Research Article

Antioxidant Capacity of Spices/Vegetables Protects Erythrocyte Toxicity *Ex-Vivo*

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ABSTRACT

Than antioxidant capacity of sixteen spices and five vegetables commonly consumed in the West region of Cameroon were evaluated in the study. The total phenolic content (TPC), Ferric Reducing Antioxidant Power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and $ABTS^+$ characterized the antioxidant capacity. Samples with best antioxidant capacity were then used for erythrocyte protection studies against cupper (II) ion induced oxidative stress. Superoxide dismutase (SOD), catalase (CAT) activities and malondialdehyde (MDA) concentration were analyzed as markers of oxidative stress in erythrocytes hemolysate. The results showed that all the samples studied possessed some antioxidant capacity irrespective of the method used though some samples had very little compared to others. Considering the best three samples with antioxidant capacity the order was *C. fructescens* (273.61±13.33 mg/g) > *D. glomerata* (94.90±2.25 mg/g) > *E. giganteus* (41.905±1.49 mg/g) for TPC while for FRAP it was *D. glomerata* (22.64±0 mg/g) > *S. zenkeri* (16.67±0.48 mg/g) > *T. tetraptera* (9.71±0.04 mg/g). The order of antioxidant capacity for DPPH was *D. glomerata* (74.25±2.36 mg/g) > *S. zenkeri* (61.61±1.29 mg/g) > *T. tetraptera* (33.04±0.53 mg/g) while for ABTS it was *S. zenkeri* (85.32±5.46 mg/g)> *D. glomerata* (82.33±9.6 mg/g) > *T. tetraptera* (65.92±11.17 mg/g). Incubation of erythrocyte with 10mM Copper II sulphate solution induced a significant (P< 0.001) increase in the erythrocyte antioxidant enzyme (SOD, CAT) activity and MDA concentration. However, co-incubation with spices extract significantly inhibited the oxidative effect of copper II ion in a dose dependent manner. 10 µM TPC was significantly (P<0.001) better than 1 µM TPC. However, catechin had a significant (P<0.001) erythrocyte protective effect than spices extracts at equimolar concentration. Overall, the order of antioxidant capacity of the first three samples are in the order *D. glomerata* > *S. zenkeri* > T. tetraptera. *D. glomerata* and *S. zenkeri* protected the erythrocyte from oxidative effect of copper II ion though not comparable to catechin at equimolar concentrations.

Keywords: Species and vegetables, oxidative stress, antioxidant activity, erythrocytes toxicity, copper II sulphate.

INTRODUCTION

Antioxidant studies of natural product is gaining grounds yearly since oxidative stress has been identified as the causative factor or a product in various disease state. Oxidative stress is defined as an imbalance between prooxidant and antioxidant factors in favor of prooxidants. The salubrious physiological activity of the cell such as host defense and neuronal transduction mechanisms produce a lot of reactive oxygen species which are responsible for oxidative stress.[1,2] The cellular physiological system produces enzymic (catalase, superoxide dismutase, glutathione peroxidase/reductase etc) and non-enzymic (Vitamin C and E, carotene, uric acid etc) antioxidants as natural defense to check the excesses of the reactive oxygen species.[3] However, in critical situations such as introduction of exogenous free radical, contamination, exposure to UV radiation, infection and disease condition the cellular antioxidant defense may not be sufficient[3,4] resulting to oxidative stress.

Increased intakes of dietary antioxidants (consumption of vegetables, spices and fruits) may help to maintain an adequate antioxidant defense status.

Spices are food adjuncts used as flavoring, coloring and seasoning agents. Spices consist of a collection of leaves and/or branches of plants, ripened fruits or seeds or root/bulbs of certain plants that are usually dried and used to season foods because of their distinctive flavor and aroma.[5] Spices have become common ingredients in every cuisine because of their properties to increase both flavor and taste.

