Asian Journal of Research in Biochemistry

8(1): 35-41, 2021; Article no.AJRB.63577
ISSN: 2582-0516

In-vitro Anti-lipid Peroxidation and Other Antioxidant Potentials of *Gongronema latifolium* Leaf Extract

Tebekeme Okoko[1*] and Daniel I. Silas-Olu[2]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.
[2]Department of Microbiology, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author TO designed the study while Author DISO performed the laboratory and statistical analysis. Author TO wrote the first draft of the manuscript including management of the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRB/2021/v8i130172
Editor(s):
(1) Dr. Héctor Manuel Mora Montes, Universidad de Guanajuato, México.
(2) Dr. Spirina Liudmila V, Siberian State Medical University, Russia.
Reviewers:
(1) M. Prasanna Rajeshkumar, KS Rangasamy College of Arts and Science (Autonomous), India.
(2) Neha Sharma, School of Life Sciences, India.
(3) Lotfollah Rezagholizadeh, Ardabil University of Medical Sciences, Iran.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63577

Original Research Article

Received 10 October 2020
Accepted 16 December 2020
Published 27 January 2021

ABSTRACT

Gongronema latifolium is a herbaceous plant consumed as a traditional folk medicine. The aim of this work is to evaluate the anti-lipid peroxidation activity and other antioxidant effects of the plant leaf extract of *Gongronema latifolium* using various *in vitro* models. Lipid peroxidation was first induced in egg yolk and bovine liver homogenate using the ascorbate-ferrous system and incubated with the plant extract at different concentrations. In another experiment, hydrogen peroxide was used to induce erythrocyte hemolysis and lipid peroxidation and those erythrocytes were incubated with the extract. Finally the potential ferrous reducing ability, hydroxyl radical and hydrogen peroxide scavenging activities of the plant extract were also analyzed. It revealed that the leaf extract significantly reduced chemically-induced lipid peroxidation in both homogenates when compared to quercetin. The extract also reduced both hydrogen peroxide-induced hemolysis and

**Corresponding author: E-mail: tebebuddy@yahoo.com;*

lipid peroxidation in erythrocytes. The extract also possessed significant abilities to reduce ferric ions, scavenge both hydroxyl radicals and hydrogen peroxide. In most cases, the responses were concentration-dependent ($p < 0.05$). The findings are ascribed to the important phytochemicals which are antioxidant in nature hence the plant could be exploited both pharmacologically and neutraceutically.

Keywords: Gongronema latifolium; *lipid peroxidation; hemolysis; erythrocytes; antioxidant.*

1. INTRODUCTION

Over the years it has been proved that the use of botanicals as alternative medicine could be more beneficial to mankind than conventional medicine. Up till date, several regions of the world rely on plants/plant-derived products as the first line treatment against many ailments [1]. One key factor is that there are less frequent side effects from the use of plants when compared to conventional chemotherapy. Plants are considered therapeutic because of the presence of bioactive phytochemicals most of which are secondary metabolites [2,3]. The use of medicinal plants in the management and treatment of diseases is as old as mankind and is still an important alternative therapy in many regions of the world [4]. The biomedical potentials of a lot of plants have been investigated using different models however the utilization of these plants does not correlate with their reported bioactivities.

Gongronema latifolium (Asclepiadaceae) is a herbaceous, non-woody plant widespread in the tropical and sub-tropical regions of the world [5]. The plant is consumed as a spice, vegetable in local desserts and as a traditional folk medicine [6]. Chemical analysis of the leaves revealed the presence of various phytochemicals, minerals, protein and fat which may contribute to the observed medicinal value of the plant [6,7]. The aim of this study was to elucidate the effect of *Gongronema latifolium* leaf extract on chemicaly-induced lipid peroxidation on egg yolk and bovine liver homogenates. Other *in vitro* antioxidant properties were also evaluated.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All chemical and reagents were commercially available and of analytical grade thus used without further purification. All buffers and solutions were prepared using double glass distilled water. Where necessary, buffers were stored at 4°C.

