Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

# Effect of Heat Treatment on the Antioxidant Capacity of Aqueous and Ethanol Extracts of *Aframomum angustifolium* Seed

Ehizuelen I. Ebhohimen [1]*, Lawrence Edemhanrhia [1], Adriel Ekozin [1], Paulinus N. Okolie [2]

[1]*Department of Chemical Sciences, College of Basic and Applied Sciences, Samuel Adegboyega University, Ogwa, Edo State Nigeria.*
[2]*Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.*

ARTICLE INFO

*Article history:*
Received 29 July 2017
Revised 01 September 2017
Accepted 08 September 2017
Published online 09 September 2017

*Keywords:*
*Aframomun angustifolium,*
*Antioxidant potential,*
*Heat treatment,*
*Spices.*

ABSTRACT

Spices are rich in phytochemicals which are responsible for their observed antioxidant property. This potential can be affected by heat treatment when spices are used as food additives. This research studied the effect of heat treatment on the antioxidant capacity of aqueous and ethanol extracts of *Aframomum angustifolium* seeds. The antioxidant capacity was measured *in vitro* by evaluating the metal chelating activity (MCA), ferric reducing ability (FRA), hydroxyl radical (•OH) scavenging activity, nitric oxide (NO) scavenging activity and total antioxidant capacity (TAC) before and after heat treatment. The percentage metal chelating activity of the extracts before heating were comparable ($p > 0.05$) to that of ascorbic acid while the percentage metal chelating activity of the ethanol extract decreased significantly ($p < 0.05$) from 65.99% to 47.62% after heat treatment for 30 min. The percentage hydroxyl radical scavenging activity of the aqueous extract was unaffected ($p > 0.05$) by heat treatment whereas the activity in ethanol extract decreased. The ferric reducing ability of the extracts decreased significantly after heat treatment while the NO scavenging activity increased with heat treatment. The total antioxidant capacity (TAC) of the extracts measured as ascorbic acid equivalents reduced but were not significantly affected by heat treatment. The results of this study suggest that the antioxidant potential of aqueous and ethanol extracts of *A. angustifolium* seeds is not totally lost by heat treatment.

## Introduction

Thermal treatment has been reported to induce changes in organoleptic, nutritional and textural properties of foods.[1] Cooking increases food safety by destroying food microbial flora, inactivating anti-nutritional factors and enhancing digestibility of foods thereby increasing bioavailability of nutrients.[2] However, cooking and other thermal processes have also been reported to reduce food quality.[3] Thermal processing affects the phytochemicals responsible for the pharmacological properties of plants.[4,5] There is a general belief that levels of phytochemicals are reduced by thermal treatment.[2] Home cooking methods have been reported to cause significant decreases in the phytochemical contents and antioxidant capacity of faba bean (*Vicia faba* L.).[6] However, an overall increase in antioxidant activity of extracts from *Citrus ushin* peels compared to the unheated samples have also been reported.[7]

*Afromomum angustifolium*, a member of the Zingiberaceae family commonly called Longoza plant is a geophyte which occurs in rain forests, ground-water forests and seasonally dry forests in Africa. Various parts of the plant are used extensively in ethnomedicine. The seeds are used as worm expeller in the Democratic Republic of Congo and the root-stock is given to alleviate postpartum pain in Liberia.[8] The medicinal use of this botanical family as laxatives, anthelmintics, antidiarrhoea and as a tonic for sexual stimulation as well as antiulcer, antimicrobial, antinociceptive, antiplasmodial, hepatoprotective and anticancer have been reported.[9] The seeds are used mainly as a traditional food spice in the South-South and South-East regions of Nigeria. Apart from nutritional and organoleptic properties, spices are recognized as active and cytoprotective agents.[5]

The antioxidant properties of spices and their health benefits are well documented. Antioxidants protect biological systems against adverse effects of free radicals.[10-12] A study on the phytochemical composition of *A. angustifolium* reported the presence of flavonoids, cardiac glycosides, terpenoids, tannins, alkaloids and saponins.[13] Epidemiological studies indicate that long term intake of diets rich in antioxidants provid some protection against development of cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases.[14] The beneficial effect of antioxidants have resulted in increased research interest in them. Hydroxyl radical is extremely reactive. This short-lived molecule is generated from oxygen ($O_2$) during cellular metabolism.[15] Hydroxyl radicals cause oxidative damage to cells because they can attack biomolecules located a few nanometers from the site of generation,[16] and are involved in cellular disorders such as neurodegeneration,[17] cardiovascular diseases[18] and cancers.[19] Hydroxyl radical generation is associated with Fenton reaction, a redox reaction in which free iron ($Fe^{2+}$) reacts with hydrogen peroxide ($H_2O_2$) and the Haber-Weiss reaction that generates $Fe^{2+}$ when superoxide reacts with ferric iron ($Fe^{3+}$). Also, the activation of $H_2O_2$ by transition metal salts, ozone and UV- light can also generate $^{\bullet}OH$ in living cells.[20,21]

