World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/
WJARR

(Research Article)

Check for updates

The effect of crude extract of *Cyathula prostrata* leaves on serum c-reactive proteins levels of normal albino Wistar rats after an administration period of 7 days

Ombu Sunita Etta [1,*], Oyom Bright Bassey [1], Uroko Emmanuel Augustine [2] and Canice Peace Jerimoth [3]

[1] *Department of Biochemistry, Cross River University of Technology, Calabar, Nigeria.*
[2] *Department of Physics, Ahmadu Bello University, Zaria, Nigeria.*
[3] *Department of Biochemistry, Bingham University, Auta-Balefi, Nigeria.*

World Journal of Advanced Research and Reviews, 2023, 20(03), 1550–1557

Publication history: Received on 08 November 2023; revised on 18 December 2023; accepted on 20 December 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.20.3.2592

Abstract

Food fortification is considered a sustainable public health strategy because it can reach wider populations through existing food delivery systems without requiring major changes in existing consumption patterns (Das et al., 2013.). C-reactive Protein (CRP) is a major acute phase reactant synthesized primarily in the liver hepatocytes. CRP shows the strongest association with cardiovascular events. It is detectable on the surface of about 4% of normal peripheral blood lymphocytes. This research was designed to study the effects of the administration of graded doses (100mg/kg and 200mg/kg) of crude extracts of *Cyathulaprotrata* leaves on albino *Wistar* rats after a period of 7 days. The animals were sacrificed after the experimental period and blood collected and prepared for analysis to access the level of blood C-reactive protein. After the study, it was observed that the blood C-reactive protein levels for groups II and III animals were elevated significantly, indicating that there was tissue damage due to toxicity of xenobiotics. This research has led to the conclusion that the consumption of *Cyathulaprostrata* leaves extract was safe though it generated oxidative stress. Results show that experimental animals of group II, and III indicated elevated C-reactive protein levels (3.21±0.19 and 3.14±1.11 mg/dL respectively) significantly at $p<0.5$ when compared to experimental animals of group 1 (control, 2.38±1.04 mg/dL) significantly at $p<0.05$.

Keywords: Cyathulaprotrata; C-reactive Protein; Oxidative stress; Oxidative stress; Xenobiotics

1. Introduction

Micronutrient deficiencies and the negative consequences of a diet lacking in essential vitamins and minerals/trace elements continue to pose significant public health problems for many low- and middle-income country (LMIC) populations. This hidden hunger is more prevalent in vulnerable populations, including women of reproductive age and young women (Yang and Huffman, 2011), and female adolescents (Thurnham, 2013).

It is estimated that at least 1.6 billion people around the world suffer from anaemia (McLean *et al.,* 2009). Globally approximately 2 billion suffer from chronic micronutrient deficiencies (Muthayya *et al.,* 2013), with the most common forms of micronutrient malnutrition being caused by a lack of iron, folate, iodine, vitamin A, and zinc overall, micronutrient malnutrition has significant health and economic consequences (Fletcher *et al.,* 2004; Horton *et al.,* 2008; Bhutta *et al.,* 2013; Black *et al.,* 2013). Micronutrient deficiencies alone have been estimated to cost an annual GDP loss of 2% - 5% in low and middle-income countries (LMIC) (Ming *et al.,* 2014; Dary, 2008; Taylor *et al.,* 2015), with direct costs estimated between US $20 to $30 billion every year (˜ = N=7.22 to 10.83 trillion). Consider anaemia, which is estimated to cause a 17% reduction in productivity in heavy manual labour, as well as an estimated 2.5% loss of earnings due to lower cognitive skills (Horton *et al.,* 2008).

* Corresponding author: Ombu Sunita Etta.

Copyright © 2023 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution Liscense 4.0.

Annually, 40-60% of children 6 – 24 months of age in LMIC are at risk of impaired cognitive development due to iron deficiency, while anaemia during pregnancy contributes to 20% of all maternal deaths, and reduced work productivity in adults (Black *et al.,* 2013). Iodine deficiency causes some 35 million newborns to be born intellectually impaired (UNICEF, 2015) as a result of poor maternal iodine status. The estimated intellectual loses for these newborns range from 7.4 to 15 IQ points (Canadian Public Health Association - CPHA – 2015).

Insufficient intake of vitamin A results in an estimated 250,000 to 500,000 cases of childhood blindness every year. An estimated 250 million preschool children are vitamin A deficient (8), leading to a compromised immune system and increased mortality risk. In 2013, it was estimated that, annually, 2.3% and 1.7% of all childhood deaths can be attributed to deficiencies in vitamin A and zinc. Approximately 300,000 children are born each year with severe birth deficits due to maternal folate deficiency.