2.2 Plant Material

The leaves of the plant were harvested from Amassoma, Bayelsa State, Nigeria. The plant was identified by Prof I Ogidi of the Department of Crop Production, Niger Delta University, Amassoma, Bayelsa State. A voucher specimen is deposited in a plant house of the University. The leaves were sundried and ground using a waring blender and soaked in absolute methanol for 3 days at room temperature in the dark. The contents were filtered and the filtrate concentrated using a rotary evaporator at 40°C. It was further dried to a paste and various concentrations (50 – 500 µg/ml) of the extract were made using distilled water.

2.3 Preparation of Homogenates

A weighed portion of fresh bovine liver (bought from the local market) was rinsed, blotted and homogenized in 0.02 M phosphate buffered saline (pH 7.4) to give a 10% homogenate. Similarly, egg yolk was also dissolved in same buffer to give 10% homogenate. Both homogenates were centrifuged at 750 x *g* for 20 min at 4°C. Supernatants (otherwise referred to as the homogenate in later sections) were used for the anti-lipid peroxidation analysis.

2.4 Anti-lipid Peroxidation Assay

The ability of the leaf extract to inhibit lipid peroxidation was performed according to the method of Yoshiyuki et al. [8] with some modifications. Initial assay mixture was made up of 0.1 ml of 0.04 M ferrous chloride, 0.1 ml of 0.1 mM ascorbic acid, 0.2 ml of 0.02 M phosphate buffer (pH 7.4), 0.5 ml of homogenate and 0.5 mL of either extract or control (quercetin monohydrate). Distilled water (1.8 ml) and 2 mL of 2% thiobarbituric acid were also added and contents were incubated for 30 min in a boiling water bath. After cooling, n-butanol (5 mL) was added and shaking vigorously. The n-butanol layer was collected via centrifugation and the

absorbance was measured at 532 nm. Results were expressed as relative activity.

2.5 Reduction of Erythrocyte Hemolysis

The ability of the extract to inhibit erythrocyte hemolysis was performed as reported by Okoko and Ere [9]. First of all, whole blood was collected from a volunteer via vein and delivered into heparinized tubes and centrifuged at 2880 x *g* for 10 min at 4°C. Plasma was removed and cells washed and finally re-suspended in 0.02 M phosphate buffer (pH 7.4) to 5% hematocrit level. Erythrocytes were immediately used for further studies.

For the assay, erythrocytes (200 µL) were first incubated with 100 µL of 100 µM hydrogen peroxide in order to induce hemolysis. The extract or control (200 µL) was added, gently swirled and incubated for 3 hrs at 37°C. The tube was allowed to cool and 8 mL of phosphate buffered saline (0.02 M, pH 7.4) was added and centrifuged at 2880 x *g* for 10 min. Absorbance of the supernatant was measured 540 nm using a UV-Vis spectrophotometer. The inhibitory activity was expressed as relative activity [9].

2.6 Reduction of Erythrocyte Lipid Peroxidation

The ability of the extract to reduce erythrocyte lipid peroxidation was performed according to Okoko and Ere [9] as modified [10]. Briefly, 200 µL of erythrocytes were incubated with 100 µL of 100 µM hydrogen peroxide and 200 µl of test solution. The contents were swirled and incubated for 1 hr at 37°C. Reaction was stopped by adding 2 ml of thiobarbituric acid stock reagent (15% w/v TCA, 0.375% TBA, 0.25 M HCl) and incubated in a boiling water bath for 15 min. After cooling, precipitate was removed via centrifugation and absorbance of the supernatant was measured at 532 nm. The inhibitory activity was expressed as relative activity [9].