Hence, their dietary contribution and functional perspective are of concern.[6] 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.[6]

Antioxidants can inhibit oxidative reactions *in vivo*, and aid in functional performance of enzyme systems for self-defence mechanisms within cells.[7]

Healthy feeding with spices, vegetables and fruits may reduce the risk of chronic diseases, including cardiovascular disease, stroke, neurodegeneration, and type II diabetes.

Spices extracts have been reported to possess cardio, hepato and reno antioxidant status with antiatherogenic activity in high cholesterol fed hamsters.[3,8] The present study evaluates spices and vegetables commonly

consumed in the Western Region of Cameroon for their antioxidant capacity using four different antioxidant assay methods.

MATERIALS AND METHODS

Reagents Used

Folin-Ciocalteu (Sigma), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) (Sigma), Catechin (Sigma), Ferric Chloride (BDH), HCl (BDH), Acetic acid (BDH), Sodium Acetate (BDH), Copper II sulphate, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), All reagents were of Analytical grade.

Preparation of Plant Material

All the samples (Spices and Vegetables) were bought from the Bafoussam and Foumban markets in the Western Region of Cameroon and are listed in Table 1. The vegetables (fresh) were washed and air dried at room temperature to a constant weight in the laboratory while the spices were bought already dried. After drying samples were crushed into powder and stored at -20°C until required.

In-vitro Antioxidant Assay

For each sample, 100 mg were weighed into test tubes to which 10 ml of distilled water were added. This was boiled at 100°C in a water bath for an hour. After cooling to room temperature the supernatants were stored at -20°C until required for the different assays.

Total Phenolic content (TPC)

The total phenolic content in the sample was determined using the Folin-Ciocalteu reagent (Sigma Chemical Co., St. Louis, MO) diluted 5 times to 0.4N concentration as earlier described.[9] The absorbance was measured at 750nm after 12 min of incubation with the aid of spectrophotometer. Catechin (10 – 100 µM) was used to prepare the standard curve.

Ferric Reducing Antioxidant Power

The ferric reducing antioxidant power (FRAP) of extracts was determined as earlier described by Benzie and Strain.[10]

The FRAP reagent consisted of ten parts of acetate buffer (300 mM, pH 3, 6), one part of TPTZ 10 mM in 400 mM of HCl, Sigma, and one part of ferric chloride (10 mM). 2 ml of solution of freshly prepared FRAP reagent were added to 75 µl extract and the optical density read after incubate for 15 min. Catechin (50-300 µM) was used as the standard and optical density read at 593 nm.

DPPH Radical Scavenging Activity

DPPH measured the ability of the extracts to scavenge free radical. This was estimated using the method described by Braca.[11] Plant extract (0.1 ml) was added to 3ml of a 0.004% methanol solution of DPPH. Optical density was read at 517 nm using a spectrophotometer after 30 min incubation in the dark. The percentage inhibition activity was calculated from $[(A_0-A_1)/A_0] \times 100$, where A_0 is the optical density of the control, and A_1 is the optical density of the extract/standard.

ABTS Radical Scavenging Effect

The 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonic acid) (ABTS+) scavenging effect of the extracts were analyzed in accordance with the method of Re et al.[12] The ABTS radical was generated by mixing equal volumes of 7mM of ABTS and 4.9 mM of potassium permanganate (KMnO4) and kept in the dark room for 24h. 8 ml of the ABTS generated radical solution was diluted further in 72 ml of distilled water. One ml of the diluted radical solution was added to 20 µl of plant extract and the optical densities read at 734 nm after 12min of incubation. $\% \, Inhibition = \frac{(Abs2-Abs1)}{Abs1} \times 100$

where Abs1 is the absorbance of the blank and Abs2 absorbance of sample.

Ex-vivo Antioxidant Enzyme Assays

Two samples (*D. glomerata* and *S. zenkeri*) with the best *in-vitro* antioxidant capacity were selected for the *ex-vivo* assay. The polyphenolic concentration of each spice sample as determined by TPC was diluted to 1 µM and 10 µM and same concentrations of the standard were prepared as well.

