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In vitro Free Radical Scavenging and Antioxidant Activities of Aqueous Extracts of *Crateva adansonii* (Three-leaf Plant)

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Authors' contributions

This work was carried out in collaboration between all authors. Author HCCM designed the study, wrote the protocol, wrote the first draft of the manuscript and carried out the bench work. Authors CEU, MSA, SSB, ANO, PNO and JT did the literature search while author CCD edited the work, performed the statistical analysis and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Ethnopharmacological Relevance: *Crateva adansonii* popularly known as Three-Leaf plant is used in some South Eastern part of Nigeria by traditional medicine practitioners to treat certain diseases such as infertility and infection.
Aim: This research work aimed at investigating the *in vitro* antioxidant activities of various parts of *Crateva adansonii* (Three-leaf plant) an Eastern Nigerian medicinal additive using various method of extraction (hot and cold extracts prepared from the root, leaf and stem bark of the plant). This is to know the best method that would give highest antioxidant properties.
Study Design: This work was designed to compare the antioxidant activities of various parts of

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Crateva adansonii under Ferric thiocyanate (FCT) assay. These include; Hot root extract, cold root extract, hot leaf extract, cold leaf extract, hot stem-bark extract and cold-stem bark extract at various durations. The study was also designed to study the in vitro antioxidative activities, including thiobabituric acid (TBA) assay, diphenyl-β-picryl- hydrazyl (DPPH) radical scavenging effects and reducing power as standard evaluation methods.

Place of Study and Duration of Study: This study took place at the Department of Biochemistry, Faculty of Science, University of Maiduguri, Borno, State Nigeria and the duration of study was eight weeks

Methodology: Parameters were analyzed spectrophotometrically using UV- VIS spectrophotometer (model 725G, China) at room temperature. Hot and cold water were used for the extraction.

Results: It was observed that the antioxidant activities using FTC assay increased as the peroxide concentration decreased with time in all the extracts. The results showed that the hot extracts inhibited lipid peroxidation more than the cold extracts. The hot root extract showed the highest reducing power ($p<0.05$) among all the other extracts. Hot root extract showed stronger antioxidant activities under FTC assay than cold root extract. Hot leaf extract did not show stronger antioxidant activities than cold leaf extract while hot stem bark extract did not show stronger antioxidant activities than cold stem bark extract. The results were analyzed by one way ANOVA using SPSS statistical soft ware version 16.

Conclusion: The extracts showed a positive DPPH test with the HLE, CRE, and CLE showing strong antioxidant actions respectively. The results demonstrated that the extracts from *C. adansonii* scavenged free radical, and reduced lipid peroxidation and could serve as a potential source of natural antioxidants. Hot water is therefore better than cold water when doing root extraction of this plant.

Keywords: Crateva adansonii; antioxidant activities and scavenging effect.

1. INTRODUCTION

Free radicals and other reactive oxygen species (ROS) are generated in living cells through different pathways [1]. Excess of ROS leads to oxidative stress, leading to oxidative DNA damages which were implicated in the pathogenesis of many disorders [2,3]. They are also considered to induce lipid peroxidation causing food deterioration [4]. The search for natural antioxidants as alternatives is therefore of great interest among researchers. This might provide leads for the development of new drugs, which may reduce the risk of chronic diseases caused by free radicals [5]. The consumption of a diet rich in vegetables and fruits has been associated with a number of health benefits including the protection of the human body from attack of free radicals and retardation of the progress of many chronic diseases [6,7]. This beneficial effect is taught to be due at least partially to the action of antioxidant compounds in plants, which reduce oxidative damage to the cells [8].

Crateva adansonii belongs to the family *Capparaceae*. It is a small tree that grows up to 3 - 10 m tall with 3- foliated leaves. The plant has recently been reported to inhibit xanthine oxidase [9] while the aqueous leave extract was reported to be safe on selected tissues of rats [10]. The plant has been claimed to have antipyretic effect and are used to treat conditions such as skin infections, rheumatoid arthritis and insect bites [10-12]. It has also been reported to have antimicrobial and antimutagenic activity [13,14]. Our resent reports [1,15] showed that *Crateva adansonii* has active principles like flavonoids, carotenoids and phenols which exhibit antioxidant properties of various potencies.

