as μg galic acid equivalents/mg dry extract (μgGAE/mgdE). **T**otal flavonoids content (TFC) was also determined by $AlCl_3$ spectrophotometric method, reported as μg quercetin equivalents/ mg dry extract (μgQE/mgdE) (18).

The antioxidant and radical scavenging profile was assessed through free radical scavenging (DPPH), reducting power (potassium ferricyanide), total antioxidant activity (phosphomolibdene) and metal chelating (Fe^{++}-Ferrozine) tests. Different doses of each fraction (1-400 μg/ml) were tested and the results were taken for constructing the respective concentration-effect curve. Quercetin, rutin, gallic acid, hesperidin, ascorbic acid and Na_2EDTA were used as references.

The IC_{50} (or EC_{50}) was calculated for each fraction or reference from concentration-effect curves using linear and non-linear regression.

The potency score (PS) was calculated individually for each substance in all tests according to the follow expression:

$$PS = \left(\frac{Ca50}{Ci50}\right);$$

Where: Ca_{50}, quercetin IC_{50} or EC_{50} value in a particular test; Ci_{50}, fraction or reference (not quercetin) IC_{50} or EC_{50} value in the same test.

Results and Discussion

Positive results were found for phenols, flavonoids, coumarins, and alkaloids in all fractions. The qualitative phytochemical analysis reveals that quinones are not present (or in a few amount only) in BF, however triterpens/steroids were detected only in this fraction but not in EF and EAF (table 1).

Table 1: Phytochemical screening of *K. africana* flowers fractions.

Classes of phytochemicals	Assay	Fractions		
		EF	**EAF**	**BF**
Phenols and tannins	$FeCl_3$	++	++	++
Flavonoids	Shinoda	+ (red[a])	+ (orange[b])	+ (red[a])
Coumarins	Baljet	+	+	+
Quinones	*Bornträger*	+	++	-
Alkaloids	Drangendorff	++	+	++
Cardiac glycosides	Kedde	-	-	-
Triterpenes and/or steroids	Lieberman-Burchard	-	-	+

+: positive, -: negative, ++: strong, a: flavonols?, b: flavones?.

Phenols and flavonoids are plant secondary products that may contribute to the natural antioxidant system against negative redox balance in human diseases (19). The total amount and particular chemical characteristics of these metabolites are relevant at this point, including the role as prooxidant agent (4, 19-22). The total phenolic content (TPC) and total flavonoids content (TFC) found for EAF (µGAE/mgdE = 523.31±23.40; µQE/mgdE = 43.57±3.46) were highest ($p<0.05$) than BF (µGAE/mgdE = 290.66±35.15; µQE/mgdE = 32.29±1.41) and EF (µAGE/mgdE = 116.02±13.47; µQE/mgdE = 14.25±1.36). According to these results, it is possible that the antioxidant potency score will show a direct relation with TPC and TFC.

Table 2: Potency score and IC_{50}, EC_{50} values for fractions and references. The values expressed as statistic mean ± standard deviation of sixth experiments.

Antioxidant	**DPPH**	**PS**	**TAA**	**PS**	**RP**	**PS**
	CI_{50}(µg/ml)		**CE_{50}(µg/ml)**		**CE_{50}(µg/ml)**	
quercetin	0.57±0.03	1	9.32±0.26	1	2.43±0.14	1
rutin	1.48±0.20	0.39	181.89±2.85	0.05	6.03±0.14	0.4
ascorbic acid	3.10±0.08	0.18	10.83±0.06	0.86	2.78±0.02	0.87
hesperidin	ND	-	> 400	-	ND	-
EAF	4.96±0.25[a,b,c,e,f]	0.11	28.99±0.62 [a,b,c,e,f]	0.32	5.16±0.26 [a,b,c,e,f]	0.47
BF	7.09±0.46[a,b,c,d,f]	0.08	99.36±1.88 [a,b,c,d,f]	0.09	5.94±0.32 [a,b,c,d,f]	0.41
EF	13.57±0.67[a,b,c,d,e]	0.04	204.55±10.14 [a,b,c,d,e]	0.05	20.02±1.19 [a,b,c,d,e]	0.12

PS: potency score, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, TAA: total antioxidant activity, RP: reducting power.

[a,b,c,d,e] statistically significant ($p<0.05$), a:quercetin, b: rutin, c: ascorbic acid, d: EAF, e: BF, f: EF.

In fact, the antioxidant profile of three fractions was in accordance with theirs TPC and TFC values (table 2). EAF exert the best antioxidant effect, which was similar to ascorbic acid and rutin. However, while DPPH scavenging, reducting power and total antioxidant activity tests revealed good results, the

metal chelating capacity was very low for all of them (~ ≤ 35%) (data not shown). The Na_2EDTA, unsurprising, showed a potent iron chelating effect (IC_{50} = 4.02±0.1 µg/ml).

