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Proximate Composition, Phenolic Content and Antioxidant Activities of Three Black Plum (*Vitex* sp.) Fruits: Preliminary Results

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Abstract: Highly reactive molecules (NO, O_2^-, HOO^-) generated in biological systems are associated with initiation of degerative diseases. The antioxidants can reduce this risk. Fruits are one of the most important sources of antioxidants such as vitamins and phenolic phytochemicals. *Vitex* plants are wild tropical plant which has found wide application in traditional medicine and human food in the west and central African regions. This study was conducted to test antioxidant capacity, to compare the values of total phenolic and flavonoid contents and to estimate the proximate composition of three different fruits of *Vitex* species. The free radicals deactivating ability was measured using DPPH, NO scavenging assays and the reducing power tested by FRAP assay. Total phenolic content and flavonoid content were evaluated according to the Folin-Ciocalteu procedure and a colorimetric method, respectively. *Vitex doniana* and *Vitex kiniensis* extract showed high total phenol (601.40 and 719.83 mg GA/100 g FW, respectively) and flavonoid (2.35 and 2.09 μg RT/g extract) content and better reducing power by FRAP at 545.71 and 402.43 μMFe^{2+}/100 g FW, respectively. The proximate compositions of the three fruits showed slight variation. The extracts demonstrated good scavenging activities against DPPH and NO assays which were not significantly different at ($p<0.05$). Thus these results suggest that extract three *Vitex* species may serve as potential source of natural antioxidant for food and nutraceautical application.

Key words: *Vitex doniana, Vitex kiniensis, Vitex fischeri*, antioxidant, DPPH, nitric oxide, FRAP

INTRODUCTION

Uncontrolled generation of free radicals together with reduced level of antioxidative vitamins and enzyemes are considered to be the main contributor to oxidative stress (Ellnain-Wojtaszek *et al*., 2003). Free radicals attack membrane lipids, protein and DNA which is believed to be involved in many health disorders such as diabetes mellitus, cancer, neurodegenerative and inflammatory diseases (Gulcin, 2007). The human body has an antioxidant defense system and it has been assumed that a diet rich in antioxidant strengthens this system (Stangeland *et al*., 2009). Fruits including berries and nuts, some seeds, vegetables and some beverages (coffee, tea, fruit juices and red wine) are good sources of antioxidants (Stangeland *et al*., 2009). The use synthetic drugs for immediate relief off some diseasemay be required, howeverlong term use of these drugs are not only causes side effects such as nausea, allergy and immunosuppression but leading themselves to be causative agents for others disorders (Nakaya *et al*., 2010). Cells and tissues of living systems may become an easy target for exposure to free radicals generated during drug metabolism. In addition, exogenous sources like ozone, exposure to UV radiations and cigarette smoke also induce biomolecular changes such as DNA damage, protein oxidation and generation of lipid peroxides leading to severity of chronic diseases (Nakaya *et al*., 2010).

Increased interest in the beneficial health effects from consuming certain foods has resulted in research aimed at determining the levels of phytonutrients and specific health benefits in a range of indigenous fruits and vegetables. Foods rich in antioxidants can play an essential role in the prevention of chronic and degenerative diseases such as cardiovascular diseases and cancer (Ames *et al*., 1993). Plant foods contain of bioactive compounds which are actively being researched for their potential in health care. Although most research on the health benefits of plant-rich diets has focused on the established vitamins, the available data are controversial and the drive towards identification of more components and plants food sources continues. Nutraceuticals are generally considered powerful instruments in maintaining and promoting health, longevity and life quality (Ferreira *et al*., 2009). The advantages of naturally occurring antioxidants for use in foods over synthetic antioxidants are numerous. The combined action of two or more components in fruits or

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vegetables often potentiates a specific therapeutic action (Liu, 2004) and with no observed secondary or collateral effects as is the case with chemically synthesized compounds (Lizcano *et al*., 2010). Folk medicine in Kenya (East Africa) is still important in several regions where many wild plants are linked to many different traditional medicinal uses.