To classify a substance as an antioxidant, it is important to assess its interaction against a wide range of species directly responsible for oxidative damage [16]. A screening of the antioxidative capacity should include the determination of the ability of a putative antioxidant to scavenge radicals and ferryl species. The present study was carried out to investigate the antioxidant efficacy of the aqueous extracts from the root, leaf, and stem bark of *Crateva adansonii* including DPPH, FCT, reducing power and TBA assays using *in vitro* models.

2. MATERIALS AND METHODS

2.1 Materials

All chemicals used were of analytical grade and were obtained from British Drug House (BDH) Limited, through their sales representatives in Lagos State, Nigeria. The leaf, stem and roots of the plant were freshly collected from Orlu town, Imo State, Nigeria. The plant material has been earlier authenticated by Mr. Ozioko of Botany Department, University of Nigeria, Nsukka (UNN), Enugu State, Nigeria, where a herbarium specimen with identification number UNN/H/67 was deposited, in the work reported by [1]. The leaves, roots and stem bark were properly washed with distilled water and dried at room temperature. Forty grams of the leaves root and stem bark were scrapped, weighed and pounded separately using mortar and pestle. The aqueous cold extracts were prepared in 1:5 w/v distilled water while the hot extracts were prepared by boiling in distilled water (1:5 w/v) for 30 min at 100°C. Each of the extract was allowed to stand for 24 hours at room temperature. The aqueous extracts were filtered and the filtrate used for analysis. This was an adaptation of the traditional method of preparing the extract by the natives.

This research has employed direct in vitro antioxidant test to investigate the antioxidant properties of *Crateva adansonii*'s aqueous extracts to confirm, justify or otherwise our earlier reports [1,15] which implicated the plant in possession of antioxidant active principles.

2.2 Methods

2.2.1 *In vitro* antioxidant activity

2.2.1.1 Ferric thiocyanate (FTC) assay

The method of [17] with slight modification from [18] was adopted to determine the amount of peroxide at the initial stage of lipid peroxidation. The peroxide reacts with ferrous chloride ($FeCl_2$) to form a reddish ferric chloride ($FeCl_3$) pigment. The concentration of peroxide decreases as the antioxidant activity increases.

Briefly, 4 ml of sample (hot/cold), standard (ascorbic acid), and control (distilled water) were each dissolved in 4 ml of absolute ethanol and 4.1 ml of 2.25% oleic acid solution added. 8 mls of 0.05 M phosphate buffer was added and placed in an oven at 40°C in the dark. To 0.1 ml of these solutions, 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate was added and allowed to stand for 30 min at room temperature and 0.1 ml of 0.02M $FeCl_3$ in 30% HCl added. The absorbance was read at 500 nm immediately and after 24 hours daily for 5 days at room temperature

2.2.1.2 Thiobarbituric acid (TBA) assay

Thiobarbituric acid (TBA) assay was determined according to the method of [19]. The formation of malondialdehyde is the basis for the TBA assay used in evaluating the extent of lipid peroxidation. At low pH and high temperature (100°C), malondialdehyde binds TBA to form red complex that can be measured at 532 nm [18]. The increase in the amount of red pigment formed correlated with the oxidative rancidity of the lipid (18). Briefly, 1.0 ml of sample prepared in step 2 of the FTC procedure, 2 ml of 20% tricarboxylic acid (TCA) was added. To the solution, 2 ml of 0.1 M TBA was added and incubated at 40°C in the dark after which the mixture was placed in boiling water for 10 min and the absorbance of the supernatant measured at 532 nm.

2.2.1.3 Reducing power

The reducing power of the aqueous extracts was determined according to the method of [20]. The extract serves as electron donor that reacts with free radical to convert them to more stable products and terminates radical chain. The extracts (2.5 ml) were mixed with phosphate buffer (5 ml, 0.2 M, pH6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2 ml, 1%); the mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 300 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml distilled water and $FeCl_3$ (0.5 ml, 0.1%). The protocol was repeated for the standard (ascorbic acid) and control (distilled water). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture showed increased reducing power.

2.2.1.4 Scavenging effect on DPPH radical

The effect of extracts on DPPH radical was estimated according to the method of [20]. The extracts (30 µl) were added to a methanol solution of DPPH (0.2M, 1 ml). The mixture was

shaken and left to stand at room temperature for 30 min and the absorbance of the resulting solution was measured spectrophotometrically at 517 nm.