2.7 Ferric Reducing Ability

The ability of the extract to reduce ferric ions was determined according to the method of Oyaizu [11] with some modifications. Assay mixture consisted of 0.5 ml of phosphate buffer (0.2 M, pH 6.6), 5 ml of test sample (extract or vitamin C) and 0.5 ml of 1% potassium ferricyanide. Contents were incubated for 20 min at 50°C. Upon cooling, 0.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 750 *x g* for 10 min. Upper layer (0.5 ml) was mixed with 0.5 ml of distilled water and 0.5 ml of ferric chloride and incubated at room temperature for 10 min. Absorbance was finally read at 700 nm. Increasing absorbance indicates increasing reducing ability.

2.8 Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging activity was investigated using the method described by Bera et al. [12]. Briefly, 0.02 ml of ferric chloride (0.02 M) and 0.05 ml of phenanthroline (0.04 M), 1 ml of phosphate buffer (0.02M, pH 7.2) and 1 ml of sample or control were introduce into a test tube. The reaction was initiated by adding 0.05 ml of hydrogen peroxide (7 mM). The tube was incubated for 5 min at room temperature and absorbance was measured at 560 nm. Hydroxyl radical scavenging activity was expressed as relative scavenging activity as reported [10].

2.9 Hydrogen Peroxide Scavenging Activity

The Hydrogen peroxide scavenging activity was determined according to the method of Ruch et al. [13] with some modifications. Briefly 0.5 ml of either extract or control was delivered into a test tube followed by 0.6 ml of 40 mM hydrogen peroxide (prepared in 0.02 M phosphate buffer, pH 7.4). Tube was incubated for 10 min at room temperature. Absorbance was measured at 230 nm against a reagent blank. The hydrogen peroxide scavenging activity was also expressed as relative activity [10].

2.10 Statistical Analysis

Representative readings were expressed as mean ± S.E.M from four replicates. Quercetin monohydrate was used as the control for the anti-lipid peroxidation assays while vitamin C was used for the other assays. Data were analyzed by analysis of variance followed by the Tukey test with $p < 0.05$ being considered indicative of a statistical difference between mean values. The Past3 package was used for the analysis. Values with different superscript letters differ significantly.

3. RESULTS

3.1 Anti-lipid Peroxidation Ability

The anti-lipid peroxidation ability of the extract is shown in Fig. 1. It revealed that the plant extract possessed significant anti-lipid peroxidation

ability on both tissue homogenates (egg yolk and liver) when compared to the reference quercetin. In both cases, the response was concentration-dependent ($p < 0.05$).

3.2 Erythrocyte Hemolysis and Lipid Peroxidation

The ability of the extract to reduce hydrogen peroxide-induced erythrocyte hemolysis and lipid peroxidation is shown in Fig. 2. The plant extract significantly inhibited both hemolysis and lipid peroxidation in erythrocytes when compared to the standard (Vitamin C). There was no significant difference in the inhibition of hemolysis when erythrocytes were treated with 50 µg/ml and 100 µg/ml of the extract ($p > 0.05$) however, the response was concentration-dependent from 100 µg/ml - 500 µg/ml. The inhibition of lipid peroxidation was concentration-dependent ($p < 0.05$).

3.3 Other Antioxidant Activities

The reducing ability, hydrogen peroxide and hydroxyl radical scavenging activities of the extract are shown in Fig. 3. It revealed that the extract possessed significant abilities to reduce ferric ions, scavenge hydrogen peroxide and hydroxyl radicals when compared to the standard. There was no significant difference in the reducing ability from 50 µg/ml to 200 µg/ml ($p > 0.05$). However, the hydrogen peroxide and hydroxyl radical scavenging activities of the extract were concentration-dependent ($p < 0.05$).