Indigenous fruits play a vital role in the livelihoods of many rural communities in Eastern Africa, especially those living in the drylands. Ethnobotanical records available on useful wild plants in Kenya highlight the importance of *Vitex* sp. medicinal roles and human consumption of their edible fruits (Kokwaro, 1993).

Plants of the genus *Vitex* from the Verbanaceae are trees or shrubs occurring in tropical and subtropical regions. In Kenya, they occur in western, central and coastal regions (Beentje, 1994). Phytochemical reports on *Vitex* sp. indicate that they are rich sources of ketosteroids, iridiods, terpenoids and flavonoid glycosides (Ono *et al*., 2000). The investigation of some *Vitex* species have resulted in the isolation of iridoid glycosides named agnuside, eurostoside, negundoside (2α-p-hydroxybenzolymussaenosidic acid), 6α-phydroxybenzoylmussaenosidic acid, nishindaside and isonishindaside from leaves and agnuside and 10-O-vanilloylaucubin from fruits; agnuside, limoniside and peduncullariside from stem barks (Kuruuzum *et al*., 2003). These plants have a wide range of biological activities. Among them in the management of menstrual disorders (Pearlsten and Steiner, 2008), induction of uterine contraction (Ladeji *et al*., 2005), anti-inflammatory and analgesic activities of *Vitex doniana* (Sweet) (Iwueke *et al*., 2006).

Yet, data related to antioxidant activity as well as phenolic content of the genus fruits and other parts are unknown. The aim of the present study was to evaluate the nutritional, phytonutritional contents and radical scavenging activities of the fruits of the three species viz *Vitex doniana* (sweet), *Vitex kiniensis* (Turrill) and *Vitex fischeri* (gurke) from Kenya.

MATERIALS AND METHODS

Plant materials: Fresh black plum fruits were collected (Fig. 1a-c) from different regions in Kenya as green unripe fruit and black ripe fruits. *Vitex doniana* fruits were collected in Maseno, Western Kenya region (0°00' 28.09"S; 34°35'46.30"E elevation 5015 m a.s.l); *Vitex fischeri* fruits were collected from Oyugis (0°29' 08.16"S; 34°44'05.47"E; elevation 4804 m a.s.l), Western Kenya regions, while *Vitex kiniensis* were collected from Kericho (0°29'07.70"S; 34°44'02.288:E; elevation 4815 m a.s.l),

Fig. 1: Fresh black fruits (a) *Vitex kiniensis*, (b) *Vitex fischeri* (gurke) black and fruits, (c) *vitex domiano* (sweet) black matur fruits

Kenya highlands rain forests. The fresh weights were recorded for both ripe and unripe fruits after collection. Different plant parts (leaves, flowers, fruits and stem bark) samples collected and identified at the Maseno University Herbarium by Mr. V. Okello (Taxonomist), where voucher specimens were prepared and deposited.

Materials and chemicals: Folin-Ciocalteu reagent, trichloro acetic acid, 1, 1-dipenyl-2-picrylhydrazyl (DPPH), 2, 4, 6-tri-2-pyridyl-s-triazine (TPTZ), sodium nitropruside, sulfanilamide, naphthyethylenediamine dihydrochliride, gallic acid, rutin and ascorbic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA)

through Kobian Kenya Ltd. All the chemicals and solvents used were analytical grade purchased from Kobian Kenya Ltd.

