Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 3(6): 174-178
Received: 06-01-2015
Accepted: 12-02-2015

Timoléon Andzi-Barhé
Laboratoire de Chimie des Substances Naturelles, Faculté des Sciences (FS), Université des sciences et techniques de MASUKU (USTM), BP: 941, Franceville - GABON.

Klo Kevin Massala
Laboratoire de Chimie des Substances Naturelles, Faculté des Sciences (FS), Université des sciences et techniques de MASUKU (USTM), BP: 941, Franceville - GABON.

Louis Clément Obame Engonga
Laboratoire de Chimie des Substances Naturelles, Faculté des Sciences (FS), Université des sciences et techniques de MASUKU (USTM), BP: 941, Franceville - GABON.

Jacques Lebibi
Laboratoire de Chimie des Substances Naturelles, Faculté des Sciences (FS), Université des sciences et techniques de Masuku (Ustm), BP: 941, Franceville - GABON.

Correspondence:
Timoléon Andzi-Barhé
Laboratoire de Chimie des Substances Naturelles, Faculté des Sciences (FS), Université des sciences et techniques de Masuku (Ustm), BP: 941, Franceville - GABON.

Phytochemical studies, total phenolic and flavonoids content and evaluation of antiradical activity of the extracts of the leaves from *Dischistocalyx sp.* (Acanthacées)

Timoléon Andzi-Barhé, Klo Kevin Massala, Louis Clément Obame Engonga, Jacques Lebibi

Abstract
The present study appears in the setting of the valorization of food and medicinal plants of Gabon. The traditional use of *Dischistocalyx sp* for the treatment of anemia and the absence of scientific works on this species incited us to achieve our survey on this plant. The phytochemical screening, The Folin-Ciocalteu method, the method of aluminum trichlorure ($AlCl_3$, 2%) and DPPH radical was used, respectively, to identify the chemical groups content of *Dischistocalyx sp,* to quantify flavonoids and polyphenols content and to measure the Antiradical activity. Four extracts (water extract, ethanolic-water extract, ethanolic-acidified extract and ethanolic extract) have been analyzed. The qualitative analysis permitted to identify the presence of polyphenols, anthocyanins, tannins, triterpenoids, reducing sugars, flavonoids, glycosides and saponins in the different extracts. Quantitative analysis showed that the content in flavonoids was raised more in ethanolic-acid extract (330.406 ± 0.021 mg EQ/g) and total polyphenols in the ethanolic-water extract (290.33 ± 0.037 mg EAG/g). Radical Scavenging Activity (%RSA) increasing in the following order: % RSA (water extract) < % RSA (ethanolic extract) < % RSA (ethanolic-acidified extract) <% RSA (ethanolic-water extract)

Keywords: *Dischistocalyx sp., phytochemical screening, polyphenols, flavonoids, antiradical activity, DPPH.*

1. Introduction
In recent years, the interest in natural antioxidants, in relation to their therapeutic properties, has increased dramatically. Previous scientific research has shown that most of natural or synthetic antioxidants have phenolic hydroxyl groups in their structures and antioxidant properties are attributed to the ability of these compounds to scavenge free radicals.
Free radicals are chemical species having one or more unpaired electrons in their outer layer. A gap in antioxidant system or an overproduction of free radicals may result in an imbalance between oxidant and antioxidant system, one of the most important factors involved in the genesis of free radicals oxidative stress [1-2]. Thus, oxidative stress is involved in several pathologies such as diabetes [3-4], atherosclerosis, Alzheimer's disease, Parkinson's disease, glaucoma and macular degeneration and age-related [5-6].
An intake of antioxidants through diet is a simple way to reduce the development of induced oxidative stress pathologies [7-8]. These are mostly extracted from medicinal and food plants as secondary metabolites.
To contribute to the valorization of medicinal plants of Gabon, our work has focused on the leaves of Dischistocalyx sp. The leaves of this plant are used in the Gabonese pharmacopoeia for the treatment of anemia.
The objective of the study is to identify, to quantify and to determine the antiradical power of the extracts of the leaves from Dischistocalyx Sp. Thus, four (04) extracts were prepared in four solvent mixtures. Phytochemical screening of each extract was performed to determine the composition of secondary metabolites.
This analysis was followed by quantification of total polyphenols and flavonoids of each extract, and a study of the antiradical activity of total extracts by reaction with the free radical 2.2-diphenyl-1-picrylhydrazyl (DPPH), to find the relationship between the composition of secondary metabolites in each extract, the amount of phenolic compounds and their ability to Scavenge free radicals in the body.