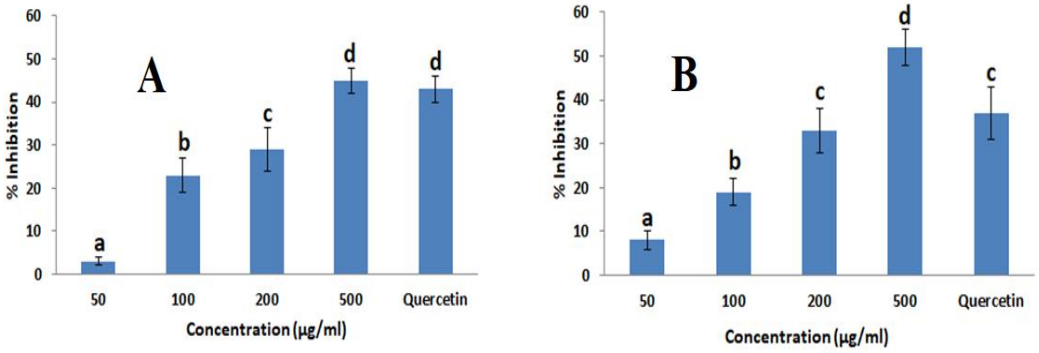

Fig. 1. Inhibitory effect of *Gongronema latifolium* leaf extract on ferrous-ascorbate induced lipid peroxidation on (A) liver homogenate (B) Egg yolk homogenate

Each bar represents mean ± SEM from six replicate experiments. Values having different superscript letters differ significantly (P < 0.05). Quercetin (control) was used at 100 µg/ml

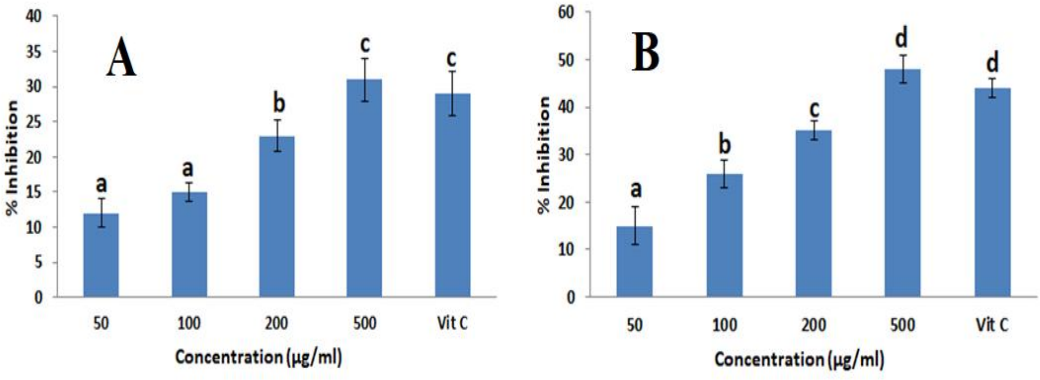

Fig. 2. Inhibitory effect of *Gongronema latifolium* leaf extract of hydrogen peroxide induced (A) hemolysis and (B) lipid peroxidation in erythrocytes

Each bar represents mean ± SEM from six replicate experiments. Values having different superscript letters differ significantly (p < 0.05). Vitamin C (control) was used at 100 µg/ml