Proximate analysis: The total moisture was determined by drying the fruits in a Memmert 40050-IP20 oven at 105°C until a constant weight was obtained (AOAC, 1990). Crude protein content was calculated by converting the nitrogen content, determined by Kjeldahl's method (6.25×N) (AOAC, 1990) in a Tecator 2020 digester and Kjeltec 1030 auto analyzer. Fat content was determined by the AOAC (1990), using the Soxhlet system (AOAC, 1990). Ash content was determined by dry ashing in Vulcan A550 muffle furnace at 525°C for 12 h. Crude fiber was estimated (Triebold and Aurand, 1982) by mixing the residue left after the extraction of crude fat with 0.5 g asbestos and 200 mL of boiling sulfuric acid for digestion, the contents were filtered and residues mixed with sodium hydroxide and again digested for 30 min in a reflux condenser, residue was dried, weighed and ignited, the difference in initial and final weights was taken as the weight of crude fiber. The phenol-sulfuric acid procedure was conducted as described by Fournier (2001) which is based on the absorbance at 490 nm of colored aromatic complex formed between phenol and the carbohydrate. The amount of sugar present was determined by comparison with a calibration curve using a spectrophotometer. A liquots 0.8 mL of the samples from known masses (1 g each) were mixed with 25 μL of 4% phenol followed by 2.5 mL 96% H_2SO_4 acid. The absorbances of the solution were then measured at 490 nm using Uv spectrophotometer against equimolar glucose-fructose (50-500 μg L^{-1}) mixture as standard sample. The sugar content were estimated from the standard calibration curve where concentration in mol g^{-1} were deduced using the formula: Concentration (mol g^{-1}) = (x) g/(y) mol.wt (g mol^{-1}) x wt (g)

Where x is the quantity deduced from the curve; mol.wt is molecular mass of the glucose; wt (g) is the mass of the sample. Carbohydrates were calculated as nitrogen free extracts according to the formula: carbohydrates = 100 - (% moisture + % protein + % crude fibre + % fat + % ash).

Preparation of the extract: About 150 fresh fruits were sorted as mature ripe and mature unripe, cleaned immediately after harvesting and the pulp of each variety was separated from the endocarp using a sterile knife. Both exocarp and mesocarp were used as the edible portion of each fruit which were homogenized using a blender and the homogenate was then stored at -4°C in the refrigerator. Methanol-acetone-distilled water (6:3:1) was used for extraction of phytonutrients using Soxhlet extraction method. The extraction was carried out for 6 h. The extracts were concentrated at 50°C using rotary evaporator (EYELA rotary evaporator N-1000) and resultant residues were then made-up 50 mL and stored under refrigerated conditions for further experiments.

Evaluation of antioxidant activity

Determination of total phenolic content: The phenolic contents were determined using Follin-Ciocalteu reagent and expressed as Gallic Acid Equivalents (GAE) (Singleton *et al*., 1999). The extracts were diluted with methanol, by taking 3 mL of methanol and 1 mL of crude fruits extract solution. To this sample solution, 1 mL of 5-fold diluted Folin-Ciocalteu's reagent was added. The contents were mixed well and kept for 5 min at room temperature followed by the addition of 1 mL of 10% aqueous sodium carbonate. After incubation at room temperature for one and half hour the absorbance of the developed blue color was read at 760 nm (Shimadzu UV-1650 PC Shimadzu Corporation, Kyoto, Japan) against reagent blank. Gallic acid (100-1000 mg mL^{-1}) was used to construct the calibration curve. Results were calculated as gallic acid equivalents (mg/100 g) of samples. The reactions were conducted in triplicates and concentrations of phenolic compounds were calculated according to the equation obtained from the standard dallic acid graph: A = 0.0044 (gallic μg) + 0.6746 (R^2: 0.9173).

Determination of total flavonoid content: Total Flavonoid Content (TFC) was determined spectrophotometrically using the method of Zhishen *et al*. (1999) based on the formation of flavonoid-aluminium complex. An aliquot (0.5 mL) of the extract solution were mixed with 2 mL double distilled water, followed by 0.15 mL of 5% $NaNO_2$ solution. After 6 min, 2 mL of $AlCl_3$ (10%) was added, followed by addition of 0.5 mL of NaOH (1N) to the mixture. The mixture was diluted by adding 2.5 mL of double distilled H_2O immediately, then mixed thoroughly. Absorbance of the mixture, pink in color, was determined at 510 nm (Shimadzu UV-1650 PC) versus the prepared blank without extract.