2. Materials and Methods

2.1. Plant material

Dischistocalyx sp. also called Idibundjélé Ndzina in Sindara's autochthons people of Gabon, is a plant belonging to the Acanthaceae family. It's a plant with red flower, composed of underground roots, aerial stem divided and opposite simple leaves two dual. In south-east of Gabon, the leaves are used in the treatment of anemia.

This is a species that grows in groups, with more or less creeping stems that have axillary buds necessary for reproduction by cuttings [9]. The Leaves of Dischistocalyx sp. were harvested in June 2013 in Mbaya neighborhood of Franceville (Gabon). After drying at room temperature and out of direct sunlight, the plant material is ground in an electric mill and kept in a closed bottle.

2.2. Preparation of extracts for phytochemical screening

Air-dried powdered fruits (10 g) of Dischistocalyx sp, were separately extracted with 150 mL of water (100%), mixture ethanol -water (EtOH-H2O, 50:50 v/v), ethanol-acidified (ETOH-Ac, 99.5: 0.5 v/v) and ethanol 100% (ETOH) by maceration for 72 h. Extracts were filtered and the filtrate was used for photochemical screening.

2.3. Preparation of extracts for polyphenols, flavonoids measure and antiradical activity

Air-dried powdered fruits (10 g) of Dischistocalyx sp, were separately extracted with 150 mL of water (100%), mixtures ethanol -water (EtOH-H2O, 50:50 v/v), ethanol-acidified (ETOH-Ac, 99.5: 0.5 v/v) and ethanol 100 % (EtOH) by maceration for 72 h. Extracts were filtered and dried under reduced pressure at 40° C. the four (04) extracts were stored in freezer at 4 °C until further tests

2.4. Phytochemical screening

The four extracts, Water (100%), EtOH-H2O (50:50 v/v), ETOH acidified and ETOH (100%) were screened for their classes of bioactive compounds using standard procedures [10-15]. The extracts were tested qualitatively for the presence of chemical constituents such as tannins, terpenes, saponins, flavonoids, polyphenols, anthocyanins, cardiac glycosides, alkaloids and reducing sugar.

2.5. Determination of total phenol and flavonoids contents

The Folin-Ciocalteu method was used to measure total amount of polyphenol content [16-18]. Aliquots of 0.25 ml of extracts (1 mg/mL) were mixed with 1.25 mL Folin–Ciocalteu reagent (0.2 N diluted in MeOH). A reagent blank using MeOH instead of sample was prepared. After 5 min incubation at room temperature, 1 mL sodium carbonate solution (75 g/L) was added. Samples were incubated at room temperature for 2 h and the absorbance was measured at 765 nm versus the prepared blank with UV GENESYS 10 Bio spectrometer. All tests were carried out in triplicate and total phenol content was expressed as mg of gallic acid equivalents (GAE) per 100 g of extract.

Flavonoids were quantified with the methods of aluminum trichlorure (AlCl3, 2%) [18]. 1 mL of extracts (1 mg/mL) is mixed with 1 mL of AlCl3 2%, after 10 minutes of incubation, the absorbance were measured at 415 nm. Quercetin was used as a standard. Results were expressed as mg of quercetin equivalents (EQ) per 100 g of extract.