Preparation of Erythrocytes

A healthy pig's blood was used in this study. Blood was drawn from a healthy pig in the Yaoundé Abattoir into an EDTA containing bottle with the aid of a veterinarian. The blood sample was transferred into 5 ml centrifugation tubes and centrifuged at 3000 rpm for 15 min to separate the plasma from the erythrocytes. The plasma (supernatant) was discarded and the erythrocytes washed four times with physiological saline.

Antioxidant Quality Studies by Incubation of Erythrocyte with Sample and Oxidants

The method earlier described by Agbor[13] for LDL-oxidation was adopted in this study with some modification. In brief the erythrocyte (500µl) was diluted with physiological saline (500µl) to which was added 20 µl of extract with adjusted polyphenolic concentrations of 1 µM and 10 µM. The reaction was spiked by the addition of 100 µL of freshly prepared copper II sulphate (10 mM oxidant) to each and incubated for 30 minutes. This same procedure was repeated with samples being replaced by the catechin standard. Another set of tubes were kept as the oxidant control (contained erythrocyte, physiological saline and oxidant only) while another set of tubes that contained erythrocyte and physiological saline were kept as normal control. After 30 min of incubation at room temperature the samples were centrifuged at 3000 rpm and washed three times with physiological saline. The resulting erythrocytes were hemolyzed with 500 µl of

cold distilled water and stored at - 20°C until required for analysis.

Measurement of Super Oxide Dismutase (SOD) Activity

SOD activity was measured as the inhibition of adrenalin oxidation to adrenochrom, according to the method of Mishra and Fridovish.[14] The change in optical density per min was monitored at 480 nm for three min. 1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrom per minute.

Measurement of Catalase (CAT) Activity

CAT activity was measured according to the method of Sinha.[15] CAT breaks down hydrogen peroxide to oxygen and water. Thus this method measures the decrease in hydrogen peroxide in solution as a function of time. Acidified potassium dichromate acted as a stopping reagent.

It reacts with the remaining hydrogen peroxide in solution to form a blue-green precipitate which dissolves on heating and has an optical density at 570 nm. A standard curve of hydrogen peroxide (10-640 µM) was prepared from which the catalase activity was calculated. 1 unit of catalase was determined as the amount required to catalyze the reduction of 1 µM of H_2O_2 in 1 minute.

Measurement of Malonedialdehyde (MDA)

The lipid peroxidation was measured by applying the method earlier described by Biswas.[16]

Lipid peroxide formed during oxidation reacts with thiobarbituric acid to produce a complex with pink color which has maximum absorbance at 532 nm.

Measurement of Total Proteins

The method used is based on the color intensity of the blue complex formed which has maximum absorbance at 750 nm and proportional to the quantity of proteins in the mixture.[17]

Statistical Analysis

Measurements were carried out in triplicate, and the results are presented as Mean ± Standard Deviation (SD). The Wilcoxon signed rank test was employed in assessing the difference between means at $P < 0.05$. The SigmaStat (Systat software, Richmond, CA) version 3.01 was used for this analysis.

