WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 2, 1610-1622. Research Article ISSN 2277– 7105

COMPARATIVE RADICAL SCAVENGING PROPERTIES OF FIVE SPICES COMMONLY CONSUMED IN CAMEROON

Armelle D Tchamgoue[1], Lauve RY Tchokouaha[1], Protus Arrey Tarkang[1], Joe A Vinson[2] and Gabriel A. Agbor[1, 2]*

[1]CRPMT, Institute of Medical Research and Medicinal Plants Studies, P.O Box 6163, Yaoundé Cameroon.

[2]Department of Chemistry, University of Scranton, Scranton, Pennsylvania, 18510 USA.

Article Received on
10 Dec 2014,

Revised on 04 Jan 2015,
Accepted on 29 Jan 2015

***Correspondence for Author**
Dr. Gabriel A. Agbor
CRPMT, Institute of Medical Research and Medicinal Plants Studies, P.O Box 6163, Yaoundé Cameroon.

ABSTRACT

The present study was designed to evaluate *R. heudelotii*, *S. zenkeri*, *A lepidophyllus*, *I. gabonensis* and *T. vulgaris*, common Cameroonian spices for radical scavenging activity. This was carried out by analysing their methanolic extracts against different assays such as DPPH, superoxide radical, hydroxyl radical, and nitric oxide and their reducing power potency. It was observed that all the plants studied had a dose dependent radical scavenging activity irrespective of the method used. *I. gabonensis* seed extract had the best DPPH radical, superoxide radical and nitric oxide scavenging activity. *I. gabonensis and T.vulgaris* were found to be more potent in reducing power meanwhile *R. heudelotii* extract presented the best hydroxyl radical scavenging activity. The results obtained confirmed that methanolic extracts of *R. heudelotii*, *S. zenkeri*, *A lepidophyllus*, *I. gabonensis* and *T. vulgaris* posses radical scavenging activity which may be accountable to their antioxidant potentials.

KEYWORDS: *R. heudelotii*, *S. zenkeri*, *A lepidophyllus*, *I. gabonensis*, *T. vulgaris*, radical scavenging activity.

INTRODUCTION

The main free radicals that generate redox processes in biological systems are reactive oxygen species (ROS) and they are the main contributors to oxidative stress.[1,2] Various forms of ROS exist with superoxide anion, hydroxyl radicals, hydrogen peroxide and singlet oxygen as examples.[3] Oxidative stress has been linked to non communicable diseases such

as atherosclerosis, Parkinson's disease, Alzheimer's disease, stroke, arthritis, chronic inflammatory diseases, cancers and other degenerative diseases.[1,2] Hence, a diet rich in antioxidant is an asset to cells exposed to oxidative stress. Because of their functions as antioxidant, antimutagenic and antitumor activities, phenolics have received considerable attention in research, all reporting on the antioxidant potentials of one extract or a mixture of extracts. [3-8] Increased consumption of fruits and vegetables has been associated with a lower risk of degenerative diseases such as cancer, cardiovascular disease, cataracts and brain and immune dysfunction.[9]

The properties of spices and herbs to increase both flavor and taste have made them common ingredients in every cuisine. Hence, their dietary contribution and functional perspective are of concern. Cameroon has a rich flora with more than 13, 000 species of plants identified. The multi-cultural background of Cameroon exposes Cameroonians to a variety of well-spiced diets that hardly go sour even in the absence of a refrigerator because of their antioxidant properties. Hence, it is important that some of the commonly consumed spices be screened for ROS scavenging activity.

Irvingia gabonensis commonly known as 'African mango' or 'bush mango' is a tree that grows up to 40 meters in height, with a slightly buttressed bole. It is largely distributed in Africa.[10, 11] It is largely used in traditional and modern medicine for the treatment of a variety of illnesses such as hunchback and infectious diseases while the seed serves as the main spice in a traditional soup known as ogbono soup.[12, 13] *Ricinodendron heudelotii* is a perennial native tree in the tropic and sub-tropic areas, reaching 40 m in height and 1.2 m of diameter, belonging to the *Euphorbiaceae* family.[14] The bark extract of *Ricinodendron heudelotii* is used for the treatment of cough, intestinal diseases, yellow fever, malaria, headache, stomach pains and as poison antidote.[15] The fruit of *R. heudelotii* commonly known as njangsang serves as spice in many traditional dishes including the ogbono soup in Africa. *Scorodophloeus zenkeri* and *Afrostyrax lepidophyllus* are tropical garlic trees from different families but with similar garlic flavor. A phytochemical study of the bark of *S zenkeri* revealed the presence of sulfur rich compounds and some essential oils with antimicrobial activity.[16,17] Traditionally, *Afrostyrax lepidophyllus* bark extract is used as a remedy for children's cough, heart beat rate, worms, constipation, hernia, abscesses, and boils.[18] Moreover, the root bark decoction is drunk as anthelmintic, against vomiting, or as enema against urinary infections.[19] Both the seeds and the stem back *A. lepidophyllus* and *S.*

