Research Article

***In Vitro* Evaluation of Free Radical Scavenging, Fe²⁺ and SNP-Induced Lipid Peroxidation (Rat Brain) Activities of Methanolic Extracts from Three (3) Northern Nigerian Plants Leaf**

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ABSTRACT

Over the years research findings have shown that free radicals are the basis for many diseases. This study sought to investigate the free radical scavenging and inhibition of lipid peroxidation activities of some three Northern Nigerian plant leaf; *Boswellia dalzielii* Hutch. (Burseraceae) (BDL), *Detarium microcarpum* Guill. and Perr. (DML), and *Daniellia oliveiri* (Rolfe) Hutch. & Dalz (DOL). The study investigated both qualitative and quantitative phytochemical screening, free radical scavenging activities of 1, 1-diphenyl-2-picrylhydrazyl DPPH, total antioxidant capacity ABTS, hydroxyl, reducing property and inhibition of lipid peroxidation. All the seven phytochemicals screened were all present in both BDL and DML plant extracts but saponins and glycosides were absent in DOL plant leaf. The result revealed that BDL methanolic extract had the highest total flavonoid and phenolic content while BDL methanolic extract had the highest alkaloid content. DOL methanolic extract had the least content in all the 3 phytochemicals quantified. BDL methanolic extract had the highest free radical scavenging activity in 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Hydroxyl and reducing power assays. While DML methanolic extract, had the highest free radical scavenging ability in 2, 2-azino-bis(3-ethylbenzazoline-6-sulphonic acid (TEAC) assays. The IC₅₀ of BDL extract (12.37 mg/mL) was not significantly different ($p > 0.05$) from DML extract (10.39 mg/mL) in the Fe²⁺-induced lipid peroxidation assay. While, the IC₅₀ of DML extract (14.83 mg/mL) was significantly higher ($p < 0.05$) than the BDL extract (19.92 mg/mL) in the SNP-induced lipid peroxidation. The DOL extract had the least scavenging and peroxidative inhibitory activity in all the assays carried out. We therefore concluded that the synergistic free radical scavenging activities and inhibition of lipid peroxidation of the three plants studied (especially, BDL and DML), provides a biochemical rationale for their usage as a medicinal plant.

Keywords: Free radicals, antioxidants, medicinal plants, lipid peroxidation

Introduction

A free radical is a resultant molecule with one or two unpaired electrons in its outer orbit. It is highly unstable and reactive; it can be either oxidants or reductants in their reaction with other biological molecules [1]. They can be derived from normal essential metabolic processes or from ex-

ternal sources, such as, exposure to X-rays, cigarette smoking, air pollutants and toxic chemicals. Examples of oxygen containing free radicals are; hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical. Biological free radicals can lead to the damage of DNA ma-

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terials and peroxidation of cellular membranes. Diseases linked to free radicals include inflammation, cancer, atherosclerosis, heart disease, stroke, diabetes mellitus, rheumatoid arthritis, osteoporosis, ulcers, and cataracts [1, 2].

Antioxidants are molecules that can donate an electron and pair up with free radical, thus incapacitating its damaging effects. It acts as radical scavengers, electron donors, hydrogen donors, peroxide decomposers, metal chelators, singlet oxygen quenchers, and enzyme inhibitors [3, 4]. The antioxidant enzymes (superoxide dismutase, catalase and glutathione systems) in the human body help to combat these free radicals. Free radicals can also be combated via the activities of exogenous antioxidants; such as, ascorbic acid, beta carotene, alpha-tocopherol, enzyme cofactors trace elements (selenium, zinc, and copper), and natural polyphenols [1, 3, 4]. Polyphenols are phytochemicals such as phenolic acids, flavonoids, lignans, and stilbenes which are naturally occurring micronutrients in plants (cannot be produced in humans). Their structural component predisposes them to be good electron donors for the free unstable radicals; this bestows their antioxidant potentials to them. The antioxidative properties of polyphenols confer on plants their medicinal properties [2, 5].