2.3 Statistical Analysis

All data were expressed as mean ± standard deviation. Statistical significance of the difference was analyzed through one way analysis of variance (ANOVA) by SPSS version 16 for windows. The least significant difference was used to determine the difference between the test group and the control, and P<0.05 was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

The effects of various extracts on the peroxidation of oleic acid by the FTC assay are shown in Table 1. In general the antioxidant activity of oleic acid was markedly inhibited by the extracts. The antioxidant activity of the hot root and cold root extracts (HRE and CRE) increased as the peroxide concentration decreased with time. From our results, the HRE showed stronger antioxidant activity (P<0.05) compared to the CRE. Also, as shown in Table 1, the results of the hot leaf and cold leaf (HLE, CLE), and hot stem bark and cold stem bark (HSBE, CSBE) extracts indicate that the antioxidant activity increased as the peroxide concentration decreases. We observed no significant difference (P<0.05) between the hot and cold leave and stem bark extracts although the hot extract showed higher activity.

The effects of the extracts on lipid peroxidation, reducing power and the scavenging of DPPH radical are presented in Table 2. The TBA assay shows that the HRE possesses more activity relative to the CRE (P<0.05) while both extracts produced significantly higher (P<0.05) activity compared with ascorbic acid. The TBA assay for the HLE and HSBE showed that they have more activity compared to the CLE, CSBE, and ascorbic acid which were not significant (P<0.05).

As shown in Table 2, the reducing power of the HRE was significantly higher than the CRE (P<0.05) and the standard. The reducing power of the HLE was significantly higher (P<0.05) compared with the CLE while the HSBE possessed a non significant (P<0.05) reducing potential compared with CSBE. The results showed that the HRE has the highest reducing power capacity (P<0.05) among all the extracts and ascorbic acid.

The scavenging activity of DPPH radical was tested by reduction of the stable radical DPPH to the yellow colored diphenylpicryl hydrazine. The experimental observations of the scavenging effects of the extracts with DPPH radical are presented in Table 2. The *in vitro* analysis reveals that the HSBE and the CSBE produced the least percentage inhibitory capacity while the HLE, CLE, and CRE produced the highest percentage inhibitory capacity. A positive DPPH test suggests that the extract is a potential free radical scavenger. However, the activity of the extracts was less when compared with the standard, ascorbic acid (Table 2).

Table 1. *In vitro* Ferric Thiocyanate (FCT) antioxidant activities of the various *Crateva adansonii* extracts

Time (days)	0	1	2	3	4	5
Standard (Ascorbic acid).	0.827±0.01	0.487±0.08	0.397±0.02	0.312±0.04	0.199±0.04	0.083±0.04
Control (water)	1.292±0.03	1.303±0.05	1.293±0.03	1.292±0.04	1.292±0.04	1.291±0.05
HRE	0.970±0.05^{b}	0.881±0.105^{b}	0.856±0.01^{b}	0.701±0.01^{b}	0.561±0.02^{b}	0.226±0.01^{b}
CRE	0.956±0.05^{b}	0.473±0.09^{b}	0.374±0.01^{b}	0.401±0.56^{b}	0.145±0.01^{b}	0.035±0.01^{b}
HLE	0.250±0.04	0.441±0.13	0.540±0.04	0.68±0.03	0.72±0.02	0.78±0.04
CLE	0.390±0.15	0.492±0.25	0.670±0.19	0.76±0.08	0.81±0.01	0.83±0.10
HSBE	1.004±0.08	0.637±0.05	0.593±0.01	0.349±0.02	0.266±0.03	0.145±0.02
CSBE	0.817±0.03	0.692±0.02	0.623±0.05	0.404±0.04	0.313±0.53	0.296±0.03

N = 6 for each group. Values are mean ± SD of triplicate readings. Values with superscripts in a column are significant (p<0.05). CRE; b = HRE vs CRE; HRE: hot root extract, CRE: cold root extract, HLE: hot leave extract, CLE: cold leave extract, HSBE: hot stem bark extract, CSBE: cold stem bark extract

Table 2. Effects of the extracts of *Crateva adasonii* on the lipid peroxidation, reducing power and scavenging of DPPH radical