zenkeri are used in the preparation of special meals for those who are believed to have spiritual attacks in traditional medicine. *Thymus vulgaris* is an aromatic plant belonging to the *Lamiaceae* family, used as medicine and as a spice. Thyme essential oil is exploited in the perfumery and cosmetic industry based on its characteristic aroma.[20] *Thymus vulgaris* has earlier been assessed for FRAP total antioxidant capacity and was found to contain 45.4 mmol/100g in the French Thyme and 95.0 mmol/100g in the English Thyme.[21] In our earlier studies, we reported the Ferric reducing antioxidant power (FRAP), total phenolic content and the antioxidative property in LDL/VLDL cholesterol oxidation of *Recinodendron heudelotii, Scorodophloeus zenkeri, Afrostyrax lepidophyllus, Irvingia gabonensis and Thymus vulgaris*.[22, 23]

The present study was designed to test the hypothesis that *Recinodendron heudelotii, Scorodophloeus zenkeri, Afrostyrax lepidophyllus, Irvingia gabonensis and Thymus vulgaris* are potential radical scavengers as a justification of their antioxidant potential in the prevention of oxidation of LDL/VLDL cholesterol.

MATERIALS AND METHODS

Reagents

Deoxyribose, thiobarbituric acid (TBA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), (TCA), Folin-Ciocalteu reagent, ferric chloride ($FeCl_3$), potassium ferricyanide, phenazine methosulphate (PMS), nitro blue tetrazolium (NBT), nicotinamide adenine dinucleotide phosphate reduced (NADPH), ascorbic acid, sodium nitroprusside, Griess reagent (sulphanilamide, phosphorus acid, n-(1-naphthyl) ethylenediamine dihydrochloride), ethylene diamine tetra acetate (EDTA), Trichloroacetic acid (TCA), methanol, H_2O_2, hydrochloric acid (HCl), phosphate buffer saline (PBS, pH 7.4), sodium acetate.

Sample preparation

Samples were bought from the local markets in Yaoundé, Cameroon. Each sample was prepared as earlier described.[24] In brief, the samples were cleaned with tap water and dried. The edible portions were chopped, weighed and blended in a high speed blender then lyophilized under liquid nitrogen. A weighed portion of the lyophilized samples were then stored at -20^{o}C until analyzed.

100 mg of the lyophilysate was accurately weighed into a 10 ml plastic screw-capped tube and extracted repeatedly with hexane to eliminate lipids. The residue was then collected and

processed as earlier described.[25, 26] 10 ml of methanol and sample were vortexed for 5 minutes and heated at 90°C for 2 hours with intermittent shaking every 30 minutes. The samples were then allowed to cool and the volume made up to 10 ml with methanol, then centrifuged for 10 minutes at 5000 rpm using a bench top centrifuge (Fisher Scientific) to remove solids. The extracts each done in duplicate, were then stored at -20 ^{0}C until analyzed.

Radical scavenging effect of extracts

The free radical scavenging activity of spice extract was assayed by DPPH radical as earlier described.[27] The percentage (%) radical scavenging effect of extract was calculated as follows:

% radical scavenging effect = $[(Abs_1-Abs_2)/Abs_1] \times 100$

Where Abs_1 is the absorbance of the control, and Abs_2 is the absorbance of plant extract.