Medicinal plants have been the mainstay of primary health interventions in many developing nations; Africa and Asia. In Malaysia, over 1300 medicinal plant species have been recorded in Peninsular Malaysia alone; such as; *Amaranthus spinosus*, *Callicarpa arborea* Roxb., *Zingiber officinale* Rosc., etc. These and others have been used in the management of ailments such as arthritis, senility, parkinson's illness, gastrointestinal disturbances, tumor promotion, and carcinogenesis [6]. The three selected plants leaf are; *Boswellia dalzielii* Hutch. (Burseraceae), *Detarium microcarpum* Guill. and Perr., and *Daniellia oliveiri* (Rolfe) Hutch. & Dalz, these have been used in the treatment of inflammation, cancers, fibrosis, anti-rheumatism in Northern Nigeria folk medicine [7]. There is little or no information about the comparative antioxidative evaluation of these three plants leaf. Therefore, this study sought to comparatively evaluate, the free radical scavenging and inhibition of lipid peroxidation activities of their methanolic extracts.

Material and Methods

Chemicals and reagents

All chemicals and solvents used were of analytical grade (purity > 98%) from Sigma (St. Louis, USA). The water used was double distilled water from Milli-Q Direct 8/16 System (Millipore SAS, 67120 Molsheim, France).

Sample collection, authentication, and preparation of plant extracts

These three plant leaves, *Detarium microcarpum* Guill and Perr. (Fabaceae) (DML) voucher no.-FHJ-225, *Boswellia dalzielii* Hutch. (Burseraceae) (BDL) voucher no.-FHJ-226, and *Daniellia oliveiri* (Rolfe) Hutch. & Dalz. (Leguminosae-Caesalpinioideae) (DOL)- voucher no.-FHJ-224, were collected, authenticated and given voucher numbers, by the Mr. Azilla in the Federal College of Forestry, Jos, Plateau State, Nigeria.

Qualitative phytochemical analysis

Phytochemical screening of the three plants leaf (DML), (DOL), and (BDL) for saponins, tannins, cardiac glycosides, alkaloids, anthraquinones and flavonoids was carried out according to standard methods [8].

Preparation of 70% methanolic extracts

As much as 20 g of powdered shade-dried plants leaf were placed differently in a 500 mL flask, and 70% methanol (70 : 30, methanol : double distilled water) was added. The extraction was performed on an orbital electric shaker (OHAUS laboratory equipment, ChemEurope, Germany) for a period of 48hrs at room temperature 25°C. After the extraction, the residues were separated from the extracts by filtering through a Whatman filter paper No. 1. This phase was carried out twice using fresh solvents, and then all the extracts were pooled together, respectively. The extracts were then concentrated to dryness under reduced pressure at 45°C, using a rotary evaporator (EYELA, N-N series, Rikakikai Co. Ltd. Tokyo, Japan). The dried methanolic extracts were stored in the refrigerator at -4°C, until it was required for the in vitro assays. All the spectrophotometric measurement was done using a Jenway UV/Visible Spectrophotometer 7315, Bibi Scientific, United Kingdom.

Quantitative Phytochemical Analysis

Determination of total phenol content

The total phenolic contents in the DML, DOL, and BDL methanolic extracts were estimated using Folin–Ciocalteu reagent [9]. Absorbance was measured at 765 nm using Jenway 7315, UK. Gallic acid was used for the standard. The Unit of measurement was in mg/GAE g.

Total phenol was calculated thus:

$$Abs_{sam} \text{ mg/g} = \frac{(Abs_{sam} \times Conc_{std})}{(Abs_{std} \times Conc_{sam})}$$

Note:

Ab_{std} :Absorbance of standard (Gallic acid)

Ab_{sam} :Absorbance of sample

$Conc_{std}$:Stock concentration of standard in mg/mL

$Conc_{sam}$:Stock concentration of sample in mg/mL

Determination of total flavonoid content

The total flavonoid content of the three extracts (DML, BDL, and DOL) was determined using a slightly modified method reported by Meda, Lamien, Romito, Millogo, Nacoulma [10]. Absorbance was subsequently measured at 415 nm, using Quercetin as a standard. The unit measurement was in mg/ QUE g. The formula for its calculation is the same one used for total phenol.