The absorbance of each blank consisting of the same mixture in which $AlCl_3$ solution was substituted with double distilled H_2O was subtracted from the test absorbance (Joubert *et al*., 2008). Rutin (0.04-2.5 μg mL^{-1}) was used as standard and TFCs from the extracts were expressed as μg-rutin equivalent RT/g dry weight of each fruit sample. The concentrations of the flavonoids were calculated according to the following equation that was obtained from the standard rutin graph: A = 1.639 [rutin μg mL^{-1}]-0.0558 (R^2 = 0.9947).

DPPH radical scavenging activity: Free radical scavenging activity of the black plums extracts were determined by using a stable 2, 2-dipheny l-1-picrylhydrazyl radical (DPPH) (Brand-Williams *et al*., 1995). DPPH is a free radical of violent color. The antioxidants in the samples scavenge the free radicals and turn it into yellow color from violet is proportional to the radical scavenging activity.

Briefly, the assay contained 1 mL of 0.1 mM DPPH in methanol and varying concentrations of extracts (50-1000 μg mL^{-1}) in methanol and standards in the same solvent and made up to 3.5 mL with methanol. The contents were mixed well immediately and then incubated for 30 min at 30°C in water bath. The degree of reduction of absorbance was recorded in UV-Vis spectrophotometer at 517 nm (Shimadzu UV-Vis 1650 PC).

The percentage of scavenging activity was calculated as:

$$A\% = (Ac-As)/Ac \times 100$$

Where:
Ac = The absorbance of control (without sample)
As = The absorbance of sample

Percentage of radical scavenging activity was plotted against the corresponding concentration of the extract to obtain IC_{50} values. IC_{50} is defined as the amount of antioxidant material required to scavenge 50% of free radical in the assay system. The IC_{50} values are inversely proportional to the antioxidant activity.

Nitrite oxide scavenging assay: The scavenging effect of the extracts on nitrite oxide was measured according to the method of Marcocci *et al*. (1994). Four milliliters of the extract was added to test tubes containing 1 mL sodium nitropruside solution (25 mM) and the tubes incubated at 37°C for 2 h. An aliquot (0.5 mL) of the incubated solution was removed and diluted with 0.3 mL Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% naphthyethylenediamine dihydrochliride). The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthyethyenediamine dihydrochloride was read at 570 nm (Shimadzu UV-Vis 1650 PC) and referred to be absorbance of standard solutions of sodium nitrite salt treated in the same way with Griess reagent using the calibration curve line of $A = 0.824[NO]-1.5709$ ($R^2 = 0.9963$). The percentage of the NO scavenged after the reactions were regressed against the increasing concentration of the extracts to estimate the IC_{50}.

Total reducing ability: Antioxidant activity in the samples using the Ferric Reducing Ability of Plasma (FRAP) assay (Benzie and Strain, 1996) following the modification by Halvorsen *et al*. (2002) in which the samples were diluted in methanol instead of water.

The procedure involved adding 1.5 of the FRAP reagent (acetate buffer, pH 3.6, $FeCl_3$, TPTZ in 40 mM HCl) to 50 μL of the samples and 150 μL of distilled water. The absorbance at 593 nm was measured after10 min of incubation at 40°C. A triplicate of each sample was analyzed using UV-Vis spectrophotometer (Shimadzu UV-Vis 1650 PC). The instrumen twas calibrated using $FeSO_4.7H_2O$ (50-1000 μmoL) with ascorbic acid and gallic acid as controls. The antioxidant activity in the samples (5 mg mL^{-1}) was calculated with reference to the reaction signal given by Fe^{2+} solution this representing a one electron exchange reaction. The results were expressed in μmoL Fe^{2+} per 100 g fresh weight (μ moL $100g^{-1}$ FW) of sample.

Statistical analysis: The experimental results were expressed as mean±Standard Deviation (SD) of three replicates. Where applicable, the data were subjected to one way Analysis of Variance (ANOVA) and the differences between samples means were determined by post-hoc using the Statistical Analysis System (SAS) programme.