*In vivo*, Nitric oxide (NO) reacts at a slow rate with $O_2$ producing an orange-brown gas; nitrogen dioxide ($^{\bullet}NO_2$), a very reactive species. Further reactions between NO and $^{\bullet}NO_2$ produce nitrite ($NO_2^-$).[22] The biological actions of NO are mediated by guanylate cyclase and cyclic guanosine monosphosphate. Nitric oxide traverses adjacent cell membranes and enters the cytosol, where it activates guanylyl cyclase by binding to the "haem" component of porphyrin ring. The cytotoxic

*Corresponding author. E mail: israel.ebhohimen@gmail.com
Tel: +2347011289286

https://doi.org/10.26538/tjnpr/v1i3.8

effects at elevated concentrations result from the inhibition of mitochondrial enzymes including ubiquinone oxidoreductases and aconitase, which are important in cellular oxidative metabolism.[23] It is thus important to ascertain if heat treatment will alter the antioxidative property of *Aframomum angustifolium* seeds.

## Materials and Methods

*Sample collection and extract preparation*

*Aframomum angustifolium* fruits were purchased in Ebelle market, Igueben Local Government Area of Edo State. They were authenticated in the Department of Biological Sciences, Samuel Adegboyega University, Ogwa, Edo State, Nigeria. The seeds obtained were sun dried for five days and thereafter pulverized using a mechanical blender. Two parts (400 g each) were macerated in 800 mL water and ethanol for 72 h with periodic agitation. After extraction, the solutions were filtered with cheese cloth and concentrated using rotary evaporator. The concentrates were dried to powder using silica gel in a desiccator and powder was stored in air-tight bottles at room temperature.[24]

*Assay for Antioxidant Activity*

*Metal chelating activity (MCA)*

The MCA was measured using the spectrophotometric method as described by Dinis *et al.*[25,26] The reaction mixture containing 0.5 mL of extract (100 µg/mL), 1.6 mL of deionized water, 0.05 mL of $FeCl_2$ (2 mM) and 0.1 mL of 1,10-phenanthroline (5 mM) was incubated at 40°C for 10 min and the absorbance measured at 562 nm. Metal chelating activity of the extracts were also measured as described above after subjecting extracts to heat treatment in a water bath (80°C) for 10 min and 30 min. Ascorbic acid was used as standard. The percentage metal chelating activity was calculated using the formula:

$$\% \text{ Metal chelating activity} = [1 - (A_1 - A_2)/A_0] \times 100$$

Where:
$A_0$ = absorbance of the control (without extract)
$A_1$ = absorbance in the presence of extract
$A_2$ = absorbance without $FeCl_2$

*Ferric reducing ability (FRA)*

The FRA was measured using the spectrophotometric principle described by Benzie and Strain,[27] by mixing reconstituted methanol solutions of extracts (1 mL, 100 µg/mL) with 2.5 mL phosphate buffer (0.2 M, *pH* 6.6) and 2.5 mL potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. Thereafter, 2.5 mL of 10% trichloroacetic acid was added to the mixtures and then centrifuged at 3000 rpm for 10 min. An aliquot (2.5 mL) of the supernatant was mixed with distilled water (2.5 mL) and 0.1% ferric chloride (0.5 mL). The absorbance of the reaction mixture was measured at 700 nm. The ferric reducing ability of the extracts were also measured as described above after subjecting extracts to heat treatment in a water bath (80°C) for 10 min and 30 min respectively. Ascorbic acid was used as standard. The percentage ferric reducing ability was calculated using the formula:

$$\% \text{ Ferric reducing ability} = (A_0 - A_1/A_0)] \times 100$$

Where: $A_0$ = absorbance of the control (all reagents without extract) and $A_1$ = absorbance of all reagent with extract