Determination of total alkaloid content

The total alkaloid content was determined according to Trease and Evans [11]. UV- vis Spectrophotometer method. The absorbance was measured at 470 nm. The unit of measurement was expressed in percentage (%).

1, 1-diphenyl–2-picrylhydrazyl (DPPH) free radical-scavenging ability

DPPH radical scavenging capacities of methanolic extracts were estimated by the reduction of the reaction colour between DPPH solution and extracts as previously described by Gyamfi, Yonamine, Aniya, [12]. The absorbance was measured at 516 nm. The DPPH free radical scavenging ability was subsequently calculated and was expressed in percentage (%).

2', 2'-azino-bis (3-ethylbenzazoline-6-sulphonic acid) (ABTS+) scavenging ability

The ABTS' scavenging ability of the extracts was determined according to the method described

by Re, Pellegrini, Protoggento, Pannala, Yang, Rice-Evans [13]. The absorbance was measured at 734 nm. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated, using Trolox as the reference and measurement of the inhibition of ABTS+ was expressed in percentage (%).

Inhibition of H_2O_2 -induced OH^* production (degradation of Deoxyribose)

The ability of the three methanolic extracts; DML, BDL, and DOL to inhibit H_2O_2 - induced decomposition of deoxyribose was determined using a modified method of Halliwell, Gutteridge, Aruoma [14]. The absorbance was measured at 532 nm and inhibition measured in percentage.

Determination of reducing power

This was done by assessing the ability of the extract to reduce $FeCl_3$ solutions described by Oyaizu [15]. Subsequently the absorbance was measured at 700 nm. The ferric reducing capacity was then calculated.

Lipid peroxidation assay

Experimental animals

Three male wistar rats weighing 150 – 160 g (4 weeks) were gotten from the University of Jos animal house. The handling of experimental animals was done in accordance with ethical clearance as approved by the Animal Ethics Committee, University of Jos. They were housed in stainless steel cages under controlled conditions of a 12 h light/dark cycle, 50% humidity, and 28°C temperature. The rats were allowed access to food and water ad libitum.

Preparation of tissue homogenates

The three wistar rats were each decapitated via cervical dislocation and the cerebral tissue (whole brain), was rapidly dissected, placed on ice and weighed. After which it was rinsed in cold saline solution and homogenized in phosphate buffer pH 7.4 (brain, 1 : 10 w/v) with about 10-up and -down strokes at approximately 1,200 rev/min in a Teflon-glass homogenizer. Each of the homogenates was centrifuged for 10 min at 3,000 g to yield a pellet that was discarded and the supernatant (S1) containing proteins and lipids was used for lipid peroxidation assay.

Table 1. Phytochemical quantitative and qualitative analysis of BDL, DML, and DOL plant leaf extract

Screened phytochemical	BDL Quantitative	BDL Qualitative	DML Quantitative	DML Qualitative	DOL Quantitative	DOL Qualitative
Total Flavonoid (mg/QUEg)	0.0289 ± 0.001 ^a	++	0.0165 ± 0.004 ^b	++	0.0235 ± 0.004 ^a	+
Total Alkaloid (%)	4.85 ± 0.07 ^c	+	10.53 ± 0.13 ^a	++	6.5 ± 0.042 ^b	+
Total Phenol (mg/GAE g)	0.1116 ± 0.001 ^a	++	0.097 ± 0.002 ^b	+	0.1054 ± 0.002 ^a	+
Tannins	ND	+	ND	+	ND	+
Saponins	ND	+	ND	+	ND	—
Terpenes	ND	+	ND	+	ND	+
Glycosides	ND	+	ND	+	ND	—

Note:

1. (-) signifies absent, (+) signifies present; (++) signifies highly present, ND- Not determined
2. Values represent mean ± standard deviation of triplicate readings. Values with different superscript along the same column (for the same parameter) are significantly different ($P < 0.05$)
3. BDL- *B. dazielli* leaf; DOL- *D. oliveiri* leaf; and DML- *D. microcarpum* leaf

Table 2. IC₅₀ values of DPPH (1, 1-diphenyl-2-picrylhydrazyl), Fe²⁺ and SNP- induced lipid peroxidation inhibition of BDL, DML, and DOL leaf methanolic extracts in the rat brain

IC ₅₀ (mg/mL)	BDL	DML	DOL
DPPH (1.1-diphenyl-2-picrylhydrazyl)	44.98 ± 0.91 ^a	58.94 ± 1.56 ^b	70.06 ± 0.90 ^c
Inhibition of Fe ²⁺ induced lipid peroxidation (Brain)	12.37 ± 0.93 ^a	10.39 ± 0.76 ^a	43.68 ± 0.79 ^b
Inhibition of SNP induced lipid peroxidation (Brain)	19.92 ± 1.01 ^a	14.83 ± 0.933 ^b	35.41 ± 1.03 ^c

Note: Values represent means ± standard deviation of triplicate readings. Values with different superscript along the same row are significantly different ($p < 0.05$).

Lipid peroxidation and thiobarbituric acid reactions

The lipid peroxidation assay was determined by a slightly modified method of Ohkawa, Ohishi, Yagi [16]. The absorbance of thiobarbituric acid reactive species (TBARS) produced was measured at 532 nm. Malondialdehyde (MDA) produced was measured and expressed in percentage (%).

Data analysis

Wherever possible, results of triplicate experiments were pooled and expressed as mean ± standard deviation [17]. One-way analysis of variance (ANOVA) and post hoc Tukey analysis were carried out. All statistical analysis was performed using IBM SPSS Statistics for windows, version 22.0 (IBM Corp., USA). A significant difference was accepted at $p < 0.05$.

Results and Discussion

Qualitative and quantitative phytochemical analysis

Plants with phytochemicals that have the largest quantity of polyphenolic have been found to be highly ameliorative in the treatment and management of diseases. Polyphenols contain phenolic rings and have structural elements that bind these rings. Also, flavonoids are the most abundant polyphenols in plants. Polyphenols possess antioxidative properties (because it cannot be found in humans) therefore makes medicinal plants a reliable source for antioxidants [5, 18]. Table 1 shows the phytochemical qualitative and quantitative analysis of BDL, DML, and DOL. Qualitatively, flavonoids were highly present in both BDL and DOL, it was present but not highly in DML, and for the alkaloid content, it was highly present in DML,

but not highly present in both BDL and DOL. Tannins and Terpenes were invariably present in all the three plant extracts, while, Saponins and Glycosides were present in both BDL and DML plant extract but absent in DOL plant extract. Quantitative analysis of the total flavonoid contents, showed that BDL (0.0289 mg/QUE g) and DOL (0.0235 mg/QUE g) were significantly higher ($p < 0.05$) than DML (0.0165 mg/QUE g) methanolic extract. Total alkaloid contents from Table 1, revealed that DML methanolic extract ($10.53 \pm 0.13\%$) was significantly higher ($p < 0.05$) than DOL ($6.5 \pm 0.042\%$), while DOL ($6.5 \pm 0.042\%$) was significantly higher ($p < 0.05$) than BDL ($4.85 \pm 0.07\%$) extract. There was a correlation between the qualitative screening and quantitative analysis determined. This result agrees with Chukwuma, and Ejikeme [19] that studied the Qualitative and Quantitative determination of phytochemical contents of Indigenous Nigerian Softwoods.