RESULTS AND DISCUSSION

Proximate composition: The proximate composition of the fruits of the three *Vitex* species are shown in Table 1. The fruits of all the three species contained a relatively high percentage of soluble sugar and fibre content. There were minor variations in the proximate values between the different ripe fruits but there were significant variations ($p = 0.05$) in the values between the ripe and the raw (Table 1). The moisture, carbohydrate and fat content values obtained for the three Vitex species fruits (Table 1) were comparable to the values stated for *Vitex doniana* by Agbede and Ibitoye (2007) while the values obtained for crude protein, ash and fibre were lower than those reported for *Vitex doniana* (Agbede and Ibitoye, 2007). Such variation might have resulted from geographic, climatic and seasonal variations. The fruit pulps were very high in moisture content and this may underscore their high perishability and susceptibity to microbial infections. Crude protein content ranged from 0.85-2.78% (Table 1) with raw fruits having relatively higher values ($p = 0.05$).

The amount of protein which is about 75% (when converted) of the total nitrogen in green plants parts is variable, ranging from 5-10% of fresh weight basis. These values were low compared to the dry milled values reported for *Vitex doniana* fruits 72.8 mg kg^{-1} (7.28%) (Agbede and Ibitoye, 2007) *Telfairia occidentalis* fruits (22.4%), *Tamarindus indica* (24.3%), *Hibiscus*

Table 1: Proximate composition of the raw and ripe three black plum *Vitex* species fruits

Samples	Moisture (%)	Ash (%)	Fibre (%)	Protein (%)	Soluble Sugar (%)	Total carbohydrate (%)	Fat (%)
VD	39.42±0.72	3.41±0.09	11.48±0.55	0.85±0.09	13.55±0.49	29.57±0.67	2.44±0.06
VK	40.56±0.77	3.40±0.13	10.42±0.47	0.87±0.05	14.45±0.50	28.04±0.56	2.35±0.06
VF	37.74±0.76	3.66±0.24	12.38±0.43	0.98±0.06	15.39±0.44	27.28±0.35	2.66±0.09
VDr	38.16±0.71	3.63±0.08	11.82±0.25	2.32±0.22	9.88±0.12	31.71±0.81	2.46±0.09
VKr	40.86±0.39	3.34±0.41	10.35±0.33	2.24±0.28	10.44±0.61	31.56±0.55	2.07±0.10
VFR	38.46±0.92	3.31±0.52	12.39±0.53	2.78±0.22	9.53±0.07	31.30±0.40	2.35±0.09
CV%	1.75	8.25	3.99	7.64	3.40	1.19	3.77
LSD p≤0.05	1.45	0.60	0.96	0.27	0.87	1.25	0.19
VD (Agbede and Ibitoye, 2007)	48.77 (487.7 mg kg^{-1})	5.27 (52.7 mg kg^{-1})	6.73 (67.3 mg kg^{-1})	7.28 (72.8 mg kg^{-1})		28.95 (289.5 mg kg^{-1})	3 (30 mg kg^{-1})

VD = *Vitex doniana*; VK = *Vitex kiniensis*; VF = *Vitex fischerii*; VDr, VKr, VFr = Respective raw fruits

esculentus (23%) (Igbal *et al*., 2006). So consumption of 100 g of black plum fruits from Kenyan soils may not be capable of providing 27 g of protein for children (FAO, 1986).

The fibre contents of the whole fruits pulp varied between the species slightly (p = 0.05) from 10.35-11.48% (Table 1) with *Vitex fischeri* both raw and ripe fruits exhibiting higher fibre content (12.38 and 12.39%, respectively).

Values were higher than the range for *Vitex doniana* reported from Nigeria as 67.3±0.7 mg kg^{-1} (6.73%) (Agbede and Ibitoye, 2007). All the *Vitex* fruits examined can be considered as rich source of crude fibre, all falls in the range of Recommended Dietary Allowance (RDA) for fibre in children, adults, pregnant and lactating mothers which are 19-25, 21-38, 28 and 29%, respectively (Ishida *et al*., 2000). Crude fibre is an important part of diet which decreases serum cholesterol levels, risk of coronary heart disease, hypertension, diabetes, colon and breast cancer (Ishida *et al*., 2000).