Scavenging of superoxide radical

The superoxide radical scavenging activity was determined as previously described.[28] The superoxide radical was generated in a PMS-NADPH system by oxidation of NADPH and assayed by the reduction of NBT.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of spice extract was determined as earlier described with modification.[29] The reaction mixture consisted of $FeCl_3$ (300 µM), EDTA (780 µM), 2-deoxiribose (2.8 mM), ascorbic acid (300 µM), H_2O_2 (4mM) and aliquots of extract in a final volume of 1 ml. All the reagents were dissolved in potassium phosphate buffer (20 mM, pH 7.4). This was then incubated at 37°C for 1 hour. After incubation, 1 ml of TCA (2.8 %) and TBA (1 %) were added to the reaction mixture and incubated at 100 °C for 20 minutes, and the absorbance at 532 nm was read spectrophotometrically.

Scavenging activity against nitric oxide (NO)

Nitric oxide scavenging activity of extract was assayed as described by Sreejayan and Rao. [30] This method measured the concentration of nitrite produced by the reaction of nitric oxide and oxygen.

Reducing power

The reducing power of the extracts was determined according to a previously described method.[31]

Statistical analysis

Measurements were carried out in triplicates and the results are presented as mean ± standard deviation (SD). Kruskal-Wallis One Way Analysis of Variance on Ranks was employed in groups and dose comparison. Student Newman-Keuls Test was used to determine significant difference between groups and doses ($P<0.001$). The SigmaStat (Systat software, Richmond, CA) version 3.01 was used for these analyses.

RESULTS AND DISCUSSION

The methanolic extracts of *R. heudoletii* (seeds), *S. zenkeri* (Seeds and bark), *A. lepidophyllus* (seeds), *I. gabonensis* (seeds) and *T. vulgaris* (seeds) were evaluated for their radical scavanging activity of DPPH, superoxide radical, hydroxyl radical, nitric oxide and reducing activity. Some extracts showed promising dose depending activities in all methods of assay used.

Table 1: Dose related DPPH free radical scavenging activity (%) of methanolic extracts of spices.

Species	Parts used	1mg/ml	2mg/ml	4mg/ml	8mg/ml	10mg/ml
R. heudelotii	Seeds	11.64±1.22	24.18±0.61[a]	37.89±0.68[b]	65.66±1.22[c]	84.49±1.12[d]
S. zenkeri	Seeds	8.57±1.13	18.67±1.69[a]	21.73±1.27[b]	35.51±0.78[c]	43.16±1.45[d]
S. zenkeri	Bark	12.30±0.99	17.15±0.94[a]	28.76±1.03[b]	45.05±1.56[c]	51.94±1.11[d]
A. lepidophyllus	Seeds	16.58±1.23	26.03±0.78[a]	42.31±0.49[b]	56.12±0.68[c]	68.57±1.02[d]
I. gabonensis	Seeds	52.55±2.34	80.53±1.52[a]	92.31±1.22[b]	92.38±1.68	93.43±0.62
T. vulgaris	Seeds	34.13±0.98	59.63±1.24[a]	90.41±1.35[b]	92.51±1.07	92.9±1.35

[a] significantly different from 1mg/ml, [b] significantly different from 1mg/ml and 2mg/ml, [c] significantly different from 1mg/ml, 2mg/ml and 4mg/ml, [d] significantly different from 1mg/ml, 2mg/ml, 4mg/ml and 8mg/ml.

The concentration dependent DDPH radical scavenging activities (%) of spices are presented in Table 1. Samples had significant ($P<0.001$) concentration dependent DPPH radical scavenging activity. Among extracts at the highest dose of 10mg/ml, *I. gabonensis* and *T. vugaris* had 90% and above radical scavenging activity and were closely followed by *R heudelotii* that had 80%. Of the herbs/spices studied, *S. zenkeri* (seeds) had the least DPPH radical scavenging activity registering only 43.16% at a dose level of 10mg/ml. At 2mg/ml, *I. gabonensis* and *T. vugaris* had radical scavenging activity above 50% making then strong antioxidant sources. One of the prerequisites to qualify an extract as antioxidant is its ability to neutralise radical induced oxidative stress. Such an extract should be reactive towards the radical and the resultant antioxidant radicals should not be reactive towards bio-molecules. In

other to study this effect, DPPH radical was used. DPPH is a stable free radical that forms a stable diamagnetic molecule on accepting an electron or hydrogen.[32] Polyaromatic hydrocarbons generate polyaromatic hydrocarbon cation free radicals *in situ* that may partly be linked to carcinogenesis.[33] Thus plants with antioxidants activity capable of scavenging free radicals may possess anti-carcinogenic and anti-mutagenic activities. In the present study, extracts of all the samples studied showed a significant (P<0.001) DPPH radical scavenging activity in a dose dependent manner. *I. gabonensis* was the most effective spice with very high radical scavenging activity while *S. zenkeri* had the least effect on DPPH radical compared to the rest of spices studied. This implies that the radical scavenging activity of extracts may be attributed to their ability to donate protons and hydrogen atom to DPPH.[34]

Table 2: Dose related Superoxide radical scavenging activity (%) of methanolic extracts of spices.