2.6. Determination of the Radical Scavenging Activity

The activity is defined by the index of reduction of the radical scavenger activity, expressed as a percentage (%RSA) where the absorbance of the reactional mixture which contains the free radical and the sample of antioxidant is compared with the absorbance of the mixture without the antioxidant (control solution).

$$\%RSA = ((AT - AS)/AT) \times 100$$

AT: absorbance of the control (DPPH° only)
AS: absorbance of the sample (antioxidant + DPPH°)

The activity is measured using the methods described by [19-23]. The radical DPPH° was dissolved in methanol with a concentration of 1.01.10-4 mol/l and kept at -20 °C away from light prior to use. Five test tubes were prepared, of which four contained increasing concentrations of the analyzed extract. 3 ml of DPPH° was added to each tube, and the absorbance was measured after 10 minutes with a GENESYS 10 Bio spectrometer at 517 nm. The total volume of each tube was 3.5 ml. 2, 2-diphenyl-1-picrylhydrazyl free radical was used to study the relationship between structure and antioxidant activity of the four extracts [24-25].

3. Results and Discussion

3.1. Phytochemical screening

Table1 show the results of phytochemical screening for four (04) extracts of the leaves from Dischistocalyx sp.

Table 1: Results of phytochemical screening of extracts of Dischistocalyx sp.

Chemical constituents		Water extract	Ethanolic-water extract	Acidified-ethanolic extract	Ethanolic extract
Saponins		+	-	-	-
Triterpenoids		+ +	+ + +	+ + +	+ + +
Alkaloids		-	-	-	-
Reducing sugars		+ +	+ + +	+ + +	+ + +
Polyphenols		+ ++	+ + +	+ + +	+ + +
Anthocyanins		+ + +	+ + +	+ + +	+ +
Tannins	*Gallic*		++	+++	+++
	Catechin		+++	++	++
Flavonoids	*Flavonols*		-	-	++
	Flavones		++	+++	+
	Flavanons		-	+	+++
Cardiac glycosides	*Digitoxin*		-	-	-
	Digitoxigenin		++	+++	++
	Gitoxin		-	-	-
	Gitoxigenin		-	-	-

Legend: -: Not detected, +: Rare, ++: Abundant, +++: Very abundant,

The phytochemical screening of the extracts was first performed to detect the major chemical groups occurring in the extracts. In view of the results in Table 1, it appears that Dischistocalyx sp contains triterpenoids, polyphenols, anthocyanins, flavonoids, tannins, reducing sugars and cardiac glycosides, especially digitoxigenin. In addition to these compounds, water and ethanolic extracts contains respectively saponins and gitoxigenin.

There is also a presence of flavonol and flavonon, especially in alcoholic extracts. This suggests that these compounds are

stable in the acidified alcohol solvents, because there are the large amounts in the acidified-ethanol extract.

The strong presence of anthocyanin and tannins are responsible for the red color [26] observed in our study and also by traditional therapists in their use leaves Dischistocalyx sp for the treatment of anemia.

The presence of glycosides and sugars in different extracts suggests that anthocyanins in the leaves of Dischistocalyx sp. are glycosylated compounds. This glycosylation increases the stability of anthocyanins [25].

All of these bioactive secondary metabolites identified in the various drugs have many pharmacological properties assigned to them [27-30]. These properties from compounds found in the extracts of this plant suggest that they can be used in pharmaceuticals.

3.2. Total phenolic and flavonoids Content

Levels of phenolic content were expressed in terms of gallic acid equivalent (GAE). The equation of the right hand side of the proportioning of total phenolic content by the method of Folin- Ciocalteu gave Y = 0.0012 X – 0.0004 with R2 = 0.9902 [31]. The total phenolic compound contents in the plant extracts are shown in Table 2.

Table 2: Phenolic and flavonoids content of four extracts tested

Plants / Extracts	Total phenols (mg GAE/100 g of extract)	Flavonoids Content (mg EQ/g of extract)
Water extract	214.5 ± 0.008	140.562 ± 0.0325
Ethanolic-water extract	290.33 ± 0.037	307.75 ± 0.0325
Acidified-ethanolic extract	222.416 ± 0.0025	330.406 ± 0.021
Ethanolic extract	217 ± 0.003	241.968 ± 0.01

It appear that hydro-ethanolic extract of Dischistocalyx sp. had the highest content of phenolic compounds (290.33±0.037 mg GAE/100 g of extract) and water extract the lowest content (214.5±0.008 mg GAE/100 g of extract). Ethanolic and acidified ethanolic extract shows intermediate phenolic content followed by ethanolic extract with 222.416±0.0025 and 217.00±0.003 mg GAE/100 g of extract, respectively.