RESULTS AND DISCUSSION

Table 1: List of some Spices and Vegetables of the Western Region of Cameroon

S. No.	Vernacular Names	Family	Scientific Names	Parts Used
1	Soh	Solanaceae	*Capsicum fructescens*	fruit
2	Ndjapche	Solanaceae	*Solanum nigrum*	leaf
3	Guege	Solanaceae	*Solanum melongena*	fruit
4	Medjock	Annonaceae	*Monodora myristica*	nought
5	Biqui	Annonaceae	*Xylopia aethiopica*	fruit
6	Ndzu-fo	Mimosaceae	*Pentadiplandra brazzeana*	fruit
7	Toun lapin	Mimosaceae	*Dichrostachys glomerata*	fruit
8	Sop	Piperaceae	*Piper guineense*	seed
9	Mbembe	Piperaceae	*Piper umbellatum*	leaf
10	Ha	Malvaceae	*Hibiscus esculentus*	leaf
11	Sa	Malvaceae	*Hibiscus sabdariffa*	leaf
12	Nga-chu	Rutaceae	*Xanthoxyloides zanthoxylum*	fruit
13	Melan	Rutaceae	*Fagara leprieurii*	fruit
14	Tchuwienga	Asteraceae	*Echinops giganteus*	root
15	Ketcho	Zingiberaceae	*Afromomun danielli*	fruit
16	Dim-te	Periplocaceae	*Mondia whitei*	root
17	Lem rond	Olaceae	*Olax subscorpioidea*	fruit
18	Tsutshop	Momosaceae	*Tetrapleura tetraptera*	fruit
19	Ngan noh	Cyperaceae	*Scleria striatinux*	Rhizome
20	Lom nkah	Caesalpiniaceae	*Scorodophloeus zenkeri*	tree bark
21	Chou	Brassicaceae	*Brassica oleracea*	leaf

Table 2: *In-vitro* antioxidant capacities of the extracts of spices and vegetables commonly consumed in the Western region of Cameroon

Samples	Polyphenols (mg/g)	FRAP (mg/g)	DPPH (% Inhibition)	ABTS (% Inhibition)
Leafy Vegetables				
H. sabdariffa	24.65 ± 5.81 (10)	7.81 ± 0.72 (4)	23.03 ± 2.51 (4)	28.73 ± 5.52 (4)
B. oleracea	3.944 ± 1.39 (18)	2.00 ± 0.07 (12)	15.79 ± 0.63 (10)	6.09 ± 7.74 (17)
H. esculentus	2.95 ± 0.36 (20)	0.58 ± 0.49 (21)	9.63 ± 0.83 (14)	14.17 ± 0.98 (10)
P. umbellatum	25.51 ± 3.32 (9)	0.78 ± 0.18 (20)	9.53 ± 0.38 (15)	3.23 ± 0.24 (21)
S. nigrum	26.86 ± 2.82 (7)	1.36 ± 0.14 (17)	8.08 ± 0.93 (16)	28.10 ± 26.42 (5)
Spices				
S. melongena	4.43 ± 0.73 (17)	1.75 ± 0.20 (14)	11.25 ± 7.05 (13)	4.72 ± 8.26 (19)
X. aethiopica	1.972 ± 1.06 (21)	1.79 ± 0.27 (13)	19.68 ± 1.26 (5)	5.22 ± 6.27 (18)
E. giganteus	41.905 ± 1.49 (3)	4.06 ± 0.06 (5)	16.68 ± 2.09 (8)	12.31 ± 2.44 (12)
S. striatinux	3.57 ± 0.76 (19)	2.62 ± 0.15 (8)	16.15 ± 0.6 (9)	7.71 ± 2.99 (15)
A. danielli	8.75 ± 8.21 (14)	1.63 ± 0.13 (15)	12.32 ± 0.26 (11)	13.68 ± 15.96 (11)
T. tetraptera	17.37 ± 1.47 (13)	9.71 ± 0.04 (3)	33.04 ± 0.53 (3)	65.92 ± 11.17 (3)
P. brazzeana	30.56 ± 2.72 (6)	0.89 ± 0.17 (19)	12.16 ± 0.54 (12)	3.98 ± 1.07 (20)
S. zenkeri	38.82 ± 2.66 (4)	16.67 ± 0.48 (2)	61.61 ± 1.29 (2)	85.32 ± 5.46 (1)
X. zanthoxylum	4.56 ± 0.56 (16)	2.46 ± 0.10 (10)	17.69 ± 0.62 (7)	11.19 ± 3.05 (13)
D. glomerata	94.90 ± 2.25 (2)	22.64 ± 0 (1)	74.25 ± 2.36 (1)	82.33 ± 9.60 (2)
O. subscorpioidea	20.58 ± 2.51 (11)	0.93 ± 0.05 (18)	19.02 ± 0.65 (6)	18.15 ± 9.54 (9)
M. myristica	5.17 ± 0.64 (15)	1.57 ± 0.18 (16)	6.87 ± 3.73 (17)	22.63 ± 17.91 (8)
F. leprieurii	19.47 ± 0.85 (12)	3.67 ± 2.28 (6)	6.78 ± 0.97 (18)	27.61 ± 16.78 (6)
C. fructescens	273.61 ± 13.33 (1)	2.41 ± 0.31 (11)	5.09 ± 0.73 (20)	26.36 ± 14.76 (7)
P. guineense	26.25 ± 3.20 (8)	2.62 ± 0.18 (9)	5.90 ± 0.67 (19)	6.84 ± 0.93 (16)
M. whitei	32.0 ± 2.82 (5)	2.69 ± 0.11 (7)	4.90 ± 0.27 (21)	10.57 ± 0.21 (14)