Extracts	TBA (mg/dl)	Reducing power (% inhibition)	DPPH (% inhibition)	DPPH % inhibition
Standard (ascorbic acid)	0.684±0.01	1.624±0.01	0.129± 0.14	80.60
Control (water)	1.151±0.01	0.041±0.01	0.667± 0.04	0.00
HRE	141.630±47.82^{a,b}	15.007±3.14^{a,b}	0.382± 0.45	42.72
CRE	132.090±38.13^{,ab}	1.320±0.65^{a,b}	0.126± 0.20	81.11
HLE	1.040±0.01	1.753±0.01^{c}	0.118± 0.01	82.30
CLE	0.826±0.03	0.580±0.02^{c}	0.127± 0.07	80.95
HSBE	0.619±0.03	1.136±0.01	0.532±0.04	39.20
CSBE	0.767±0.02	0.994±0.02	0.568±0.02	35.08

N = 6 for each group. Values are mean ± SD of triplicate readings. Values with superscripts in a column are significant (p<0.05). a = standard vs HRE, CRE; b = HRE vs CRE; c = HLE vs CLE. HRE: hot root extract, CRE: cold root extract, HLE: hot leave extract, CLE: cold leave extract, HSBE: hot stem bark extract, CSBE: cold stem bark extract

3.2 Discussion

Medicinal plants contain large amounts of antioxidants such as polyphenols that are known to have significant antioxidant potentials associated with lower occurrence and lower mortality rates of several human diseases [21]. There is an increasing interest in finding natural antioxidants from plants because they can protect the human body from the attack of free radicals and retard the progress of many chronic diseases [6,7,15] as well as retarding lipid peroxidation and oxidative rancidity in foods.

Antioxidants exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements which are involved in free radical generation, scavenging reactive species and augmenting the activity of antioxidant enzymes [22,23]. The antioxidant activity by the use of ferric thiocyanate method measures the amount of peroxides produced at the initial stage of lipid peroxidation which is indicated by a decrease in absorbance, indicating an increased level of antioxidant activity [23]. Therefore, the antioxidant activity may be due to hydroperoxide inhibition, of free radical or complex formation with metal ions or combinations [24]. The results showed that the extracts exhibited good antioxidant activity which might be attributed to the presence of flavonoid like phytoconstituents. The results suggest that HRE has stronger antioxidant capacity when compared to CRE and this decreases with time.

Oxidative stress can lead to peroxidation of cellular lipids and can be measured by estimating the levels of thiobarbituric acid-reactive substances. Lipid peroxidation has been thought of for a long time in the biological system as a toxicological phenomenon, as it resulted in many pathological consequences [25]. Quantification of MDA, one of the products of lipid peroxidation, with TBA at low pH and high temperature (100°C), resulted in the formation of a red complex, which is measured at 532 nm. This is a most common assay used for the determination of the rate of extent of lipid peroxidation and the concentration of lipid peroxidation product which may reflect the degree of oxidative stress in any disorder [26,27]. The extracts inhibited the rate of lipid peroxidation by a reduction in the red color complex formed reflecting its anti-lipid peroxidation potential. The MDA generated as a result of lipid peroxidation reacts with thiobabituric acid and was found to be inhibited in the presence of the extracts.

The reducing ability of a compound is related to its electron transfer ability and may serve as a significant indicator of its potential activity [28]. The presence of reductants in the extracts cause the reduction of Fe^{3+} - ferricyanide to the ferrous form which was monitored by measuring the formation of Perl's Prussian blue at 700 nm (15). Greater absorbance at 700 nm indicated greater reducing power. Table 2 depicts the reductive capabilities of the extracts. The extracts demonstrated reducing power which was more pronounced with the hot extracts. This suggests that hot extracts may have stronger reducing capacities than cold extracts. The extracts exerted antioxidant activity by breaking free radical chains and their hydrogen- donating ability [29,30]. The extracts might contain

reductants, which could react with free radicals to stabilize and terminate radical chain reaction [28].

The proton-radical scavenging action has been known as an important mechanism of antioxidation [16]. DPPH was used to determine the proton-radical scavenging action of the extracts because it possesses a proton free radical and shows a characteristic absorption at 517 nm [31], but upon reduction with antioxidant, its absorption decreases due to the formation of its nonadical form, DPPH-H [32]. Therefore, the radical scavenging capacity in the presence of a hydrogen donating antioxidant can be monitored as a decrease in absorbance of DPPH solution [33]. The effects of antioxidants on DPPH are considered to be due to their hydrogen donating ability [34]. Table 2 also shows the free radical scavenging capacity of the various aqueous extracts. The leaf extracts showed the highest percentage inhibitory activity while the stem bark extract possessed the least percentage DPPH inhibitory activity. The results indicated that the extracts exhibited the ability to quench the DPPH radical, which showed that the extracts have proton-donating capacity and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The results suggest that HRE was more active than CLE. This finding is collerborated with the report of [15,35].