Species	Parts used	1mg/ml	2mg/ml	4mg/ml	8mg/ml
R. heudelotii	Seeds	12.39±1.70	19.25±1.46[a]	17.71±2.28[a]	20.15±1.52[c]
S. zenkeri	Seeds	15.24±0.84	14.78±1.35	13.00±2.05	18.07±1.11[c]
S. zenkeri	Bark	8.32±0.87	7.47±0.62	10.37±0.40[b]	15.94±0.99[c]
A. lepidophyllus	Seeds	12.28±0.63	8.59±0.64	10.76±1.15	15.96±0.84[c]
I. gabonensis	Seeds	8.89±0.99	14.40±0.71[a]	17.39±0.59[b]	40.78±1.23[c]
T. vulgaris	Seeds	22.18±0.92	23.21±1.17	29.94±0.97[b]	40.47±1.97[c]

[a] significantly different from 1mg/ml, [b] significantly different from 1mg/ml and 2mg/ml, [c] significantly different from 1mg/ml, 2mg/ml and 4mg/ml.

Table 2 presents the percentage (%) superoxide radical scavenging activity of spices extracts. The superoxide radical scavenging activity was also concentration dependent with the highest concentration of 8mg/ml having the highest scavenging activity. As in the DPPH radical scavenging activity, the highest superoxide radical scavenging activity (40 %) was registered by *I. gabonensis* and *T. vulgaris* at a concentration of 8mg/ml. This was significantly (P<0.001) higher than the scavenging activity of the other extracts. Superoxide radicals are formed when oxygen is reduced by accepting a single electron to its outer electron shell. Endogenously superoxides are produced by flavoenzymes such as xanthine oxidase activated in ischemia-reperfusion.[35] Exogenous substances such as opioid peptides have been reported to stimulate superoxide radical production by human polymorphonuclear leukocytes and macrophages.[36] The superoxide radical is very harmful to cellular components as a precursor of most reactive oxygen species.[37] Formation of superoxide radicals leads to a cascade of

other reactive oxygen species (H_2O_2 and $OH^{\cdot}$) in the cell, thus removal of superoxide radical in the cell is important.[38] A reduction in absorbance of the reaction mixture in the presence of antioxidant is an indication of the consumption of superoxide radical. Superoxide radical was generated in a non-enzymic system of PMS/NADPH-NBT. Extracts of all the samples had significant ($P<0.001$) dose dependent superoxide radical scavenging activity with *I. gabonensis* having the highest effect and *A. lepidophyllus* the least.

Table 3: Dose related Hydroxyl radical scavenging activity (%) of methanolic extracts of spices.

Species	Parts used	2.5mg/ml	5mg/ml	10mg/ml
R. heudelotii	Seeds	43.41±2.58	52.56±2.35[a]	62.35±1.64
S. zenkeri	Seeds	19.67±2.69	22.33±1.98	30.67±2.49[b]
S. zenkeri	Bark	5.33±1.94	14.67±2.05[a]	20.00±2.45[b]
A. lepidophyllus	Seeds	7.89±0.84	10.67±1.25[a]	15.42±2.81[b]
I. gabonensis	Seeds	6.21±1.63	8.54±2.14	11.68±3.64[a]
T. vulgaris	Seeds	11.67±1.69	15.33±1.47[a]	27.67±2.06[b]

[a]*significantly different from 2.5mg/ml,* [b]*significantly different from 2.5mg/ml and 5mg/ml.*