*Hydroxyl radical scavenging activity (HRSA)*

The HRSA was measured using the spectrophotometric method as described by Elizabeth and Rao.[28,29] The final solution was diluted before reading absorbance. The reaction mixture (3.0 mL) containing 1.0 mL of 1.5 mM $FeSO_4.7H_2O$, 0.7 mL of 6 mM hydrogen peroxide, 0.3 mL of 20 mM sodium salicylate and 1 mL of the extract solution (100 µg/mL) was incubated for 1 h at 37°C. The absorbance of the hydroxylated salicylate complex was measured at 562 nm. The hydroxyl radical scavenging activity of the extracts were also measured as described above after subjecting extracts to heat treatment in a water bath (80°C) for 10 min and 30 min respectively and was calculated using the formula:

$$\% \text{ Hydroxyl radical scavenging activity} = [1 - (A_1 - A_2) / A_0] \times 100$$

Where: $A_0$ is absorbance of the control (without extract),
$A_1$ is the absorbance in the presence of the extract,
$A_2$ is the absorbance without sodium salicylate.

*Nitric oxide (NO) scavenging assay*

The NO scavenging activity was measured using the spectrophotometric method as described by Green *et al.*[30] The extract (1 mL, 100 µg/mL) was mixed with 1 mL of sodium nitroprusside solution (5 mM) and incubated at 25°C for 2 h. An aliquot (1 mL) of the reaction mixture was mixed with 1 mL Griess reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% napthylethylene diamine dihydrochloride) and the absorbance measured at 546 nm. The hydroxyl radical scavenging activity of the extracts were also measured as described above after subjecting extracts to heat treatment in a water bath (80°C) for 10 min and 30 min respectively. Ascorbic acid was used as standard. Percentage NO scavenging activity was calculated using the formula:

$$\% \text{ NO scavenging activity} = (A_0 - A_1/A_0) \times 100$$

Where: $A_0$ is the absorbance of control (without extract/standard) and $A_1$ is absorbance in the presence of extract

*Total antioxidant capacity (TAC)*

The TAC was measured using the spectrophotometric method as described by Srinivasan *et al.*[31]. Aliquots of the test extracts (100 µg/mL) were mixed with 3 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) in test tubes. The tubes were capped with aluminium foil and incubated in a boiling water bath at 95˚C for 90 min. The reaction mixture was cooled to room temperature and the absorbance of the solution measured at 695 nm. The total antioxidant capacity of the plant extract was extrapolated as ascorbic acid equivalent within the range 10 - 100 µg/mL. The effect of heat treatment on the TAC of the extracts were also measured as described above after subjecting extracts to heat treatment in a water bath (80°C) for 10 min and 30 min.

*Statistical analysis*

All assays were performed in triplicates and results expressed as Mean ± SEM. Experimental data were analysed by one-way analysis of variance (ANOVA) to evaluate significant difference at $p < 0.05$.

## Results and Discussion

Apart from improving the organoleptic property of food, phytochemical components of spices also have antioxidant capacity.[3] In this study, the effect of heat treatment on the antioxidant capacity of aqueous and ethanol extracts of *A. angustifolium* seeds were investigated *in vitro.*

The metal chelating activity of the aqueous extract after heat treatment for 30 min was not significantly ($p > 0.05$) affected while that of the ethanol extract reduced. Metals catalyze oxidative reactions *in vivo.* Reduction/chelation prevents them from participating in redox reactions. Both extracts were observed to be effective chelators of ferrous ion radical compared to ascorbic acid (Table 1). Also, the ferric reducing ability of the aqueous and ethanol extracts were comparable to ascorbic acid before and after heat treatment for 10 min. Heat treatment for 30 min, significantly reduced ($p < 0.05$) this ability (Table 2). This suggests that prolonged heat treatment reduces the ferric reducing ability of the extracts. This may negatively impact the ability to inhibit the Fenton reaction. However, a significant increase in the ferric reducing power of citrus peel extracts have been reported after heating for 30 min.[7]

Hydroxyl radical participates actively in oxidative reactions *in vivo.* It has been reported as the most reactive oxygen species.[30] In this study, the $^{\bullet}OH$ scavenging activity was determined by the ability of extracts to bind $^{\bullet}OH$ preferentially in the presence of salicyclic acid. The scavenging activity of the extracts were significantly lower than ascorbic acid before heat treatment. The activity of the aqueous extract increased after heat treatment but the ethanol extract was not significantly affected (Table 3). This sustained *in vitro* $^{\bullet}OH$ scavenging activity after thermal processing suggests that the extracts may be available *in vivo* to support metabolic processes the neutralize the deleterious effect of $^{\bullet}OH$.