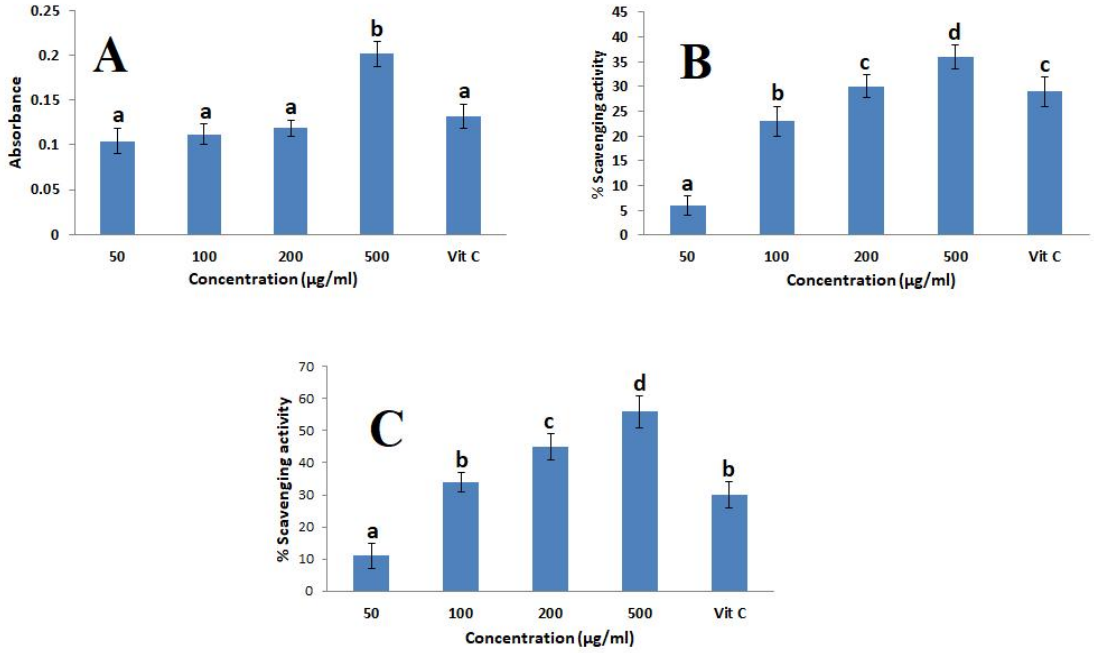

Fig. 3. (A) Reducing ability, (B) hydroxyl radical scavenging activity and (C) hydrogen peroxide scavenging activities of *Gongronema latifolium* leaf extract

Each bar represents mean ± SEM from six replicate experiments. Values having different superscript letters differ significantly (P < 0.05). Vitamin C (control) was used at 100 µg/ml

4. DISCUSSION

The production of oxidants in biological system is indispensable since they are produced as a result of normal cellular metabolism and environmental factors. However their activities are kept in check by robust antioxidant systems which are either enzymatic or non-enzymatic. Should an imbalance occur in favour of the oxidants, oxidative stress occurs which has been implicated in various disorders such as diabetes, cardiovascular disorders, cancer, inflammation, ageing etc [14,15]. Reactive oxygen species target DNA, proteins, lipids and other components of the cell thereby causing cumulative cellular damage which could be irreversible [16,17]. In membranes, the peroxidation of lipids (called lipid peroxidation) alters the integrity of the cell which plays a major role in several disease conditions [18].

In the current work, chemically-induced lipid peroxidation in tissue homogenates was reduced by the *Gongronema latifolium* leaf extract. In the experiment, lipid peroxidation in egg yolk and liver tissue homogenate was induced by the ferrous/ascorbate system. Ferrous ions are the most powerful pro-oxidant redox active metals that accelerate lipid peroxidation via the Fenton reaction thus minimizing ferrous ions may afford protection against oxidative damage by inhibiting production of ROS and lipid peroxidation [19]. The extract also reduced erythrocyte hemolysis and lipid peroxidation induced by hydrogen peroxide. Erythrocytes have high amounts of polyunsaturated fatty acids, high cell concentration of oxygen and hemoglobin thus are highly susceptible to oxidative attack [20,21]. Erythrocyte hemolysis has been implicated in some disorders such as β-thalassaemia, sickle cell anemia, paroxysmal nocturnal hemoglobinuria, hereditary spherocytosis and glucose-6-phosphate dehydrogenase deficiency and this can increase the susceptibility of the erythrocyte to peroxidation [22,23]. Hydrogen peroxide is an important reactive oxygen species that easily diffuses into cells and tissues. Though a signal molecule, it can be also be converted to the hydroxyl radical in the presence of redox active metals [24,25]. The hydroxyl radical is considered the most potent oxidant and one of the most reactive biologically active free radical [26].