Results are presented as mean ± standard deviation, samples analyzed in triplicates, ( ) represents the ranking of antioxidant capacity

Table 3: Evaluation of antioxidant quality of selected spices on protection of erythrocyte exposed to oxidation.

Sample	Conc. (µM)	CAT (unit/mg protein)	SOD (unit/mg protein)	MDA (µmole/ml)
Normal control	-	2950.11 ± 20^{c}	2874.20 ± 21.66^{b}	0.05 ± 0.00^{c}
Stress control	-	3439.91 ± 10.43^{a}	5042.01 ± 30^{a}	0.11 ± 0.00^{a}
D. glomerata	1	3243.69 ± 14.97^{b}	2709.57 ± 17.62^{b}	0.12 ± 0.01^{a}
	10	2847.63 ± 20.53^{c}	2911.25 ± 42.57^{c}	0.08 ± 0.00^{b}
S. zenkeri	1	3218.18 ± 36.24^{b}	3067.38 ± 78.49^{c}	0.072 ± 0.01^{b}
	10	2844.13 ± 58.56^{c}	3657.88 ± 65.50^{d}	0.052 ± 0.00^{c}
Catechin control	1	2760.35 ± 68.77^{c}	3645.61 ± 90.25^{d}	0.042 ± 0.00^{d}
	10	2535.48 ± 41.43^{d}	3101.94 ± 12.74^{e}	0.027 ± 0.00^{e}

Results are presented as mean ± standard deviation, samples analyzed in triplicates. Means with different letters (a, b, c, d and e) within a column are significantly different from one another ($P < 0.001$).

The screening of plants be it herbs, spices or foods for antioxidant capacity is gaining more grounds in biomedical research. This may be because of continuous increase in exposure of humans to oxidative agents in their environment. Assessing the antioxidant capacity of foods, herbs or spices give necessary information of the benefits of these and guides the choice of the consumer. This also serves as potential information as to which plants can better serve as pharmaceutical agent in drug discovery against oxidative stress related disorders

including cardiovascular diseases. The various tests carried out within the framework of the present study characterized the antioxidant capacity of some vegetables and spices, regularly consumed in the Western Region (Bafoussam, Foumban) of Cameroon listed in Table 1. The samples were twenty-one some purchased from the regional markets while others were harvested from their natural habitat. The scientific identification and validation of the samples was done by an Ethnobotanist. Of the twenty one samples identified five were leafy vegetables while sixteen were classified as spices (naught, fruit, seed, rhizome, tree bark). In the frequency of occurrence, solanaceae is the family that is highly consumed because it appeared three times followed by annonaceae, mimosaceae, malvaceae and rutaceae that appeared twice.