Table 3 presents the hydroxyl radical scavenging activity of extracts of spices. At 2.5mg/ml concentration of extracts, *R. heudelotii* had a significantly higher (rough 50%, $P<0.001$) hydroxyl radical scavenging activity than the rest of the extracts. Surprisingly, *I. gabonensis* had the least effect on the hydroxyl radical irrespective of the concentration used. Hydroxyl radicals are the most reactive of the ROS and initiates the peroxidation of cell membrane lipids.[39] The product of cell membrane peroxidation (lipid peroxides) break down is malondialdehyde which is mutagenic and carcinogenic.[40] The hydroxyl radical is formed in vivo by high energy irradiation leading to homolytic cleavage of water or from H_2O_2 in a metal catalysed process.[38, 39] Hydroxyl radicals interact with the purine and pyrimidine bases of deoxyribonucleic acid (DNA) bringing about DNA damage. It can also abstract hydrogen atoms from biological molecules, including thiols leading to the formation of sulphur radicals capable of combining with oxygen to generate oxysulphur radicals which also damage biological molecules.[39] The protection of DNA against hydroxyl radical was investigated by the Fenton reaction using the deoxyribose assay.[29] A mixture of Fe^{3+}, EDTA, H_2O_2, and ascorbic acid was used to generate hydroxyl radical which breakdown deoxyribose into fragments that react with thiobarbituric acids at low pH upon heating to produce a pink chromogen with maximum absorbance at 532nm. All extracts of samples had a significant ($P<0.001$) dose related increase in their hydroxyl radical scavenging activity. There was

increase in activity up to 5mg/ml and the activity dropped at 10mg/ml, which may imply that, the reaction reached saturation at 5mg/ml dose. All the plants extracts were able to reduce the intensity of the chromogen and hence prevented the breakdown of deoxyribose. A surprising high hydroxyl radical scavenging activity was registered for *R. heudelotii* that did not show very high DPPH or superoxide radical scavenging activity. On the other hand, *I. gabonensis* which had very high DPPH and superoxide radical scavenging activity was not very effective in the scavenging of hydroxyl radical. Thus, these extracts can be useful in minimising the effect of hydroxyl radical against biological molecules.

Table 4: Dose related Nitric oxide scavenging activity (%) of methanolic extracts of spices.

Species	Parts used	2.5mg/ml	5mg/ml	10mg/ml
R. heudelotii	Seeds	8.01±1.63	22.33±2.00[a]	37.67±1.94[b]
S. zenkeri	Seeds	5.67±1.25	4.67±2.05	6.69±2.18[b]
S. zenkeri	Bark	5.13±0.94	5.68±1.25	7.33±1.52[b]
A. lepidophyllus	Seeds	6.23±1.54	10.13±1.63[a]	12.33±1.71[b]
I. gabonensis	Seeds	29.23±1.56	41.33±2.05[a]	62.33±2.13[b]
T. vulgaris	Seeds	8.12±1.78	16.11±1.95[a]	35.67±1.94[b]

[a] significantly different from 2.5mg/ml, [b] significantly different from 2.5mg/ml and 5mg/ml.

Extracts of spices showed some scavenging activity against nitric oxide (Table 4) in a concentration dependent manner. At 10mg/ml, *I. gabonensis* had above 62% nitric oxide scavenging activity (62%). Concentration did not have any significant ($P>0.05$) effect on the scavenging activity of *S. zenkeri* (seeds and bark) and *A. lepidophyllus* extracts. Nitric oxide (NO) reacts with oxygen to produce nitrite and nitrate which are potential carcinogens. Thus, NO scavengers compete with oxygen. Several reactive nitrogen species are relevant to the food matrix and to the gastrointestinal tract. Nitrogen is present in foods as nitrates, nitrites, peptides, proteins and amino acids and its metabolites in vivo include nitric oxide, higher oxides of nitrogen and peroxynitrite.[41, 42] Generation of oxides of nitrogen in the stomach by reaction of salivary (and dietary) NO_2^- with gastric acid, initially to form HNO_2, may be an important antibacterial mechanism since $NO^·$ and its derivatives are toxic to many bacterial strains.[43] However, excess production of reactive nitrogen species e.g. as a result of *H. pylori* infection, chronic inflammation or excessive consumption of NO_2^--rich foods, may enhance the risk of gastric cancer by mechanisms involving formation of N-nitroso-compounds and possibly deamination. [44] Similarly, excess production of oxides of nitrogen may be a risk factor for cancer development in patients infected with hepatic parasites and in

hepatitis and other chronic inflammations.[45] Activity of *T. vulgaris* were found to significantly inhibit the production of NO induced by lypoxigenase and INF in a murine macrophage cell line mediated by inhibition of inducible nitric oxide synthase (iNOS) mRNA expression and/or by NO scavenging.[46] The scavenging activity of plant extracts was measured as the ability to prevent the formation of nitrite. All of the plants extracts had a significant (P<0.001) dose related effect on the scavenging of NO. *I. gabonensis* had the highest scavenging activity.