This difference can be explained by the fact that hydro-alcoholic extracts and Acidified-alcoholic possess large amounts of Gallic tannins (Table 1) that the aqueous and alcoholic extracts. It's also seen in the color of the extracts. Indeed, the hydro-alcoholic extract showed a sharper red color as the Acidified-alcoholic, alcoholic and aqueous extract. There is showed that the extract contains more tannins and anthocyanins, pigments responsible for the red color of plants and possessing more OH groups.

These results confirm those obtained from the phytochemical screening of leaves from Dischistocalyx sp. (Table 1). They show that leaves of Dischistocalyx sp are a significant source of polyphenols.

Phenolic substances have been suggested to play a preventive role in the development of chronic diseases such as cancer and heart disease [32]. Phenolic extracts have been reported to retard lipid oxidation in oils and fatty foods [33], decrease the risk of heart diseases by inhibiting the oxidation of low-density lipoproteins. They are also known to possess antibacterial, antiviral, anti-mutagenic and anti-carcinogenic properties [28, 34]

Levels of flavonoids were expressed in terms of quercetin equivalent (EQ). The equation of the right hand side of the proportioning of the quercetin by the Aluminum trichlorure method gave Y=0.0032X + 0.0077 with R2 = 1 [18]. Among extracts, flavonoids contents had ranged between 140.562 ± 0.032 and 330.406 ± 0.021 mg EQ/ g of extract (Table 2). Acidified ethanolic extract contain the highest amount of flavonoids (330.406 ± 0.021 mg EQ/g) and water extract the lowest amount (140.562 ± 0.032 mg EQ/g). Ethanolic and ethanolic-water extracts had revealed weak flavonoids contents (241.968 ± 0.01 mg EQ/g) and (307, 75 ± 0, 0325 mg EQ/g) respectively.

We note that the lowest level is always obtained in the aqueous extract. This could suggest that water is not a good solvent for extraction of polyphenols and flavonoids in leaves of Dischistocalyx sp.

However, there is an inversion between the hydro-alcoholic extract and acid-alcohol compared to the determination of polyphenols. These results are in agreement with those obtained in the phytochemical screening (Table 1).

Indeed, we find that the Acidified-alcoholic extract contains high amounts of flavonoids analyzed, including Flavonols, flavones and Flavanons, while the hydro-alcoholic extract is devoid of Flavonols. In the case of aqueous and alcoholic extracts, the first is devoid of Flavonols and Flavanons, while the second contains a small quantity.

These results show that the studied plant is rich in flavonoids. The importance of drug rich in flavonoids is related to their antiviral properties, anti-tumor, anti-inflammatory, anti-allergic and anticancer [27, 29]. As in the case of total polyphenols, the antioxidant activity of phenolic groups, gives flavonoids the power to fight against free radicals in the body [21, 35-36].

3.3. Radical-scavenging activity

2.2-diphenyl-1-picrylhydrazyl radical was used to measure the antiradical capacity of our extracts. Table 3 summarizes the results of absorbance in function of concentration for four (04) extracts from the leaves of Dischistocalyx sp.

Table 3: Absorbance of the four extracts tested according to the concentration

Tubes	Concentration (µg/ml)	Absorbance (517 nm)			
		Extracts			
		Water	Ethanolic-water	Acidified-ethanolic	Ethanolic
1	0	0,98	0,98	0,98	0,98
2	20	0,90	0,43	0,66	0,87
3	40	0,84	0,24	0,59	0,78
4	60	0,73	0,22	0,50	0,68
5	80	0,65	0,18	0,45	0,53

Figures 1 (a, b, c, d) gives the Radical-scavenging activity (% RSA) of the various extracts according to the concentration. The four curves show the strong dependent of the RSA to the concentration. So, if the concentration of the extract is higher,

the decrease in absorbance of DPPH ° is greater. Thus, Radical-scavenging activity (% RSA) is high.