The carbohydrate level of the three *Vitex* species (Table 1) ranged from 27.28% in *V. fischerii* to 31.71% in raw *V. kiniensis*. From the result of soluble sugar contents (Table 1) ripe fruits showed high sugar content than raw fruits (p = 0.05) indicating that ripping process mobilized the soluble sugars characteristic of fruits (hydrolysis of the polysaccharides to simple monosaccharides). The results also indicated that soluble sugar form the bulk of the total carbohydrate content (Table 1).

Antioxidant activity: All the fruits and vegetables materials used as sources of nutrition and medicine contain some degree of antioxidants. Free radicals are critically involved in various pathological conditions such as cancer, cardiovascular disorder, arthritis, inflammation and liver diseases (Gulçin, 2007). Many phytochemicals exhibits significant antioxidant activities that are associated with lower occurrence of such ailments (Ames *et al*., 1993). The results of the antioxidant activity of are shown in Table 2.

Total phenolic and flavonoid content: Polyphenols are gaining acceptance as being responsible for the health benefits associated with fruits and vegetables (Nisha *et al*., 2009). Due to their chemical structure that undergo redox process which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, nitric oxides or decomposing peroxides (Zheng and Wang, 2001). The level of the phenolic compounds in *Vitex kiniensis* both ripe and raw fruits were considerable (Table 2), though the values significantly varied (p<0.05) from 290.18-719.83 mg GAE 100 g^{-1} FW of the fruit sample. The highest concentration was recorded in *Vitex kiniensis* extract, followed by *Vitex doniana* raw fruits, ripe fruits *and Vitex fischerii* raw fruits and ripe, respectively (Table 2).

Flavonoids are very effective anti-oxidants (Ismail *et al*., 2010). The total flavonoid content of the methanolic extract of the three *Vitex* species did not vary much from 1.01-2.35 μg RE g^{-1} FW though the values were statistically significant (p<0.05). This implies flavonoids are not the major phenolic compounds in these fruits. The highest flavonoid content of 2.35 μg RE g^{-1} FW was observed in the *Vitex doniana* and the lowest content in the *Vitex fischerii* ripe fruits (1.01 μg RE g^{-1} FW) (p<0.05). The total phenolic content and the flavonoid content were moderately correlated (r<0.5058) which indicated that flavonoids are not the major phenolic compounds in the fruits. However, black plum fruits have high sugar content (Table 1) mostly fructose and glucose, the presence of fructose interfere with Folin-Ciocalteu's assay (Singleton *et al*., 1999) such interference could have escalated the results for phenolic content. This is evidenced by the high phenolic content for the ripe fruits as opposed to the low levels raw fruits intraspecies.

Scavenging assay effect of 1, 1-diphenyl-2-picrylhydrazyl: Table 2 shows scavenging activity of different *Vitex* species examined. The scavenging activity was increased with increasing concentration (Fig. 1). IC_{50} values (the amount of antioxidant material required to scavenge 50% of free radical in the assay system) of the standard Ascorbic acid, gallic acid and rutin were observed at 0.249±0.006, 0.413±0.041 and

Table 2: Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total Reducing Power (FRAP), IC_{50} values of DPPH and NO scavenging activity of the fruits {raw (VDr, VKr, VFr) and ripe VD, VK, VF) three *Vitex* species

Samples	TPC (mgGA/100 g FW)^T	TFC (µg RT g^{-1} FW)^T	DPPH (IC_{50} in µg mL^{-1})^T	FRAP (µMFe^{2+}/100 g FW)^T	NO scangenging (IC_{50} in µg mL^{-1})^T
VD	601.40±25.2^b	2.35±0.035^a	0.55±0.008^a	545.71±3.29^a	11.4±1.61^a
VK	719.83±46.2^a	2.09±0.178^{ac}	0.42±0.009^a	402.43±1.11^c	10.9±0.57^a
VF	572.27±49.6^c	2.08±0.177^{ac}	0.43±0.013^a	203.57±2.27^d	11.3±0.18^a
VDr	371.15±14.6^d	1.68±0.269^{bc}	0.58±0.029^a	134.89±1.35^e	25.4±1.60^b
VKr	370.20±53.9^d	2.03±0.141^{ac}	0.47±0.017^a	72.76±0.80^f	26.5±2.29^b
VFr	290.18±10.9^e	1.01±0.033^b	0.46±0.006^a	89.16±1.82^g	29.5±1.33^b
Gallic acid	-	-	0.413±0.041^a	442.80±1.58^b	ND
Ascorbic	-	-	0.249±0.006^b	417.71±1.58^c	7.59±0.79^c
Rutin	-	-	0.158±0.011^b	ND	ND
ANOVA	***	***	**	***	-