Table 5: Dose related reducing activity (optical density) of methanolic extracts of spices.

Species	Parts used	1mg/ml	2mg/ml	4mg/ml	8mg/ml
R. heudelotii	Seeds	0.22±0.01	0.23±0.01	0.26±0.01^{b}	0.34±0.01^{c}
S. zenkeri	Seeds	0.22±0.01	0.22±0.01	0.24±0.00^{b}	0.28±0.01^{c}
S. zenkeri	Bark	0.20±0.01	0.22±0.01	0.26±0.01^{a}	0.32±0.01^{c}
A. lepidophyllus	Seeds	0.21±0.01	0.24±0.01^{a}	0.28±0.01^{b}	0.35±0.01^{c}
I. gabonensis	Seeds	0.28±0.01	0.35±0.01^{a}	0.48±0.02^{b}	0.73±0.01^{c}
T. vulgaris	Seeds	0.25±0.01	0.33±0.01^{a}	0.46±0.01^{b}	0.72±0.01^{c}

^{a} *significantly different from 1mg/ml,* ^{b} *significantly different from 1mg/ml and 2mg/ml,* ^{c} *significantly different from 1mg/ml, 2mg/ml and 4mg/ml.*

The reducing power of extracts of spices measured as increase in optical density is presented in Table 5. A significant (P<0.001) concentration dependent reducing power was observed in extracts of spices with exception of *S. zenkeri* (seeds) (P>0.05) which had low reducing power. It was observed that only *I. gabonensis* and *T. vulgaris* had reducing power with optical density above 0.5 at the highest concentration (8mg/ml). The reducing power of a compound may count as a strong indicator of antioxidant potential. The reducing power has been applied in most of the methods available for the determination of antioxidant potential as the mechanism of action. Examples include the Folin and Ferric reducing antioxidant power methods though they differ in the metal being reduced.[26, 47] In measuring the reducing power, the transformation of Fe^{3+} to Fe^{2+} was investigated in the presence of the plants extracts as earlier described.[31] Increased absorbance of the reaction mixture at 700nm in the presence of extracts is an indication of a strong reducing power. The extracts showed a moderate (P<0.001) dose dependent reducing power with *I. gabonensis* and *T. vulgaris* being the strongest. The reducing ability of plant extracts has been linked to electrons donation and reaction with free radicals converting them into stable metabolites and terminates the radical chain reaction.[33] Antioxidant activity of *T. vulgaris* has also been reported. [48]

CONCLUSION

In concluding, it is important to mention that all the spices show some radical scavenging activity irrespective of the method of assay. It is possible for an antioxidant to protect in one system and fails to protect or even cause damage in another. This can be seen in the different trend of the radical scavenging activity when different methods were used. In overall, *I gabonensis* stood out as the best radical scavenger.