The antioxidant methods applied in this study are governed by three major mechanisms: These include the hydrogen atom transfer (HAT), the electron transfer (ET) and the combination of both HAT and ET.[18] The ability of an antioxidant to quench free radicals by hydrogen donation is measured by HAT while the ability of antioxidant to transfer one electron to reduce radicals, metals and carbonyls is measured by ET.[19] FRAP is measured through the ET mechanism and the Folin-Ciocalteu reagent measures the TPC through the ET mechanism.[20] The ABTS and DPPH are two free radicals that are commonly used in the assessment of antioxidant capacity of samples *in-vitro*. The antioxidant capacity was characterized by total phenolic content (TPC), ferric reducing antioxidant power (FRAP) and radical scavenging activity as measured by DPPH and ABTS. And the results obtained permit us to classify the spices and vegetables according to their antioxidant capacity as presented in Table 2.

Total Polyphenolic Content

The Folin method for determination of total polyphenolic content is based on the reduction of phosphomolybdic-tungstic chromogen by an antioxidant, and the resulting color change has a maximum absorbance at 750 nm.[21] The principal antioxidant constituent of natural products is the polyphenolic compound which constitutes phenolic acids, tannins and flavonoids. They mediate their antioxidant activity as potent free radical terminators. Of the twenty one samples studied *C. fructescens* had the highest TPC (P<0.001) of 273.61±13.33mg/g followed by *D. glomerata* (94.90 ± 2.25 mg/g) compared to the rest of the samples studied. The next sample that followed was *E. giganteus* (41.90 ± 1.49 mg/g) and *S. zenkeri* (38.82 ± 2.66 mg/g).

The sample with the least polyphenolic concentration was *X. aethiopica* that presented a concentration of 1.972±1.06 mg/g. In an earlier study it has been reported that the phenolic, flavonoid and capsaicinoid contents as seen in the hexane and acetonitrile extracts of *Capsicum fructescens* contribute to its high TPC.[22] However though high TPC *Capsicum fructescens* had poor FRAP, ABTS and DPPH activities.

The TPC of the spices and vegetables in the present study were higher than those earlier reported by Agbor[23] on extracts of some spices and vegetables consumed in Cameroon. This difference may probably be due to the processing, market sources or the duration of the spices in the market after harvesting. It is also important to mention that in the earlier work of Agbor,[23] the samples were purchased in the markets of Yaoundé (Centre Region) and Mamfe (South West Region) which are temperate regions with high temperatures and sun rays that will bleach exposed spices compared to the Western region. Earlier research results had shown that duration and exposure of spices to temperature significantly decreased their TPC due to enzymatic degradation of the phytochemicals.[24-27]

Ferric Reducing Antioxidant Content

The FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe^{2+}-TPTZ.[10] In the FRAP assay, *D. glomerata* appeared as the best spices with 22.64±0 mg/g antioxidant capacity which was significantly (P<0.001) different from the second that closely followed *S. zenkeri* (16.67±0.48 mg/g) and *H. sabdariffa* (10.87±0.36 mg/g) while *H. esculentus* had the least FRAP of 0.58±0.49 mg/g. This implies that extracts of spices and vegetables were able to donate electrons to reduce Fe^{3+} to Fe^{2+} functioning as antioxidant. This may apply in biological systems bringing about chain breaking reactions against free radicals or reactive oxygen species. *D. glomerata* fruits are commonly used as spices in a traditional soup of the Western Region of Cameroon called ''Nah po'', eaten along with taro.[28] The bark of *D. glomerata* finds application in traditional medicine as analgesic against headache and toothache and as an antibiotic against dysentery and elephantiasis. The leaves of *D. glomerata* are used for the treatment of epilepsy, diuretic and laxative.[29]

DPPH Radical Scavenging Activity

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule and is widely used to assess the radical scavenging activity of antioxidant compounds.[30,31] Some cellular free radicals are the polyaromatic hydrocarbon cations which have been linked with carcinogenesis.[32] Other in situ radicals are superoxides and hydroxyls produced in large amounts by various metabolic and physiological processes.[33-37]

They initiates the peroxidation of cell membrane lipids[34,37] yielding malondialdehyde, which is mutagenic and carcinogenic.[38] Hence radical scavenging activity is an important assay for assessing chain breaking antioxidant activity. The products that will scavenge DPPH *in vitro* may also scavenge polyaromatic hydrocarbon cations and other radicals *in vivo*.[39] *D. glomerata* (74.25±2.36mg/g)

also topped the chart for DPPH radical scavenging activity while *S zenkeri* came second.