^{T}Values are the means±S (n = 3). Data were analyzed by ANOVA (**p<0.001 and ***p<0.0001) and within each column different letters indicate statistically different values according to post-hoc comparison (LSD-test) at p<0.05. VD = *Vitex doniana*; VK = *Vitex kiniensis*; VF = *Vitex fischerii*; r = raw fruits

0.158±0.011 mg mL^{-1} (Table 2). The result indicated that there was no significant difference between the IC_{50} values between the three fruit extracts (Table 2). However, both raw and ripe fruits of *Vitex doniana* showed a relatively low activity (high IC_{50}). There was an inverse relationship between IC_{50} and antioxidant activity. Weak negative correlation (r = -0.15739) between total phenolic content and IC_{50} values of the different extracts on DPPH provide some evidence for the role of phenolic compound towards scavenging activities. This is contrary to the many published data where antioxidant capacity depends strongly on polyphenols content (Kiselova *et al*., 2006; Slusarcyk *et al*., 2009).

Nitric Oxide (NO) radical scavenging activity: Suppression of released NO may be partially attributed to direct NO scavenging, as the extracts of the black plums decreased the amount of nitrite generated from the decomposition of Sodium Nitroprusside (SNP) *in vitro*. The scavenging of NO by the extracts was increased in dose dependent manner (Fig. 2). The percentage of NO scavenged values were regressed against the sample concentration to obtained IC_{50} value (Table 2). The IC_{50} values indicates non significant difference between the species though values of raw and ripe fruits vary significantly (p<0.05). The ripe fruits displayed low IC_{50} inferring high NO scavenging activity than the raw fruits extracts.

Reducing ability assay (FRAP): The reducing ability of the extracts was in the range of 72.76-545.71 µM Fe^{2+}/100 g FW (Table 2). The antioxidant potentials of the extracts of the fruits were estimated from their ability to reduce TPRZ-Fe^{3+} complex to TPTZ-Fe^{2+}. The FRAP values for ripe fruit of *Vitex doniana* was significantly higher than that of ascorbic acid and gallic acid at 442.80 and 417.71 µMFe^{2+}, respectively. Whereas FRAP values for *V. fischerii* ripe fruits was the lowest and these values showed weak correspondence to the phenolic content and to other antioxidant assays (Fig. 3).