The antiradical activity of these extracts is due to the presence of polyphenols, particularly tannins, flavonoids and anthocyanins (Table 1). Indeed, these compounds are free radical scavengers.

Previous studies [17-18, 24-25] have shown that these compounds may have an interest in the health care due to their reactivity with the free radicals in the body.

For the four extracts tested, it appears that, for the same concentrations, Radical-scavenging activity (% RSA) increasing in the following order: % RSA (aqueous extract) <% RSA (alcoholic extract) <% RSA (from acid-alcoholic) <% RSA (hydro-alcoholic extract). This development follows the same trend as that of the composition of total polyphenols. These results confirmed the relationship between phenolic content and antiradical activity [19, 21-25]. Thus, antiradical capacity is a function of the total polyphenol content of an extract.

So, hydro-alcoholic extract has the highest antiradical activity than three (03) other extracts. These results are in agreement with the results of the phytochemical screening and the amount of polyphenols and flavonoids in our extracts.

This activity could justify the use of this plant in the treatment of anemia in Gabon, including iron anemia [37]. Indeed, the anemia is due to inadequate oxygen transport in the body, a diet rich in polyphenolics compounds provides additional oxygen to the body, preventing the use of the blood oxygen by disease. This promotes a good oxygen transport and better iron binding.

4. Conclusion

The phytochemical screening showed that the leaves of Dischistocalyx sp. contain polyphenols, anthocyanins, tannins, triterpenes, reducing sugars, flavonoids and digitoxigenins in the four extracts analyzed. Alkaloids, glycosides and saponins are almost absent in all extracts, except that saponins and gitoxigenins respectively found in the water extract and in the ethanolic-water extract.

The quantitative analysis of total polyphenols and flavonoids has confirmed the presence of these compounds in the different extracts analyzed. It appears that the flavonoid content is higher in acidified-ethanolic extract (330.406 ± 0.021 mg EQ / g of extract) and the content of total polyphenols in the ethanolic-water extract (290.33 ± 0.037 EAG mg / g of extract).

The test results of the DPPH ° for four extracts analyzed show that these extracts have significant antiradical activity. For the same concentrations, the highest inhibitory activity of free radicals is observed in the hydro-ethanolic extract and the lowest in the aqueous extract. Indeed, for concentrations of 20 µg/mL, we are obtained, respectively, 56.12% inhibition rate in the hydro-ethanolic extract and 8.10% in the aqueous extract.

This work gives us a first mapping of the extracts of the leaves from Dischistocalyx sp and allows us to observe the relationship between the content of phenolic compound and antiradical activity. It contributes to the valorization of this plant and gives an indication of his contribution in the fight against free radicals, responsible for several pathologies. Structural characterization of the compounds responsible for this activity and their applications on the anemic strains will make clear his role and use in the treatment of anemia in Gabon.

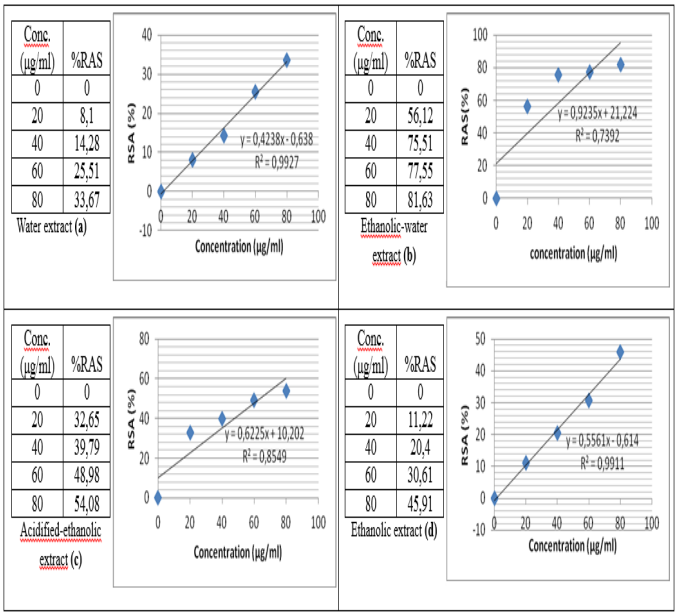

Fig 1: (a, b, c, d): Evolution of Radical-Scavenging Activity (% RSA) according to the concentration