4. CONCLUSION

The results demonstrate that the extracts from *Crateva adasonii* have promising antioxidant effects. The results therefore portray the potential medicinal value of the plant *Crateva adasonii* used in the traditional system of medicine in Imo State Nigeria and demands further detailed studies of this plant in order to justify its use in the traditional treatment of several diseases. The results also demonstrate that HRE is better than CRE in terms of antioxidant activities against DPPH, TBA and FTC. Hot leaf extract did not show stronger antioxidant actions than CLE. Also, HSBE did not show stronger antioxidant actions than CSBE.

5. FURTHER STUDIES

In vitro studies should be directed towards the hepatoprotective and hepatoceurative potentials of *Crateva adansonii* leaf extracts on Albino rats induced hepatotoxicity using carbontetrachloride (CCL_4) as model and the effect of the extracts on serum antioxidant status of the affected rats.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Maduka HCC, Maduka AA, Aguoru CU, Myke-Mbata BK, Ikwue JO, Okpogba AN, Ugwu CE, Dike CC, Ogueche PN, Ochalefu DO, Ufaryna N, Yakuby J, Sha E. Phytochemical screening, spectrophotometric determination of total carotenoids, chlorophyll a and b components of *Crateva adansonii* (Three-leaf Plant). Journal of Advances in Medical and Pharmaceutical Sciences. 2017;15(2): 1-6.
2. Kong KW. Nutritional constituents and antioxidant properties of indigenous *Dacroydes rostrata* fruits. Fd Res Int. 2013;44(7):2332-2338.
3. Adedokun II, Onuegbu NC. The physical properties of pulp and chemical characteristics of edible oil extracted from the pulp of African pear (*Dacroyodes edulis*). Pakistan J Nutri. 2011;10(6):558-560.
4. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44-84.
5. Duthie GG. Lipid peroxidation. Eur J Clin Nutr. 1993;47:759-764.
6. Kim KS, Lee S, Lee Y, Jung SH, Park P, Shim K, Kim BK. Antioxidant activities of the extracts from the herbs of *Artemisia apiacea*. J Ethanpharmacol. 2013;85:69-72.