The nitric oxide scavenging assay is based on the principle that sodium nitroprusside in aqueous solution at physiological *pH* spontaneously generates nitric oxide which reacts with oxygen to yield nitrite ions that can be estimated using Griess reagent. The percentage nitric oxide scavenging activity of the extracts increased after heat treatment for 10 min. Similar observations have been reported.[30] The percentage increase

was significant ($p < 0.05$) for the aqueous extract. After 30 min, the percentage nitric oxide scavenging activity were not significantly different from that observed for ascorbic acid (Table 4). This suggests that heating may improve the NO scavenging activity of the extract. This possible

**Table 1:** Percentage metal chelating activity before and after heat treatment.

| Time (min) | Aqueous extract (%) | Ethanol extract (%) | Ascorbic acid (%) |
|---|---|---|---|
| 0 | 66.82 ± 0.92 | 65.99 ± 0.06 | 80.61 ± 1.1.64 |
| 10 | 58.37 ± 0.72 | 63.56 ± 2.66 | |
| 30 | 67.44 ± 0.46 | 47.62[a] ± 1.55 | |

*Values are mean ± SEM of 3 assays.*
[a]*significantly lower relative to values at 0 and 10 min ($p < 0.05$).*

**Table 2:** Percentage ferric reducing ability before and after heat treatment.

| Time (min) | Aqueous extract (%) | Ethanol extract (%) | Ascorbic acid (%) |
|---|---|---|---|
| 0 | 70.89 ± 0.40 | 75.48 ± 0.77 | 96.44 ± 0.64 |
| 10 | 76.22 ± 0.75 | 82.07 ± 0.98 | |
| 30 | 7.83[b] ± 0.41 | [b]4.48 ± 0.22 | |

*Values are mean ± SEM of 3 assays.*
[a]*significantly lower than corresponding values at 0 and 10min ($p < 0.05$)*

**Table 3:** Percentage hydroxyl radical scavenging activity before and after heat treatment.

| Time (min) | Aqueous extracts (%) | Ethanol extracts (%) | Ascorbic acid (%) |
|---|---|---|---|
| 0 | 51.85 ± 0.57 | 44.64 ± 0.80 | 76.23 ± 0.11 |
| 10 | 53.32 ± 0.62 | 49.56 ± 0.99 | |
| 30 | 65.58 [a] ± 0.75 | 39.25 ± 0.36 | |

*Values are mean ± SEM of 3 assays.*
[a]*significantly higher relative to values at 0 and 10min ($p < 0.05$).*

**Table 4:** Percentage NO scavenging activity before and after heat treatment.

| Time (min) | Aqueous Ext. (%) | Ethanol Ext. (%) | Asc. Acid (%) |
|---|---|---|---|
| 0 | 33.11[c] ± 0.53 | 28.92[c] ± 0.39 | 80.08 ± 0.24 |
| 10 | 90.94[a] ± 1.13 | 46.71 ± 0.84 | |
| 30 | 86.33 ± 0.23 | 83.56 ± 0.42 | |

*Values are mean ± SEM of 3 assays.*
[a]*significantly higher than value before heat treatment ($p < 0.05$).*
[c]*significantly lower than value for ascorbic acid ($p < 0.05$).*

improvement in NO scavenging activity suggests thermal induction of modifications in the bioactive components with improved NO scavenging ability. High concentrations of NO *in vivo* is associated with nitrosative stress. This observed improved ability to remove NO *in vitro* may be replicated *in vivo* thus preventing its deleterious reactions.

The total antioxidant capacity (TAC) of the extracts of *A. angustifolium* were measured as ascorbic acid equivalents. The overall TAC of the extracts decreased but was not significant ($p > 0.05$) after heat treatment (Table 5). A decrease in total antioxidant capacity was also observed in a study on the effect of heat treatment on fada beans.[5] TAC is a unifying