1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity

From Table 2, the IC_{50} values of DPPH free radical scavenging activities of (BDL), (DML), and (DOL) leaf extracts in the rat brain were represented. The IC_{50} (mg/mL) from Table 2, showed that BDL (44.98 mg/mL) had a higher radical scavenging ability ($p < 0.05$) than DML (58.9 mg/mL) methanolic extract. While, DML (58.94 mg/mL) was significantly higher ($p < 0.05$) than DOL (70.06 mg/mL). Antioxidants are very ubiquitous in the world of medicinal plants because it can mitigate the chain initiation step by scavenging free radicals [18, 20]. The reason for the higher free radical scavenging ability may be its higher polyphenolic content. This finding agrees with earlier reports where there existed a positive correlation between the total phenol content and DPPH free radical scavenging activity of microalgae [21].

2', 2'-azino-bis (3-ethylbenzazoline-6-sulphonic acid) (ABTS+) scavenging activity

Due to the unstable free radical formed by the disintegration of DPPH, free hydrogen radical is formed but not stable. But the ABTS forms a stable free product even if this cannot be obtained in any living physiological system [22]. The ABTS* scavenging ability reported as trolox equi-

Figure 1. Total antioxidant capacity of BDL, DML, and DOL leaf. Error bars represent the standard error of three replicate analyses.

Figure 2. Hydroxyl radical scavenging ability of BDL, DML, and DOL methanolic extracts

Figure 3. Reducing the power of methanolic extract of BDL, DML, and DOL leaf

valent antioxidant capacity (TEAC) is represented in Figure 1. The result revealed that DML methanolic extract (3.81 mmol/TEAC g; 3.86 mmol/TEAC g) at both dose levels (100 µg/mL and 200 µg/mL) respectively was significantly higher ($p < 0.05$) than BDL methanolic extract (3.58 mmol/TEAC g; 3.75 mmol/TEAC g), followed with DOL methanolic extract (3.53 mmol/TEAC g; 3.68 mmol/TEAC g) with the least value respectively at both dose levels. This ABTS free radical scavenging activity is relatively proportionate to their total phenolic content.

Inhibition of H_2O_2 -induced OH^* production (degradation of Deoxyribose) activity

Hydroxyl radicals are highly reactive oxygen species usually produced during the iron-catalyzed reaction (Fenton reaction). It is one of the potent reactive oxygen species that reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids in the biological system and can cause damage to the attacked cell [22]. From Figure 2, all three extracts had OH^* free radical scavenging abilities, but BDL methanolic extract (34.5%, 45.51%, and 57.55%) had the highest scavenging activity at the three dose levels (22.27 mg/mL, 45.45 mg/mL, and 68.18 mg/mL); this was followed by the OH^* scavenging activities of DML and DOL methanolic extract respectively. This high OH^* scavenging activity can be attributed to its high phytochemical contents. This result, in comparison to that of Karuna, Dey, Das, Kundu, Bhakta, [23], was higher than the hydroxyl scavenging ability of *Asparagus racemosus* Linn. This result reveals that BDL extract can prevent the damaging effect of ROS, because it can scavenge hydroxyl radicals directly and this is one of the attributes of antioxidants.

Reducing power activity

Reducing power is an essential antioxidant defense mechanism, which is ascertained by electron or hydrogen atom transfer by the antioxidant molecules [24]. The reducing power from Figure 3, revealed that all three methanolic extracts were able to reduce Fe(III) to Fe(II) in a dose dependent manner. BDL extract had the highest reducing ability 9.92 mg/AAE g and

As much as 18.29 mg/AAE at both dose levels (0.113 mg/mL and 0.23 mg/mL) respectively. This was followed by DML methanolic extract reduc-

ing the ability, with DOL methanolic extract reducing ability with the least. This implies that that BDL extract is a much better electron or hydrogen donor than both DML and DOL.