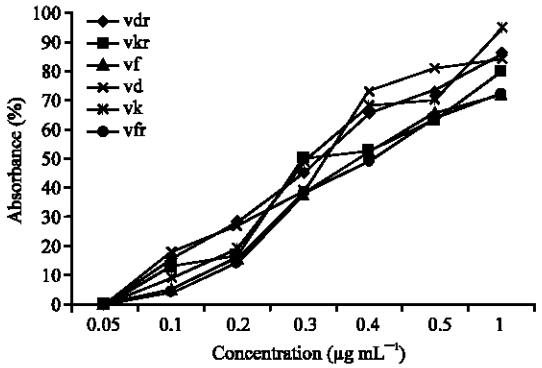

Fig. 2: DPPH scavengng activity of three extracts of three *Vitex* fruits

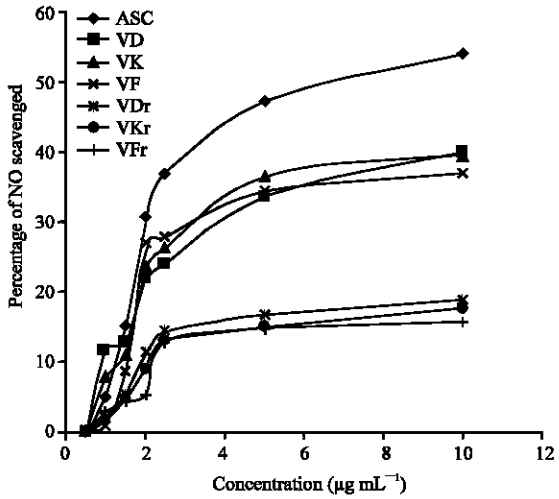

Fig. 3: Nitric Oxide (NO) scavenging activity of methanolic extracts of the three *Vitex* fruits and ascorbic acid

Generally there is moderate positive correlation between the antioxidant assays and the quantitative phytochemical screening, i.e., the total polyphenolics and the flavonoid content (r = 0.7218). This concurred with

many published data where measured antioxidant capacity depends on the polyphenol content (Kiselova *et al*., 2006; Silva *et al*., 2007). There are also many reports on the ambiguous or even adverse relationships between polyphenols and antioxidant capacity (Slusarcyk *et al*., 2009). In Verbanaceae (*Vitex* sp.), several compounds occur that can add to the polyphenol-based antioxidant potential such as hydroxlated ecdysteroids and iridoids (Kuruuzum *et al*., 2003). The apparent lack of strong correlation between the results of each assay is frequently reported as resulting from the difference of mechanism involved in the antioxidant activity (Aruoma, 2003; Halliwell, 1995). Similarly, the characteristic of a particular test reaction can influence the outcome from the analysis. Some tests are preferential towards hydrophilic or hydrophobic compounds, whilst others are insentive in this matter. Solubility of the test compounds in the assay environment is another important factor influencing the outcome. In case of polyphenols, some aglycons can be actually water-insoluble, hence the incompatibility with some water-based methods such as nitric oxide and deoxyribose assay (Aruoma, 2003; Halliwell, 1995). On the other hand, the lipophilic antioxidants can do better in fatty acid peroxidation assays (Slusarcyk *et al*., 2009).

In this study, the Methanol-acetone-water extract can contain a substantial amounts of both hydrophibic and lipophilic antioxidants exhibiting the relatively higher activities in free radical scavenging (NO and DPPH). The non-critical role of polyphenols in nitric oxide scavenging has been reported (Slusarcyk *et al*., 2009) as such activity may be observed that does not correspond to the polyphenol content. However, the assay is carried out in aqueous buffer, the nitric oxide molecule itself is a lipophilic species, hence the higher compatibility to such compounds that could disperse well in the buffer and interact with the free radical. Whether the ecysteroid, iridoids glycoside or flavonoid glycosides present in the extracts are responsible for the activity requires further studies on pure compounds. Both DPPH and NO assays measure the free radical-scavenging activity but DPPH is carried out in an organic medium (methanol) while NO assay requires buffered aqueous solution. This can account for the discrepancies between the two assays. FRAP assays on the other hand is carried out in methanol and not in aqueous medium. Hence, the lack of correlation to both of the other assay can result from the difference in mechanism involved.

CONCLUSION

In this study, the fruits of the three *Vitex* sp. containing considerable polyphenol phytochemical are efficient free radical scavenger. This provides a complementary preventive value and supports their gaining popularity as under-utilized botanical food supplement. The crude extract from the different *Vitex* species differed in response to the three *in vitro* assays, most likely due to the difference in phytochemical content and composition. The results from this study showed that methanol/acetone/water extract of three fruits demonstrated comparable DPPH and NO radical scangenging activity to the standard. *Vitex doniana* and *Vitex kiniensis* (ripe fruits) demonstrated better reducing ability than the other samples which may be attributed to the higher phenolic and flavonoid content. The identification of the antioxidant compounds from the fruits of *Vitex doniana*, *Vitex kiniensis* and *Vitex fischerii* is necessary for getting the complete insight into the relations between various compounds or antioxidants in the respective fruits.

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