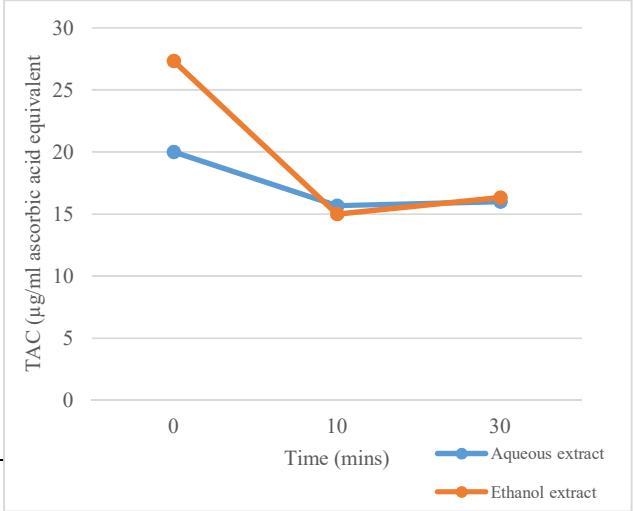


**Figure 1:** Total antioxidant capacity of extracts before and after heat treatment.

concept used to access the antioxidant status of biological samples thus providing an integrated parameter rather than the simple sum of measurable antioxidants.[32] A non-significant reduction of the TAC supports the observation that the antioxidant capacity is not completely lost.

The effect of heat treatment on the antioxidative property of plants differ.[33] The observed antioxidant effect is a product of structural modifications of existing compounds or the new compounds formed as a result of thermal processing. [6,34,35]

## Conclusion

The results of this study indicated that the antioxidant potential of *A. angustifolium* is not totally lost by heat treatment. These findings are crucial as it provides insight to the use of this spice as food flavourant and the expected benefits of the antioxidative property after thermal processing. Further research into structural changes of bioactive components induced by heat treatment of the extracts as well as *in vivo* studies to ascertain the effect of metabolic reactions is suggested.

## Conflict of Interest

There are no competing financial interests.

## Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

## Acknowledgements

The authors sincerely appreciate the Department of Chemical Sciences, Samuel Adegboyega University, Ogwa, Edo State, Nigeria for the facilities and laboratory space provided.

## References

1. Borowski J, Narwojsz A, Borowska EJ, Majewska K. The effect of thermal processing on sensory properties, texture attributes and pectic changes in broccoli. Czech J Food Sci. 2015; 33: 254–260.

2. Palermo M, Pellegrini N, Fogliano V. The effect of cooking on the phytochemical content of vegetables. J Sci Food Agric. 2014; 94: 1057–1070.