The World Health Organization (WHO) and the Food Agriculture Organization of the United Nations (FAO) have identified four main strategies for improving micronutrient malnutrition: Nutrition education leading to diets that are more diverse and better quality, Food fortification and bio-fortification, Supplementation, Disease control measures.

Each of these strategies has a place in eliminating micronutrient malnutrition. To achieve maximum impact, the appropriate mix of these strategies should be in place simultaneously to promote consumption and utilization of an adequate diet for all people in the world (WHO/FAO, 2006).

Food fortification is considered a sustainable public health strategy because it can reach wider at-risk populations through existing food delivery systems without requiring major changes in existing consumption patterns (Das *et al.,* 2013). Compared to other interventions, food fortification is likely to be more cost-effective, and – if fortified foods are regularly consumed – it has the advantage of maintaining steady body stores (WHO/FAO, 2006).

In 2006, the WHO published evidence-informed guidelines for various aspects of fortification. These guidelines included the appropriate selection of vehicles and fortificants; how to determine fortification levels; and the implementation of effective and sustainable food fortification programmes (WHO/FAO, 2006). Today, 10 years after the WHO guidelines were published, this report will provide an objective overview of the state of large-scale food fortification in the world, with particular emphasis on the fortification of staple foods. Typically, the fortification of staple foods is mandatory, but voluntary programmes are included where appropriate. The report will describe essential components of successful fortification programmes and describe remaining technical challenges and barriers. This will inform how to prioritize recommendations and next steps, which will be discussed and conclusions drawn. The report focuses on mass fortification and does not address home fortification, bio-fortification or specialized fortified foods for specific target groups (for example, fortified complementary foods for infants and young children who typically cannot consume enough of fortified family foods to match their dietary requirements (Dewey, 2013). An initial expanded version of this report was presented at the #Future Fortified Global Summit on Food Fortification, which took place in Arusha, Tanzania in September, 2015. This synopsis report summarizes the initial version and includes some of the recommendations from the summit, which have been described in details in other publications (Sight & Life, 2016).

2. Materials and methods

2.1. Materials

Mature leaves of *Cyathulaprostrata* were collected washed and further dried under ambient conditions and then crushed and blended with an electric blender and subjected through Soxhlet extraction method using petroleum ether as solvent. The extract was subsequently concentrated to about 10 percent of its original volume.

2.1.1. Chemicals

All chemicals and reagent kits were standard chemicals from the Cross-River University of Technology laboratory and of appropriate concentrations purchased from No. 121 Dan Archibong Street, Calabar, Cross River State.

2.1.2. Collection and preparation of plant materials

The whole plants of *Cyathulaporstrata* were collected from the Botanic Garden, Department of Biological Science, Cross River University of Technology, Calabar, during the months of August – September, 2017. The plant was identified by the taxonomist in the Biological Science Department, Cross River University of Technology, Calabar.

The leaves of *Cyathulaprostrata* were washed thoroughly, separated and dried under ambient conditions. The dried materials were shredded and further pulverized to very fine powder by pounding with the aid of a mortar and pistol. The powdered leaves samples obtained were stored separately in airtight containers until needed.

The powdered leaves were extracted with petroleum ether using a Soxhlet extractor. The extracts were filtered to obtain particulate free filtrates. The filtrates obtained were concentrated using a Rotary vacuum evaporator. The resulting dark semisolid material obtained was stored at a temperature (4 ^{o}C), until use (Vijayakumar, 2014).

2.2. Methods

2.2.1. Animal experimentation

30 (thirty) albino rats of *Wistar* strain weighing between 150-250 grams, obtained from the animal house of the Department of Biological Sciences, Cross River University of Technology, Calabar, were used. The animals were acclimatized for one week and their weights noted before, during and on the last day of experimental treatments. Groups of 10 animals each were housed in cages with the normal day lighting pattern of about 12 hours light (0630 – 1830 hours) and 12 dark. Animals had free access to standard livestock feed and tap water and *libitum* throughout the experimental period of 7 days.