ABTS Radical Scavenging Activity

ABTS is generated in an aqueous phase by reaction between a strong oxidizing agent (potassium permanganate or potassium persulfate) and the ABTS salt. A hydrogen donating antioxidant will reduce the blue-green ABTS radical color.[40,41]

The entire samples studied possessed radical scavenging activity against ABTS by donating hydrogen atom that reduces the blue-green coloration of ABTS with *S. zenkeri* the most effective followed by *D. glomerata*. *S. zenkeri is a* tropical garlic trees with garlic flavor.

A phytochemical study of the bark of *S. zenkeri* revealed the presence of sulfur rich compounds[42] and some essential oils with antimicrobial activity[43] which may have contributed to its radical scavenging activity.

Antioxidant Quality in Erythrocyte Protection from Oxidant

The antioxidant quality of two spices with the best antioxidant capacity was assessed by incubation of erythrocyte with copper II sulphate (oxidant) in the presence or absence of spices extract (Polyphenolic concentration of 1 and 10 µM) while catechin was used as standard control.

Copper is a redox active metal that can participate in electron transfer reactions with the consequent production of oxidant species capable of oxidizing cell components.[44]

Copper can catalyze the formation of the highly reactive hydroxyl radicals from hydrogen peroxide via the Haber-Weiss reaction and decompose lipid peroxides to peroxyl and alkoxyl radicals, which favor the propagation of lipid peroxidation.[45]

The redox chemistry of copper II ion (Eq 1 & 2) has been greatly exploited in studying *in vitro* antioxidant potential against lipid oxidation.[13,44-46]

However, this is the first study to use copper II ion for the oxidation of erythrocytes.

$Cu^{2+} + O_2^{-} \rightarrow Cu^{+} + O_2$ Eq 1

$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{\cdot} + OH^{-}$ Eq 2

Incubation of erythrocyte alone with the oxidant induced a significant increase ($P < 0.001$) in the antioxidant enzymes (CAT and SOD) activities and lipid peroxidation product (MDA) as compared to the normal control.

However, co-incubation of erythrocytes with spices extracts or catechin standard significantly ($P < 0.001$) reduced the effect of the oxidant.

The effect of the spices extracts and catechin were concentration related with 10 µM being significantly ($P < 0.001$) better than 1 µM.

D. glomerata had a significant ($P<0.001$) effect in preventing an increase in SOD activity better than *S. zenkeri* in the two concentrations studied.

However there was no significant difference ($P > 0.05$) between same concentrations of the spices extract for the CAT activity.

The effect of catechin standard was better than both *D. glomerata* and *S. zenkeri* at same concentration level.

Incubation of erythrocyte with catechin, *D. glomerata* and *S. zenkeri* also prevented lipid peroxidation as measured by the MDA concentration.

CONCLUSION

Overall, *D. glomerata* that topped the chart twice (FRAP and DPPH) and came second twice (TPC and ABTS) had the best antioxidant capacity followed by *S. zenkeri* that topped the chart once (ABTS), came second twice (FRAP and DPPH) and fourth once (TPC).

Meanwhile the sample with the least antioxidant capacity were *H. esculentus* (21st for FRAP, 20th for TPC, 14th for DPPH and 10th for ABTS) and *P. umbellatum* (21st for ABTS, 20th for FRAP, 15th for DPPH and 9th for TPC).

Hence the antioxidant capacity data obtained from different methods of assay may not necessarily be the same.

And since antioxidant activity operates through different mechanisms it is always important to use different methods for a better appraisal of the antioxidant capacity of a sample as was the case in this study.

The selected samples with best antioxidant capacity inhibited the oxidative effect of copper II ion on erythrocytes though their activity was not comparable to that of catechin at equivalent concentration.

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