Conclusions

This study reveals for the first time the antioxidant and free radical scavenging properties of *K. africana* flower fractions on cell-free systems. TPC and TFC for these fractions were also reported.

References

1. Liwa AC, Barton EN, Cole WC, Nwokocha CR. Bioactive Plant Molecules, Sources and Mechanism of Action in the Treatment of Cardiovascular Disease. In: Badal McCreath S, Delgoda R, editors. Pharmacognosy: Fundamentals, Applications and Strategies. 1st Edition ed: Academic Press; 2016. p. 315-36.
2. World Health Organization. Cardiovascular diseases (CVDs). WHO; 2017; Available from: http://www.who.int/mediacentre/factsheets/fs317/en/.
3. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiological Reviews. 2014;94:329-54.
4. Carocho M, Ferreira I. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food and Chemical Toxicology. 2013;51:15-25.
5. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. Journal of Clinical Investigation. 2004;114(12).
6. Ho E, Galougahi KK, Liu C-C, Bhindi R, Figtree GA. Biological markers of oxidative stress: Applications to cardiovascular research and practice. Redox Biology. 2013;1:483-91.
7. Hwang O. Role of Oxidative Stress in Parkinson's Disease. Experimental Neurobiology 2013;22(1):11-7.
8. Indo HP, Yen H-C, Nakanishi I, Matsumoto K-i, Tamura M, Nagano Y, et al. A mitochondrial superoxide theory for oxidative stress diseases and aging. Journal of Clinical Biochemistry and Nutrition. 2015;56(1):1-7.
9. Majzunova M, Dovinova I, Barancik M, Chan JY. Redox signaling in pathophysiology of hypertension. Journal of Biomedical Science. 2013;20(69).
10. Siti Hawa N, Yusof K, Kamsiah J. The Role of Oxidative Stress, Antioxidants and Vascular Inflammaton in Cardiovascular Disease (A Review). Vascular Pharmacology. 2015.
11. Small DM, Coombes JS, Bennett N, Johnson DW, Gobe GC. Oxidative stress, anti-oxidant therapies and chronic kidney disease. Nephrology. 2012;17:311-21.
12. Wenzel P, Kossmann S, Münzel T, Daiber A. Redox regulation of cardiovascular inflammation – Immunomodulatory function of mitochondrial and Nox-derived reactive oxygen and nitrogen species. Free Radical Biology and Medicine. 2017;109:48-60.
13. Idris Bello, Mustapha W. Shehu, Mustapha Musa, Mohd. Zaini Asmawi, Mahmud R. Kigelia africana (Lam.) Benth. (Sausage tree): Phytochemistry and pharmacological review of a quintessential African traditional medicinal plant. Journal of Ethnopharmacology. 2016;189:253-76.
14. Saini S, Kaur H, Verma B, Ripudaman, K SS. Kigelia africana (Lam.) Benth.- An overview. Natural Product Radiance. 2009;8(2):190-7.
15. Akanni OO, Owumi SE, Adaramoye OA. In vitro studies to assess the antioxidative, radical scavenging and arginase inhibitory potentials of extracts from Artocarpus altilis, Ficus exasperate and Kigelia africana. Asian Pacific Journal of Tropical Biomedicine. 2014;4(Suppl 1):S492-S9.
16. Nasiru A, Oluwasegun A. In Vitro Free Radical Scavenging Activity and Total Phenolic Content of Kigelia Africana (Lam). International Journal of Science and Research. 2014;3(1):368-70.
17. Villanueva CEB, Castillo FD, Estrada HG. Preliminary phytochemical screening of promising plant species of the Colombian Atlantic coast. Revista Cubana de Plantas Medicinales. 2013;18(4):619-31.
18. Pękal A, Pyrzynska K. Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay. Food Analytical Methods. 2014;7:1776–82.
19. Wang T-y, Li Q, Bi K-s. Bioactive flavonoids in medicinal plants: structure, activity and biological fate. Asian Journal of Pharmaceutical Sciences. 2017.

20. Banjarnahor SDS, Artanti N. Antioxidant properties of flavonoids. Medical Journal of Indonesia. 2014;23(4):239-44.
21. de Queiroz Ferreira R, Greco SJ, Delarmelina M, Weber KC. Electrochemical quantification of the structure/antioxidant activity relationship of flavonoids. Electrochimica Acta. 2015;163:161-6.
22. Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 2011;82:513-23.