2.2.2. Experimental procedures

The thirty albino *Wistar* rats weighing 150-250 g were randomly grouped into three (3) experimental groups of ten (10) rats each. Rats were fed 1.0ml crude extracts of 100 mg/kg body weight (F1) and 200mg/kg body weight (F2) in normal saline by gavage. The experimental animals for a period of 7 days as follows:

Groups	Administration
I	Control, 1.0ml 0.9 normal saline (n = 10)
II	100mg/kg body weight 1.0ml F1 (n=10)
III	200mg/kg body weight 1.0ml F2 (n=10)

The experimental treatment lasted for a period of seven (7) days. On the eight (8th) day, the animals were sacrificed and the tissue of interest were collected and stored accordingly for analysis.

2.2.3. Collection and preparation of tissues for analyses

At the end of the experimental treatments the animals were sacrificed and the blood tissue collected were thereafter centrifuged at 2000g for 5 minutes and the serum decanted for analysis.

2.3. Principles and procedures for biochemical estimation

2.3.1. Estimation of plasma C-reactive proteins levels

Principles

The Cohesion Bioscience Human C-Reactive Protein ELISA (Enzyme-Linked Immunisorbent Assay) kit is an in vitro enzymelinked immunosorbent assay for the quantitative measurement of Human C- Reactive Protein in Cell Culture Supernatants, Serum, Plasma. This assay employs an antibody specific for Human C-Reactive Protein coated on a 96 well plate. Standards and samples are pipette into the wells by the immobilized antibody. The wellsa re washed and biotinylated anti-Human C-reactive protein antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipette to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of C-reactive protein bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450nm.

- **Component** **Volume**
- 96 well Plate Coated with Anti-Human C-Reactive Protein 12x8 stripes
- Antibody

- Human C-Reactive Protein Standard 10 ng x 2
- Biotin-Labelled Detection Antibody (100x) 120 pl
- Streptavidin – HRP (100x) 120pl
- Standard/Sample Diluent 30 ml
- Detection Antibody Diluent 12 ml
- Streptavidin HRP Diluent 12 ml
- Wash Buffer (20x) 30 ml
- TMB Substrate Solution 12 ml
- Stop solution 12 ml
- Plate Adhesive Strips 3 Stips
- Technical Manual 1 Manual

2.3.2. Storage and Stability

All kit components are stable at 2 to 8^{0}C. Standard (recombinant protein) should be stored at -20 ^{0}C or -80 ^{0}C (recommended at -80 or 0C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2 to 80 ^{0}C. return unused wells to the pouch containing desiccant pack, reseal along entire edge. Note: the kit can be used within one year if the whole kit is stored at -20 ^{0}C. avoid repeated freeze-thaw cycles.

2.4. Statistical Analysis

The data obtained were analyzed statistically using analysis of variance (ANOVA) and students' T-test. Data were expressed as ± mean standard deviation. Values of $p<0.05$ were regarded as being significant.

3. Results

Table 1 shows the C-reactive protein levels in the serum of experimental animals challenged with acute toxicity from fumes of pesticides.

Table 1 C-reactive protein levels in the serum of experimental animals challenged with acute toxicity from fumes of pesticides.

Experimental Animal groups	**Group I**	**Group II**	**Group III**
C-reactive protein levels (mg/dl)	2.38±1.04	3.21±0.19	3.14±1.11

4. Discussion

Results show that experimental animals of groups II, and III indicated elevated C-reactive protein levels (3.21±0.19 and 3.14±1.11 mg/dl respectively) significantly at $p<0.5$ when compared to experimental animals of group I (control, 2.38±1.04 mg/dL) significantly at $p<0.05$.

C-Reactive protein (CRP) is a major acute phase reactant synthesized primarily in the liver hepatocytes. CRP shows the strongest association with cardiovascular events. It is detectable on the surface of about 4% of normal peripheral blood lymphocytes. Acute phase reactant CRP is produced in the liver. Displays several functions associated with host defense: it promotes agglutination, bacterial capsular swelling, phagocytosis and complement fixation through its calcium-dependent binding to phosphorylcholine. Can interact with DNA and histones and may scavenge nuclear material released from damaged circulating cells.

4.1. Summary

This research was designed to study the effects of the administration of graded doses (100mg/kg and 200mg/kg) of crude extracts of *Cyathulaprostrata* leaves on albino *Wistar* rats after a period of 7 days. The animals were sacrificed after the experimental period and blood collected and prepared for analysis to access the level of blood C-reactive protein. After the study, it was observed that the blood C-reactive protein levels for groups II and III animals were elevated significantly, indicating that there was tissue damage due to toxicity. This assumption is rife because high levels of C-reactive protein are associated with necrosis and myocardial infection, probably due to oxidative stress emanating from toxicity of xenobiotics.