7. Pryor WA. The antioxidant nutrients and disease prevention. What do we know and what do we need to find out? Am J Clin Nutr. 1991;53:391-s393.
8. Kinsella JE, Frankel E, German B, Kanner J. Possible mechanisms for the protective role of antioxidant in wine and plant foods. Food Technol. 1993;47:85-89.
9. Lana MM, Tijskens LMM. Effect of cutting and maturity on antioxidant activity of fresh-cut tomatoes. Food Chem. 2016;97: 203-211.
10. Abdullahi A, Hamzah RU, Jigam AA, Yahya A, Kabiru AY, Muhammad H, Sakpe S, Adefolalu FS, Isah MC, Kolo MZ. Inhibitory activity of xanthine oxidase by fractions *Crateva adansonii*. Journal of Acute Disease. 2012;126-129.
DOI: 10.1016/S2221-6189(13)60072-4
11. Akanji MA, Salau AK, Yakubu MT. Safety evaluation of aqueous extract of *Crateva adansonii* leaves on selected tissues of rats. Fountain Journal of Natural and Applied Sciences. 2013;2(1):17–28.
12. Maruthupandian M, Mohan VR. Observations of ethnomedicinal plants from Sirumalai Hills in Western Ghats of Tamilnadu, India. Journal of Herbal Medicine and Toxicology. 2010;4(2):89-92.
13. Ogbole OO, Gbolade AA, Ajaiyeoba EO. Ethnobotanical survey of plants used in treatment of inflammatory diseases in Ogun State, Nigeria. European Journal of Scientific Research. 2010;43(2):183-191.
14. Lucy BJ, Tijani AY, Christianah YI. Anti-ulcer and wound healing activities of *Sida corymbosa* in rats. Afr J Tradit. Complement Altern. Med. 2014;11(1):87-92.
15. Maduka HCC, Akubugwo EI, Olorunnipe O, Okpogba AN, Maduka AA, Aguoru CU, Ugwu CE, Dike CC, Ogueche PN. Elemental composition and phytochemical screening of aqueous leaf extract and stem bark extract of *Crateva adansonii.* Int J Biochem Res Rev. 2016;10(4):1-7.
16. Lucy BT, Mercy A. Evaluation of the haemostatic activities of *Sida corymbosa* in rats. British J Pharm Res. 2015;5(6): 431-436.
17. Sharma S, Kumar A. Introduction to traditional medicines of semi-arid regions. Int J Life Sci Pharm Res. 2012;2(3):293-303.
18. Dike IP, Obembe OO, Adeiyi FE. Ethnobotanical survey for potential anti-malaria plants in South-Western Nigeria. J Ethnophamacol. 2012;144(3):618-626.
19. Mrinmay D, Ashok KD. Phyto-pharmacological review of *Alternanthera sessilis* linn. Int J Inn Res Pharm Biol Sci. 2014;1:9-15.
20. Kong KW. Nutritional constituents and antioxidant properties of indigenous *Dacroydes rostrata* fruits. Fd Res Int. 2011;44(7):2332-2338.
21. Adedokun II, Onuegbu NC. The physical properties of pulp and chemical characteristics of edible oil extracted from the pulp of African pear (*Dacroyodes edulis*). Pakistan J Nutri. 2013;10(6):558-560.
22. Hatano T, Takagi M, Ito H, Yoshida T. Phenolic constituents of lico rice root: Their relative astringency and radical scavenging effects. Chemical and Pharmacology Bulletin. 2012;36:1090-2097.
23. Rosa MPG, Yoja TGN. Antioxidant and hepatoprotective effects of the methanol extract of the leaves of *Satureja macrostema*. Pharmacognosy Magazine. 2010;6(22):125-131.
24. Hanson GK, Hermansson A. The immune system in atherosclerosis. National Immunology. 2011;12:204-212.
25. Azlan A. Comparison of fatty acids, vitamin E and physiochemical properties of *Canarium adontophyillum* Miq, olive and palm oils. Journal of Food Composition and Analysis. 2010;23(8):772-776.
26. Gutierrez RMP, Luna HH, Garrido SH. Antioxidant activity of *Tagetes erecta* essential oil. J Chilean Chem Soc. 2006;51:883-886.
27. Ezekwesili CN, Eneh FU. Evaluation of dietary supplementation with *Dacryodes edulis* G. Don pulp oil on serum lipid parameters in wistar albino rats. Pakistan J Biol Sci. 2014;17:910-914.
28. Ejezie FE, Ikekpeazu JE. Fundamentals of metabolism in: Metabolism of protein and amino acids. 1st ed. Ezu Books Ltd: New Haven, Enugu. 2010;253-258.
29. Zin M, Abdul-Hamed A, Ojman A. Antioxidant activity of extracts from mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. Food Chem. 2012;48:5068-5073.
30. Kalava SV, Menon S. *In vitro* free radical scavenging activity of aqueous extract from the mycella of *Volvariella volvacea* (Bulliard Ex Fries) *Singer.* Journal of

Current Pharmaceutical Research. 2013;4(3):94-100.
31. Stademaye B. Nutrient composition of selected indigenous fruits from sub-Saharan Africa. Journal of the Science of Food and Agriculture. 2013;93(11):2627-2636.
32. Wipperman MF, Sampson NS, Thomas ST. Pathogen roid rage: Chalesterd utilization by mycobacterium tuberculosis. Crit Rev Biochem Mol Biol. 2014;49(4): 269-293.
33. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Tech. 1995;28:25-30.
34. Okafor IA, Ejimofor OC, Ezejindu DN, Chukwujekwu IE, Dike CC. The renoprotective effect of *Portulaca oileracea* in cisplatin- induced nephrotoxicity in wistar rats. Advances in Life Sciences and Technology. 2014;19:52-54.
35. Jongkwon S, Soojung L, Marcus LE, Sarah AJ, Jonghoon K, Bahram HAS. Study to find the best extraction solvent for use with guava leaves (*Psidium guajava*) for high antioxidant efficacy. Food Sci Nutr. 2014;2(2):174-180.

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