Lipid peroxidation activity

One of the major mechanisms of cell and tissue damage in aerobic organisms subjected to oxidative stress is lipid peroxidation. The chain reaction of lipid peroxidation ensures a continuous supply of free radicals which will only initiate further peroxidation activities [25, 26]. The results showed that incubation of the rat's brain in the presence of 25 µM/L of Fe^{2+} and SNP caused a significant increase ($p < 0.05$) in the MDA contents of the rat brain Fe^{2+} (72.41%) and also in SNP-induced (83.41%). (Figure not included) However, all three methanolic extracts exhibited high inhibitory effects on brain homogenate MDA produced in a dose-dependent trend (15.625 125 µg/mL). For the Fe^{2+} -induced lipid peroxidation activity, there was no significant difference in the ability to inhibit the MDA produced, between the BDL and DML methanolic extract, with DOL with the least inhibitory activity and consequently produced the most MDA. Same trend was followed but for the SNP-induced, DML extract (26.90 µg/mL) had a higher inhibition with the lowest MDA produced, followed by BDL extract (30.81 µg/mL) and DOL extract (51.42 µg/mL) with the least inhibitory effect but had the most of MDA produced at the 15.526 µg/mL dose level. Table 2, also represents the IC_{50} values of the inhibition of Fe^{2+} and SNP-induced lipid peroxidation in isolated rat's brain homogenates by the methanolic extracts of BDL, DML, and DOL. It reveals that the IC_{50} of DML methanolic extract (10.39 mg/mL) and BDL methanolic extract (12.37 mg/mL) had a significant effective inhibition of malondialdehyde (MDA) production with a minimum concentration ($p < 0.05$) than DOL methanolic extract (43.68 mg/mL). The mode of inhibition of Fe^{2+} -induced lipid peroxidation may be due to either, the formation of complexes with Fe^{2+} thereby preventing them from catalyzing the initiation of any peroxidative action or the polyphenolic could have scavenged the free radicals produced by the Fe^{2+} -catalyzed reaction. Likewise, incubation of the rat's brain tissue homogenates in the presence of Sodium nitroprusside (SNP) also caused a significant increase ($p < 0.05$)

in the rat brain MDA content. Both DML (14.83 mg/mL) and BDL methanolic extract (19.92 mg/mL) had a significant effective inhibition concentration ($p < 0.05$) than DOL methanolic extract (35.41 mg/mL), judging from the IC_{50} values. This finding agrees with the inhibitory lipid peroxidation activities of two varieties of Ginger (*Zingiber officinale*) by Akinyemi, Ademiluyi, Oboh [27].

Conclusion

Overall the results of this study revealed that all three methanolic extracts from the plant leaf, have a considerable free radical scavenging activity, *B. dalzielli* with the highest scavenging ability. This gives one of the biochemical bases for their use in folk- medicine management of degenerative diseases.