5. References

1. Punitha ISR, Rajendran K, Shirwaikar A, Shirwaikar A. Alcoholic stem extract of *Coscinium fenestratum* regulates carbohydrate metabolism and improves antioxidant status in streptozoto cinnicotinamide induced diabetic rats. ECAM. 2005; 2(3):375-381.
2. Long AC, Colitz CM, Bomser JA. Apoptotic and necrotic mechanisms of stress-induced human lens epithelial cell death. Exp Biol Med (Maywood) 2004; 229:1072-1080.
3. Pincemail J, Meurisse M, Limet R, Defraigne JO. L'évaluation du stress oxydatif d'un individu: une réalité pour le médecin. Vaisseaux, Coeur, Poumons. 1999; 4(5):12-23.
4. Huang DJ, Lin CD, Chen HJ, Lin YH. Antioxidant and antiproliferative activities of sweet potato (*Ipomoea batatas* [L.]Lam 'Tainong 57') constituents. Bot. Bull. Acad. Sin 2004; 45:179-186.
5. Drobek-Slowik M, Karczewicz DK. The potential role of oxidative stress in the pathogenesis of the age-related macular degeneration (AMD). Postepy Hig Med Dosw 2007; 61:28-37.
6. Bonne C, Muller A. Role of oxidative stress in age-related macular degeneration. J Fr. Ophtalmol. 2000; 23:835-840.
7. Zafra-Stone S, Yasmin T, Bagchi M, Chattejee A, Vinson JA, Bagchi D. Berry anthocyanins as novel antioxidants in human health and disease prevention. Mol. Nutri. Food. Res 2007; 51:675-683.
8. Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology 2000; 148:187-1897.
9. UNESCO. Pharmacopée ET médecine traditionnelles chez les Pygmées du Gabon 2009, 75.
10. Trease GE, Evans WC. Pharma-cognosy. Edn 15, Saunders, 2002, 214-393.
11. Parekh J, Karathia N, Chanda S. Eval-uation of antibacterial activity and phyto-chemical analysis of *Bauhinia variegata* L. bark. Afr J Biomed Res 2006; 9(1):53-56.