Form the above, any set of conditions that scavenge hydrogen peroxide, hydroxyl radicals

and probably reduce redox active metals could be protective. The findings in the current experiment reveal that the plant extract scavenged both hydrogen peroxide and hydroxyl radical in addition to possessing significant ability to reduce redox active metal ion (Fig. 3).

It has been reported that various phytochemicals protect cells by chelating and/or reducing redox active metals in addition to donating hydrogen and electrons to quench biologically active free radicals [27,28]. Flavonoids, polyphenols, carotenoids, alkaloids and terpenoids are some important phytochemicals that possess immense antioxidant activities [27,29-31] which have been detected in the plant [32,33].

5. CONCLUSION

In conclusion, the observed bioactivity of the plant extract is attributed to the antioxidant phytochemicals that may have acted in synergy and this may be the basis of the reported traditional uses. Further experiments to elucidate the antioxidant nature of the plant in cell based systems is ongoing with the aim to characterize important bioactive compounds.

ETHICAL APPROVAL

The study protocol was performed according to the Helsinki declaration of 1964 and approved by the Institutional Ethical Committee.

COMETING INTERESTS

Authors have declared that no competing interests exits.

REFERENCES

1. Iweala EEJ, Uhegbu FO, Adesanoye OA. Biochemical effects of leaf extracts of *Gongronema latifolium* and selenium supplementation in alloxan induced diabetic rats. J Pharmacog Phytother. 2013;5:91-97.
2. Kennedy DO, Wightman EL. Herbal extracts and phytochemicals: Plant secondary metabolites and the enhancement of human brain function. Adv Nutr. 2011;2:32-50.
3. Savithramma N, Rao ML, Suhrulatha D. Screening of medicinal plants for secondary metabolites. Middle-East J Sci Res. 2011;8:579-584.
4. Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. Medicinal plants: Past history and future perspective. J Herb Pharmacol. 2018;7: 1-7.
5. Opara MN. Some edible spices of southeastern Nigeria with antiparasitic properties: A review. Am J Ethnomed. 2014;1:2348-9502.
6. Eleyinmi AF. Chemical composition and antibacterial activity of *Gongronema latifolium*. J Zhejiang University Sci B. 2007;8:352-358.
7. Imo C, Uhegbu FO. Phytochemical analysis of *Gongronema latifolium* Benth leaf using gas chromatographic flame ionization detector. Int J Chem Biomolec Sci. 2015;1:60-68.
8. Yoshiyuki K, Michinori K, Tadato T, Shigeru A, Hiromichi O. Studies on *Scutellariae radix*. IV. Effects on lipid peroxidation in rat liver. Chem Pharm Bull. 1981;29:2610–7.
9. Okoko T, Ere D. Reduction of hydrogen peroxide-induced erythrocyte damage by *Carica papaya* leaf extract. Asian Pacific J Trop Biomed. 2012;2:449-453.
10. Gbenenee TJ, Boisa N, Okoko T. Phytochemical screening and evaluation of the antioxidant activity of *Ficus capreifolia* leaf extract via *in-vitro* models. Intern J Biochem Res Rev. 2017;17:1-7.
11. Oyaizu M. Studies on the products of browning reaction prepared from glucose amine. Jpn J Nutr. 1986;44:307-315.
12. Bera TK, Chatterjee K, Ghosh D. *In-vitro* antioxidant properties of the hydro-methanol extract of the seeds of *Swietenia mahagoni* (L.) Jacq. Biomarkers Genomic Med. 2015;7:18-24.
13. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989;10:1003-1008.
14. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organization J. 2012;5:9-19.
15. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P. Oxidative stress, aging, and diseases. Clin Interven Aging. 2018;13:757-772.
16. Sugamura K, Keaney Jr JF. Reactive oxygen species in cardiovascular disease. Free Rad Biol Med. 2011;51:978-992.

17. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. Free Rad Biol Med. 2010;48: 749-762.
18. Kamat JP, Devasagayam TPA, Priyadarsini KI, Mohan H. Reactive oxygen species mediated membrane damage induced by fullerene derivatives and its possible biological implications. Toxicol. 2000;155:55-61.
19. Kada S, Bouriche H, Senator A, Demirtaş I, Ozen T, Çeken Toptanci B, Kizil G, Kızıl M. Protective activity of Hertia cheirifolia extracts against DNA damage, lipid peroxidation and protein oxidation. Pharm Biol. 2017;55:330-337.
20. Pandey KB, Rizvi SI. Biomarkers of oxidative stress in red blood cells. Biomedical Papers of the Medical Faculty of Palacky University in Olomouc. 2011; 155:131-136.
21. Bhatti JS, Sidhu IP, Bhatti GK. Ameliorative action of melatonin on oxidative damage induced by atrazine toxicity in rat erythrocytes. Mol Cell Biochem. 2011;353:139-149.
22. Zhu QY, Holt RR, Lazarus SA, Orozco TJ. Inhibitory effects of cocoa flavanols and procyanidin oligomers on free radical-induced erythrocyte hemolysis. Exp Biol Med. 2002;227:321-329.
23. Woollard KJ, Sturgeon S, Chin-Dusting JP, Salem HH, Jackson SP. Erythrocyte hemolysis and hemoglobin oxidation promote ferric chloride-induced vascular injury. J Biol Chem. 2009;284:13110-13118.
24. Rojkind M, Dominguez-Rosales JA, Nieto N, Greenwel P. Role of hydrogen peroxide and oxidative stress in healing responses. Cell Mol Life Sci. 2002;59: 1872-1891.
25. Dash R, Acharya C, Bindu PC, Kundu SC. Antioxidant potential of silk protein sericin against hydrogen peroxide-induced oxidative stress in skin fibroblasts. BMB Rep. 2008;41:236-241.
26. Treml J, Smejkal K. Flavonoids as potent scavengers of hydroxyl radicals. Comprehen Rev Food Sci Food Safety. 2016;15:720-738.
27. Ramful D, Tarnus E, Aruoma OI, Bourdon E, Bahorun T. Polyphenol composition, vitamin C content and antioxidant capacity of *Mauritian citrus* fruit pulps. Food Res Intern. 2011;44:2088-2099.
28. Bursal E, Koksal E, Gulçin I, Bilsel G, Goren AC. Antioxidant activity and polyphenol content of cherry stem (*Cerasus avium* L.) determined by LC–MS/MS. Food Res Intern. 2013;51:66-74.
29. Sindhu ER, Preethi KC, Kuttan, R. Antioxidant activity of carotenoid lutein *in vitro* and *in vivo*. Indian J Exp Biology. 2010;48:843-848.
30. Yousefbeyk F, Gohari AR, Hashemighahderijani Z, Ostad SN, Sourmaghi MHS, Amini M, Golfakhrabadi F, Jamalifar H, Amin G. Bioactive terpenoids and flavonoids from Daucus littoralis Smith subsp. hyrcanicus Rech. f, an endemic species of Iran. DARU J Pharm Sci. 2014;22:12.
31. Costa EV, da Cruz PEO, de Lourenço CC, de Souza Moraes VR, de Lima Nogueira PC, Salvador MJ. Antioxidant and antimicrobial activities of aporphinoids and other alkaloids from the bark of Annona salzmannii A. DC. (Annonaceae). Nat Prod Res. 2013;27:1002-1006.
32. Imo C, Uhegbu FO. Phytochemical analysis of *Gongronema latifolium* Benth leaf using gas chromatographic flame ionization detector. Int J Chem Biomolec Sci. 2015;1:60-68.
33. Balogun ME, Besong EE, Obimma JN, Mbamalu OS, Djobissie SF. *Gongronema latifolium*: A phytochemical, nutritional and pharmacological review. J Physiol Pharmacol Adv. 2016;6:811-824.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63577