5. Conclusion

This research has led to the conclusion that the consumption of *Cyathulaprostrata* leaves extract was safe though it generated oxidative stress. This is due to the inherent route of metabolism of xenobiotics which generates free radicals.

Recommendation for further studies

Further research should be conducted to understudy the active ingredients within the extract that may be responsible for the observed elevation.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval for the treatment and handling of experimental animal and human subjects was obtained from the Faculty Animal Research Ethics Committee on Use and Care of Experimental Animals, Faculty of Basic Medical Sciences, Cross River University of Technology, Okuku Campus with the approval number/code; CRUTECH/FBMS/IREC/2023-C1002.

References

[1] Addo LSADAL Hypertension in SubSaharan Africa: A systematic review. Journal of The American heart Association 2007; 50:1012-1018. 7. Adeoye B. Adebiyi Tayo. Prevalence of hypertension and association with increased body mass in a semi-urban settlement in Nigeria, Journal of Hypertension, 2014.

[2] Afolabi SNC, Emeka EJ Iweala, Olubanke O. Ogunlana, Dominic E. Azuh. Body mass index and blood pressure in a semi-urban community in Ota, Nigeria Food and Public health 2014;5 (5): 157-163.

[3] Agyemang BAO-D. Factors associated with hypertension awareness, treatment, and control in Ghana, West Africa, Journal of Human Hypertension. 2006:20;67-71.

[4] Ajayi G, et al. Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of Plumbagozeylanica, Linn. J Med plants Res. 2011;5(9); 1756-61.

[5] Anthony BOUAADWPIJAJAO. Adrenaline induced elevated blood pressure in Wistar rats is not reversed by oral administration of Archachatinamarginatahemolymph. British Journal of Medicine & Medical Research. 2015;7(4); 318-326.

[6] Appel LJ. Lifestyle modification as a means to prevent and treat high blood pressure. Journal of the American Society of nephrology. 2003;14 (suppl 2): S99S102.

[7] Asgary N. Sarrafzadegan Mohammadifard, MostafavisVakili. Antihypertensive and antihyperlipidemic effects of ACHILLEA WILHELMSII; 2000.

[8] Association WM. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bulletin of the World health organization, 2001;79(4): 373.

[9] Australia HFO. Guide to management of hypertension Journal of Heart Foundation, 2010;1-30.

[10] Backer, C.A 1949, Cyathula. In: Van Steenis, C.G.G.J. (Editor) Flora Malesiana Series 1, Vol. 4. Noordhoff-Kolff, Djakarta, Indonesia. Pp. 81-83.

[11] Ballou SP. Kusher I. C-reactive protein and the execute phase Response 1992, 23; 1118-24.

[12] Bhardwaj A, et al. Screening of nutritional, phytochemical, antioxidant and antibacterial activity of underutilized seeds of Scirpusarticulatus: The basis of Khubahi Ramdana industry. Journal of Pharmacognosy and Phytochemistry 2014;3(4): 11-20.

[13] Bhutta ZA, Das JK, Rizvi A, Gaffrey MF, Walker N, Horton S, et al, Evidence – based interventions for improvement of maternal and child nutrition; what can be done and at what cost? Lancet. 2013;382 (9890); 452-77.

[14] Black RE, Victoria CG, Walker SP. Bhutta ZA, Christian P, de Onis M, et al. maternal and child under nutrition and overweight in low-income and middle-income countries. Lancet 2013;382(9890):427-51.

[15] Bloch CE. Klinische Untersuchungenuber Dystrophie und Xerophthalmiebeijungen Kidern, Jahrb Kinderheilkphys Erziehung. 1919:89:405-41.

[16] Bolanle IAS, Anzel Van Rooyen, Maryna Van de Venter Antiinflamatory analgesic and antioxidant activities of Cyathulaprostrata (Linn.) Blume (Amaranthaceae): Journal of Ethnopharmacology 2012;141;282-289.

[17] Canadian Public Health Association (CPHA), Food fortification with vitamins and minerals 2015 (Available from.

[18] Chen Z, et al. Chinese herbal medicine combined with conventional therapy for blood pressure variability in hypertension patients. A systematic review of randomized controlled trials. Evidence Based Coplementary and Alternative Medicine, 2015.

[19] Dary O. Establishing safe and potentially efficacious fortification contents for folic acid and vitamin B12. Food Nutr Bull.