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References

- Ahmadinejad F, Møller SG, Hashemzadeh-Chaleshtori M et al. (2017) Molecular mechanisms behind free radical scavengers function against oxidative stress. *Antioxidants* 6 (3): 51. doi:10.3390/antiox6030051.
- Di-Meo F, Lemaure V, Cornil J et al. (2013) Radical scavenging by natural polyphenols: Atom versus electron transfer. *The Journal of Physical Chemistry A* 117 (10): 2082 – 2092. doi: 10.1021/jp3116319.
- Aurelia MP, Aneta P (2015) The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry* 97: 55 – 74. doi: 10.1016/j.ejmech.
- Zhang H, Tsao R (2016) Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science* 8:33 – 42. doi: 10.1016/j.cofs.2016.02.002.
- Losada-Barreiro S, Bravo-Díaz C (2017) Free radicals and polyphenols: The redox chemistry of neurodegenerative diseases. *European Journal of Medicinal Chemistry* 133: 379 – 402. doi: 10.1016/j.ejmech.2017.03.061.
- Abubakar MS, Musab AM, Ahmeda A, Hussaini IM (2007) The perception and practice of traditional medicine in the treatment of cancers and inflammations by the Hausa and Fulani tribes of Northern Nigeria. *Journal of Ethnopharmacology* 111: 625 – 629. doi: 10.1016/j.jep.2007.01.011.
- Ali A, Naznin S, Ahmed A, Mohammed RA (2014) Review on Some Malaysian Traditional Medicinal Plants with Therapeutic Properties. *Journal of Basic and Applied Sciences* 10: 149 – 159. doi: 10.6000/1927-5129.2014.10.20.
- Evans WC, eds. (2009) *Trease and Evans's pharmacognosy*. 16th Edition. Edinburgh, Elsevier. pp 353 – 415.
- Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology* 29: 152 – 178. doi:10.1016/S0076-6879.
- Meda A, Lamien CE, Compaoré MYY et al. (2008) Polyphenol Content and Antioxidant Activity of Fourteen Wild Edible Fruits from Burkina Faso. *Molecules* 13: 581 – 594. doi: 10.3390/molecules13030581.
- Trease GE, Evans WC (2002) *Pharmacognosy* 15th ed. Philadelphia: WB Saunders, Elsevier Science Limited. pp 336.
- Gyamfi MA, Yonamine M, Aniya Y (1999) Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. *General Pharmacology* 32: 661 – 667. doi: 10.1016/S0306-3623(98)00238-9.
- Re R, Pellegrini N, Proteggente A et al (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology Medicine* 26: 1231 – 1237. doi: 10.1016/S0891-5849(98)00315-3.
- Halliwell B, Gutteridge JMC (1999) Free radicals in biology and medicine. In: Halliwell B, Gutteridge JMC eds., *Free radicals in biology and medicine* 3rd Edition. Oxford, Oxford University Press. pp 1 – 25.
- Oyaizu M (1986) Studies on products of browning reaction: antioxidant activity of products of browning reaction prepared from glucosamine. *Japan Journal of Nutrition* 44: 307 – 315. doi: 10.5264/eiyogakuzashi.44.307.
- Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95: 351 – 358. doi: 10.1016/0003-2697(79)90738-3.
- Zar J (1984) *Biostatistical analysis*. 2nd Edition. Englewood Cliffs, Prentice-Hall, Inc.
- Tsao R (2010) Chemistry and biochemistry of dietary polyphenols. *Nutrients* 2: 1231 – 1246. doi: 10.3390/nu2121231.
- Chukwuma SE, Ejikeme CM (2016) Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. *New Journal of Science*. doi: 10.1155/2016/5601327.
- Zhang H, Tsao R (2016) Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science* 8: 33 – 42. doi: 10.1016/j.cofs.2016.02.002.
- Shetty V, Sibi G (2015) Relationship between total phenolics content and antioxidant activities of microalgae under autotrophic, heterotrophic and mixotrophic growth. *Journal of Food Resource Science* 4 (1): 1 – 9. doi: 10.3923/jfrs.2015.1.9.

22. Alam N, Nusrat JB, Rafiquzzaman (2012) Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal* 21 (2): 143 – 152. doi: 10.1016/j.jsps.2012.05.002.
23. Karuna DS, Dey P, Das S et al. (2018) *In vitro* antioxidant activities of root extract of *Asparagus racemosus* Linn. *Journal of Traditional and Complementary Medicine* 8 (1): 60 – 65. doi: 10.1016/j.jtcme.2017.02.004.
24. Pulido R, Bravo L, Saura-Calixto (2000) Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural Food Chemistry* 48 (8): 3396 – 3402. doi: 10.1021/jf9913458.
25. Oboh G, Rocha JBT eds (2007) Antioxidants in foods: a new challenge for food processors: Leading edge antioxidants research. 1st Edition. New York, Nova Science Publishers Inc. pp 35 – 64.
26. Puntel RL, Nogueira CW, Rocha JBT (2005) Krebs cycle intermediates Modulate Thiobarbituric Acid Reactive Species (TBARS) production in rat brain *in vitro*. *Neurochemical Research* 30: 225. doi: 10.1007/s11064-004-2445-7.
27. Akinyemi AJ, Ademiluyi AO, Oboh G (2013) Aqueous extracts of two varieties of ginger (*Zingiber officinale*) inhibit Angiotensin i-converting enzyme, iron(II), and sodium nitroprusside-induced lipid peroxidation in the rat heart *in vitro*. *Journal of Medicinal Food*. doi: 16. 641 – 646. doi: 10.1089/jmf.2012.0022.