12. Harbone JB. Phytochemical methods. Edn 2, London: Champion and Hall Publishers, 1984, 84-196.
13. Ayoola GA, Ipav SS, Solidiya MO, Adepoju-Bello AA, Coker HAB, Odugbemi TO. Phytochemical screening and free radical scavenging activities of the fruits and leaves of *Allanblackia floribunda* oliv (Guttiferae). International Journal of health Research 2008; 1(2):81-93.
14. Diallo D, Sanogo R, Yasambou H, Traré A, Coulibaly K, Maïga A. Etude des constituants des feuilles de *Ziziphus mauritianalam* (Rhamnaceae) utilisées traditionnellement dans le traitement du diabète au Mali. C.R. Chimie 2004; 7:1073-1080.
15. Dohou N, Yani K, Thahrouch S, Idrissi Hassani L-M, Badoc A, Gmira N. Screeming phytochimique d'une endémique ibéro- Marocaine, Thynelaealythroides. Bull. Soc. Pharm. Bordeaux 2003; 142:61-78.
16. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidant substrates and antioxidents by means of Folin-ciocalteu reagent, Methods Eneymol. 1999; 299:152-178.
17. Ondo JP, Obame LC, Andzi Barhé T, Nsi GA, Nsi EE, Lébibi J. Phytochemical screening, total phénolic content and antiradical activity of *Asplenium africanum* (Aspleniaceae) and fruit of *Megaphrinium macrostachyum* (Marantaceae), Applied Pharmaceutical Science 2013; 3(08):092-096.
18. Obame Engonga LC, Andzi Barhé T, Ondo JP, Mewono L, Ndong Atome GR, Abdoul-Latif F *et al.*, Total Phenolic Composition, Antibacterial and Antioxidant Activities of Fagaraheitzii Aubr & Pellegr Medicinal Plant of Gabon", Vedic Research International Phytomedicine 2013; 1(3)64-69.
19. Awika JM. Screening method to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. J Agric Food Chem 2003; 51:6657-6662.
20. Bakkalbas E, Yemis O, Aslanova D, Artik N. Major flavan-3-ol composition and antioxidant activity of seeds from different grape cultivars grown in Turkey. Eur Food Res Technol 2005; 221:792-797.
21. Makris DP, Boskou G, Andrikopoulos NK. Recovery of antioxidant phenolics from white vinification solid by-products employing water/ethanol mixtures. Bioresource Technology 2007; 98:2963-2967.
22. Hua L, Xiaoyu W, Peihong L, Yong L, Hua W. Comparative Study of Antioxidant Activity of Grape (*Vitis vinifera*) Seed Powder Assessed by Different Methods. Journal of Food and Drug Analysis 2008; 16(6):67-73.
23. Berrin B, Goksel T, Ozcan D. Study on polyphenol content in the seeds of red grape (*Vitis vinifera* L.) varieties cultivated in Turkey and their antioxydant activity. Food chemistry 2008; 209:426-430.
24. Andzi BT, Obame ELC, Ondo JP. Characterization of Anthocyanic compounds and evaluation of the radical scanvenging power of *Glycine max* L. Merr. Seed. International Journal of Current Chemical Sciences 2013; 3(5):11-16.
25. Andzi Barhe´T, Feuya TGR. Comparative study of the anti-oxidant ac-tivity of the total polyphenols extracted from *Hibiscus Sabdariffa* L., *Glycine max* L. Merr., yellow tea and red wine through reaction with DPPH free radicals. Arabian Journal of Chemistry 2015.
26. Someurs TC. Wine tannins: Isolation of condenses flavonoid pigments by gel filtration. Nature 1966; 209:368-370.
27. Kim J. Protective effect of Epillagocatechin-3-gallate on UVA and UVB-induced skin damage, Skin pharmacol. Appl. Skin physiol 2001; 14:11-19.
28. Okouda T. Natural polyphénols as antioxydant and their potential use in cancer prevention, in polyphénolic phenomena. Ed. INRA, Paris 1993; 221-231.
29. Chang WS, Lee YJ, Lu FJ, Chiang HC. Inhibitory Effect of Flavonoids on Xanthine Oxidase. Anticancer Research. 1993; 13:2165.
30. Bourzeix M. Influence des proanthocyanidines du vin sur la santé, dans polyphénolic phénoména, Ed. INRA, Paris. 1993; 1987-1997.
31. Abdoul-latif FM, Bayili RG, Obame LC, Bassolé IHN, Dicko MH. Comparison of phenolic compounds and antioxidant capacities of traditional sorghum beers with other alcoholic beverages. Afr J Biotechnol 2012; 11(81):14671-14678.
32. Njintang YN, Tatsadjieu NL, Ngakou A, Danra D, Tchuenguem-Fohouo FN. Antiradical activity and polyphenol content of ethanolic extracts of Propolis. Int J Biosci 2012; 2(4):56-63.
33. Rumbaoa RGO, Cornago DF, Geroni-mo IM. Phenolic content and antioxidant capacity of phillipine potato (*Solanum tuberosum*) tubers. J Food Compos Anal 2009; 22:546-550.
34. Toufektsian MC, Lorgeril M, Nagy N, Salen P, Donati MB, Giordano L *et al.* Chronic dietary intake of plant-derived anthocyanins protects the rat heart against ischemia-reperfusion injury. Journal of Nutrition. 2008; 138:747-752.
35. Blois MS. Antioxidant determinations by the use of stable free radical, Nature 1958; 181:1199-1200.
36. Sanchez-Moreno C, Larrauri JA, Saura-Calixto F. A Procedure to Measure the Antiradical Efficiency of Polyphenols. Journal of the Science of Food and Agriculture 1998; 76(2):270-276.
37. Bilodeau VK, L' Anemie. http:// www. Institut de l'anemie.ca 2007,