REFERENCES

1. Grootveld M, Halliwell B. Measurement of allantoin and uric acid in human body fluids. A potential index of free-radical reactions in vivo? Biochem. J., 1987; 243 803–808.
2. McDermott JH. Antioxidant nutrients: current dietary recommendations and research update. J. Am. Pharm. Assoc, 2000; 40:785-799.
3. Rashmi A. Comparative study of antioxidant activity of methanolic and ethanolic extracts of *Stevia Rebaudiana* Bertoni. World J Pharmaceut Res, 2015; 4: 1474-1488.
4. Othman A, Ismail A, Ghani AN, Adenan. Antioxidant capacity and phenolic content of cocoa beans. Food Chemistry, 2007; 100: 1523–1530.
5. Abbood KW, Ibraheem RM, Ad'hiah AH. Antioxidant activity of *Hypericum triquetrifolium* Turra methanol extract, World J Pharmaceut Res, 2015; 4: 405-413.
6. Imam S, Azhar I, Perveen S, Hussain SG, Mohmood ZA. Studies on *in vitro* antioxidant activity and total flavonoid contents of a cream formulation to correlate its anti-aging effect. World J Pharmaceut Res, 2015; 4: 1646-1655.
7. Agbor GA, Vinson JA, Oben JE., Ngogang JY. Comparative analysis of the in vitro antioxidant activity of white and black pepper. Nutr. Res, 2006; 26: 659-663.
8. Agbor GA, Kotué TC, Mouotsouo JP, Nanfack P, Nkam M, Ngogang YJ. "Hémodya": A Phytomedecine For Sickle Cell Disease Management In Cameroon. World J Pharmaceut Res, 2015; 4:1.
9. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA, 1993; 90: 7915-7922.
10. Abbiw D. Useful Plants of Ghana. Intermediate Technology Publications and the Royal Botanic Gardens Kew, London, 1990.
11. Burkill HM. Useful Plants of West Tropical Africa (Families E-I). Royal Botanical Gardens, Kew, 1994.
12. Adjanohoun JE, Aboubakar N, Dramane K, Ebot ME, Ekpere JA, Enow-Orock EG, Focho D, Gbile ZE, Kamanyi A, Kamsu Kom J, Keita A, Mbenkum T, Mbi CN, Mbiele

AL, Mbome IL, Miburu NK, Nancy WL. Traditional medicine and pharmacopoeia: contribution to ethnopharmacological and floristic studies in Cameroon. OAU/STRC. Lagos (Nigeria), 1996.

13. CE-FAO. Données statistiques des produits forestiers non-ligneux du Cameroun. Information site: http://www.fao. org/docrep/003/X6699F/X6699F01.htm. [09/06/2009].
14. Noumi E, Yomi A. Medicinal plants used in intestinal diseases in Mbalmayo Region. Fitoterapia, 2001; 246–254.
15. Momeni J, Djoulde RD, Akam MT, Kimbu SF. Chemical constituents and antibacterial activities of the stem bark extracts of R heudelotii (Euphorbiaceae). Indian J. Pharm. Sci., 2005; 67:386-399.
16. Kouokam JC, Zapp J, Becker H. Oxygen-containing sulfur-rich compounds from the bark of the tropical garlic tree *S zenkeri* Harms. *Phytochem.*, 2002a; 60: 403-407.
17. Kouokam JC, Jahns T, Becker H.. Antimicrobial activity of the essential oil and some isolated sulfur-rich compounds from *S zenkeri*. *Planta Med*., 2002b; 68: 1082-1087.
18. Duncan WT, Jane MT, A Wendy AB, Fonki TM. Korup Ethnobotany Survey: Final Report to the World Wide Fund for Nature, Panda House, Weyside Park, Godalming, Surrey, 1989.
19. Musuyu DM, Fruth BI, Lami JN, Mesia GK, Kambu OK, Tona GL, Kanyanga RC, Cos P, Maes L, Apers S, Pieters L. In vitro antiprotozoal and cytotoxic activity of 33 ethonopharmacologically selected medicinal plants from Democratic Republic of Congo. J. Ethnopharmacol, 2012; 141: 301-308
20. Grigore A, Ina Paraschiv, Colceru-Mihu S, Bubueanu C, Draghici E, Ichim M. Chemical composition and antioxidant activity of Thymus vulgaris L. volatile oil obtained by two different methods. Romanian Biotechnol Lett, 2010; 15: 5436-5443.
21. Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. Several culinary and medicinal herbs are important sources of dietary antioxidants. J. Nutr., 2003; 133: 1286-1290.
22. Agbor GA, Oben JE, Ngogang JY, Xinxing C, Vinson JA. Antioxidant Capacity of Some Herbs/Spices from Cameroon: A Comparative Study of Two Methods. J. Agric. Food Chem, 2005**;** 53: 6819-6824.
23. Agbor GA, Vinson JA, Oben JE, Ngogang JY. Antioxidant effect of herbs and spices on copper mediated oxidation of lower and very low density lipoprotein. Chinese J Natural Med, 2010; 8: 0114-0120.
24. Vinson JA, Hao Y, Su X, Zubik L, Bose P. Phenol antioxidant quantity and quality in foods: fruits. J. Agric. Food Chem, 2001a; 49: 5315-5321.