3. van Boekel M, Fogliano V, Pellegrini N, Stanton C, Scholz G, Lalljie S. A review on the beneficial aspects of food processing. Nutr Food Res. 2003; 54: 1215–1247.
4. Odukoya OA, Houghton PJ, Ramarr A. Lipoxygenase inhibitors in the seeds of *Aframomum danielli* K. Schum (Zingiberaceae). Phytomed. 1999; 6: 251-256.
5. Dawidowicza AL, Wianowska D, Baraniak B. The antioxidant properties of alcoholic extracts from *Sambucus nigra* L. (antioxidant properties of extracts). LWT. 2006; 39: 308–315.
6. Boukhanouf S, Louaileche H, Perrin D. Phytochemical content and *in vitro* antioxidant activity of faba bean (*Vicia faba* L.) as affected by maturity stage and cooking practice. Int Food Res J. 2016; 23: 954–961.
7. Jeong S, Kim S, Kim D, Jo S, Nam KC, Ahn DU. Effect of heat treatment on the antioxidant activity of extracts from citrus peels. J Agric Food Chem. 2004; 52: 3389–3393.
8. Crook V. *Aframomum angustifolium*. The IUCN Red List of Threatened Species 2013. [online]. 2013 [cited February 9, 2016]. Available from: http://www.iucnredlist.org/details/biblio/44392428/0.
9. Amadi SW, Zhang Y, Wu G. Research progress in phytochemistry and biology of *Aframomum* species. Pharm Bio. 2016.
10. Zhang KZ, Deng K, Luo HB, Zhou J, Wu ZY, Zhang, WX. Antioxidant properties and phenolic profiles of four Chinese Za wines produced from hull-less barley or maize. J Inst Brewing 2013; 119: 182–190.
11. Jegadeeswari P, Daffodil, ED, Mohan VR. Quantification of total phenolics, flavonoid and *in vitro* antioxidant activity of *Aristolochia bracteata* Retz. Int J Pharm Pharm Sci. 2014; 6: 747–752.
12. Nimse SB, Pal D. Free radicals, natural antioxidants and their reaction mechanisms. RSC Adv. 2015; 5: 279-286.
13. Anywar UG, Kirimuhuzya C. Phytochemical profile and antibacterial activity of crude extracts of the pods of *Aframomum angustifolium* (Sonn.) k. Schum. Euro J Biol Res. 2015; 5: 36-41.
14. Pandey KB, Rizvi SI. Plant polypenohls as dietary antioxidants in human health and disease. Oxid Med Cellul Long. 2009; 2: 270–278.
15. Ayala A, Muñoz FM, Argüelles S. Lipid Peroxidation: Production, Metabolism, and SignalingMechanisms of Malondialdehyde and 4-Hydroxy-2- Nonenal. Oxid Med Cellul Long. 2014; 2014: 1-31.
16. Halliwell B, Gutteridge JMC. "Oxygen toxicity, oxygen radicals, transition metals and disease," Biochem J. 1984; 219: 1–14.
17. Castellani RJ, Honda K, Zhu X, Cash AD, Nunomura A, Perry G, Smith MA. Contribution of redox-active iron and copper to oxidative damage in Alzheimer disease. Ageing Res Rev. 2004; 3: 319-326.
18. Lipinski B, Pretorius E. Hydroxyl radical-modified fibrinogen as a marker of thrombosis: the role of iron. Hematol. 2012; 17: 241–247.
19. Dizdaroglu M, Jaruga P. Mechanisms of free radical induced damage to DNA. Free Rad Res. 2012; 4: 382–419.
20. Neyens E, Baeyens J. A review of classic Fenton's peroxidation as an advanced oxidation technique. J Haz Mat. 2003; 98: 33-50.
21. Hermes-Lima M. Oxygen in biology and biochemistry: role of free radicals. In K. B. Storey (Ed.), Functionl metabolism: regulation and adaptation. John Wiley and sons, Canada 2004; 245-274p.
22. Correa- Aragunde N, Foresi N, Lamattina L. Structure diversity of nitric oxide synthetases (NOS): the emergence of new forms in photosynthetic organisms. Front Plant Sci. 2013; 4:32-37.
23. Cuzzocrea S, Riley DP, Caputi AP, Salvemini D. Antioxidant therapy: a new pharmacological approach in shock,inflammation, and ischemia/reperfusion injury. Pharmacol Rev. 2001; 53:135–159.
24. Prajapati DD, Patel NM. Assessment of antioxidant potential of *Holoptelia integrifolia* Roxb leaves by *in vitro*. Am J Food Sci. Health 2015; 1: 1-9.
25. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and peroxyl radical scavengers. Arch Biochem Biophys. 1994; 315: 161-169.
26. Okolie NP, Israel EEJ, Falodun A. *In-vitro* evaluation of antioxidant potential of *Rauwolfia vomitoria* and its inhibitory effect on lipid peroxidation as indication of aphrodisiac properties. Pharm Chem J 2011; 45: 476–480.
27. 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.
28. Elizabeth K, Rao MWA. Oxygen radical scavenging activity of curcumin. Int J Pharm. 1990; 58: 237-240.
29. Sudha G, Priya, MS, Shree RI, Vadivukkarasi S. *In vitro* free radical scavenging activity of raw pepino fruit (*Solanum muricatum*). Int J Curr Pharm Res. 2011; 3:137-140.
30. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS. Analysis of nitrite, nitrate and ($^{15}$N) nitrate in biological fluids. Anal Biochem 1982; 126: 131-138.
31. Srinivasan S, Wankhar W, Rathinasamy S. Free radical scavenging potential and HPTLC analysis of *Indigofera tinctoria* Linn ( Fabaceae ). J Pharm Anal. 2015; 2015: 1–7.
32. Rubio PC, Hernández-Ruiz J, Martinez-Subiela S, Tvarijonaviciute A, Ceron, JJ. Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum: an update. BMC Vet Res. 2016; 12: 166-173.
33. Chipurura B, Muchuweti M, Mandiseraa F. Effects of thermal treatment on the phenolic contents and antioxidant activity of some vegetables. Asia J Clin Nutr. 2010; 2: 93-100.
34. Chumyam A, Whangchai K, Jungklang J, Faiyue B, Seangnil K. Effects of heat treatments on antioxidant capacity and total phenolic content of four cultivars of purple skin eggplants. Sci Asia 2013; 39: 246-251.
35. Sengkhamparn N, Phonkerd N. Effect of heat treatment on free radical scavenging capacities and phenolic compounds in *Tyopilus alboater* wild edible mushrooms. Chaing Mai J Sci. 2014; 41: 1241-1249.