25. Vinson JA, Yousef A, Debbagh MA. Tea phenols: antioxidant effectiveness of teas, tea components, tea fractions and their binding with lipoproteins. Nutr. Res, 1998; 18: 1067-1075.
26. Vinson JA, Proch J, Bose P. Determination of the quantity and quality of polyphenol antioxidants in food and beverages. Methods Enzymol. 2001b; 335: 103-114.
27. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem. Pharm. Bull., 1988; 36: 2090-2097.
28. Nishikimi M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. Biochem. Biophys. Res. Commun., 1972; 46: 849-853.
29. Halliwell B, Gutteridge JM, Aruoma OI. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Anal Biochem, 1987; 165: 215-219.
30. Sreejayan, Rao MNA. Nitric oxide scavenging by curcuminoids. J. Pharm. Pharmacol., 1997; 49: 105-107.
31. Oyaizu M. Studies on products of browning reaction: Anti-oxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr, 1986; 44: 307-315.
32. Jun M, Tohru U, Jianzhang L, Takeshi F. Identification and evaluation of antioxidant activities of Bamboo Extracts. Forestry Studies in China, 2004; 6: 1-5.
33. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem, 1995; 46: 849-854.
34. Hou WC, Chen YC, Chen HJ, Liu YH, Yang LL, Lee MH. Antioxidant activities of a 33 KDa root storage protein of sweet potato (Ipomoea batatas [L.] Lam cv. Tainong 57). J. Agric. Food Chem, 2001; 49: 2978-2981.
35. Gülçin I, Beydemir S, Alici HA, Elmastaş M, Büyükokuroğlu ME. In vitro antioxidant properties of morphine. Pharmacol. Res, 2004; 49: 59-66.
36. Blaszczyk J, Kedziora J, Luciak M, Sibinska E, Trznadel K, Pawlicki L. Effect of morphine and naloxone on oxidative metabolism during experimental renal ischemia and reperfusion. Exp. Nephrol, 1994; 2: 364-370.
37. Halliwell B, Gutteridge JMC. In: Free radicals ageing and disease, Free radicals in Biology and Medicine, 2nd edition, Clarendron Press Oxford, 1985; 279-315.
38. Cheeseman KH, Slater TF. An introduction to free radical biochemistry. Brit. Med. Bull, 1993; 49: 481-493.

39. Halliwell B. Reactive oxygen species in living systems: source, Biochemistry and role in human disease. Am. J. Med, 1991; 9(3): 14-22.
40. Miyake T, Shibamoto T. Antioxidant activities of natural compounds found in plants. J. Agric. Food Chem, 1997; 45: 1819-1822.
41. Beckman JS, Chen J, Ischiropoulos H, Crow JP. Oxidative chemistry of peroxynitrite. Meth. Enzymol,1994; 233: 229-240.
42. Tamir S, Tannenbaum SR. The role of nitric oxide (NO˙) in the carcinogenic process. Biochim. Biophys. Acta, 1996; 1288: 31-36.
43. McNight GM, Smith LM, Drummond RS, Duncan CW, Golden M, Benjamin N. Chemical synthesis of nitric oxide in the stomach from dietary nitrate in humans. Gut, 1997; 40: 211-214.
44. Dallinga JW, Pachen DMFA, Lousberg AHP, van Geel JAM, Houben GMP, Stockbrugger RW, Van Maanen JMS, Kleinjans JCS. Volatile N-nitrosoamines in gastric juice of patients with various conditions of the gastrointestinal tract determined by gas chromatography-mass spectrometry and related to intragastric pH and nitrate and nitrite levels. Cancer Lett, 1998; 124: 119-125.
45. Ohshima H, Bartsch H. Quantitative assessment of endogenous nitration in humans by measuring excretion of N-nitrosoproline in urine. Cancer Res, 1981; 41: 3658-3662.
46. Vigo E, Cepeda A, Gualillo O, Perez-Fernandez R. *In-vitro* anti-inflammatory effect of *Eucalyptus globules* and *Thymus vulgaris*: nitric oxide inhibition in J774A.1 murine macrophages. JPP, 2004; 56: 257-263.
47. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem, 1996; 239: 70-76.
48. Prakash HS, Beidokhti MN. Antioxidant and anti-inflammatory potential of selected medicinal plants of lamiaceae family. Int. J. Pharm. Pharm. Sci., 2013; 5: 100-104.