[20] Das JK, Salam RA, Kumar R, Bhutta ZA. Micronutrient fortification of food and its impact on woman and child health: a systematic review. Syst Rev. 2013;2:67.

[21] Dewey KG. The challenge of meeting nutrient needs of infants and young children during the period of complementary feeding: an evolutionary perspective. J Nutr. 2013; 143(12): 2050-4.

[22] Diosady LL, Mannar MGV. Vitamin A fortification of cooking oils. In: Preddy VR, Srirajaskanthan. R, Patel VB, editors. Handbook of food fortification and health from concepts to public health application Vol 2. New York: Springer: Humana Press; 2013. . 275-90.

[23] Duarte J, et al. Antihyperrensive effects of the flavonoid quercetin in spontaneously hypertensive rats. British Journal of Pharmacology. 2001; 133(1): 117-124.

[24] Dwyer JT. Wuemer KL, Dary O. Keen CL, King JC, Miller KB, et al.Fortification and health: challenges and opportunities. Adv fortification in public health: consultation rationale and summary. Ann NY Acad Sci. 2014; 1312:1-7.

[25] Elhakim N. Laillou A, El Nakeeb A, Yacoub R, Shehata M. fortifying baladi bread of Egypt: reaching more than 50 million people through the subsidy program. Food Nutr Bull. 2012;33 (4 Suppl): S260-71.

[26] Erhirhie EOE, Nwoke E, Ajaghaku Daniel Lotanna. Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. Journal of Natural Sciences Research. 2014;4.

[27] Farnsworth RN. Review on biological and phytochemical screening of plants. J. Pharm. Sci. 1966;55;2255-276.

[28] Flour Fortification Initiative (FFI). Global Progress. Enhancing Grains for Healthier Lives Atlanta (GA) 2015 [Available from: http://www.cpha.ca/en/programs/history/achievements/09-shf/fortification.aspx.

[29] Forestieri A, et al. Antiinflamatory, analgesic, and antipyretic activity in rodents of plant extracts used in African medicine. Phytotherapy Research. 1996;10(2): 100106.

[30] Forestieri, A.M., Monforte, M.T., Ragusa, S., Trovato, A. & Lauk L., 1996. Antiinflamatory, analgesic and antipyretic activity in rodents of plant extracts used in African medicine. Phytotherapy Research 10(2): 100-106.

[31] Forestieri, A.M., Pizzimenti, F.C., Monforte, M.T. & Bisignano, G., 1988. Antibacterial activity of some African medicinal plants. Pharmacological Research Communications Suppplement 20(5): 33-36.

[32] Gera T, Sachdev HS, and Boy E. Effect of iron-fortified foods on hematologic and biological outcomes; systematic review of fortification project: experiences from Morocco, Uzbekistan, and Vietnam. Food Nutr bull. 2012l33 (4 Suppl): S281-92.

[33] Gideon IOWOTPUNBEI. Antimicrobial and phytochemical evaluation of the leaf, stem bark and root extracts of Cyathulaprostrata (L) Blume against some human pathogens. Journal of Intercultural Ethnopharmacology. 2012;35-43.

[34] Gilani J, Ghayur, Janbaz, Akhtar. Studies on the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the Carumcopticum seed extract. Journal of Ethnopharmocology. 2005;98:127-135.

[35] Harbone JB. Phytochemical methods: A guide to modern techniques of plant analysis 3rd Edition. Chapman and Hall, London. 1998;235.

[36] Hennessy A, Walton J, Flynn A. The impact of voluntary food fortification on micronutrient intakes and status in European countries: a review. Proc Nutr Soc. 2013; 72 (4): 433-40.

[37] Horton S, Alderman H, Rivera J. Copenhagen Consensus 2008. Malnutrition and hunger. Copenhagen Consensus Center; 2008.

[38] Horton S. The economics of food fortification. J Nutr. 2006;136(4):1068-71.

[39] Ismail, N.A., Okasha, S.H., Dhawan, A., Abdel Rahman, A.M.O., Shaker, O.G. and Sadik, N.A.H. (2010) Antioxi-dant enzyme activities in hepatic tissue from children with chronic cholestatic liver disease. *Saudi Journal of Gastroenterology,* **16,** 90-94. Doi:10.4103/1319-3767.61234.

[40] Ismail, N.A., Okasha, S.H., Dhawan, A., Abdel, A.M.O., Rahman, A.M.O., Shaker, O.G. and Sadik, N.A.H. (2009) Glutathione peroxidase, superoxide dismutase and cata-lase activities in hepatic tissue from children with glycol-gen storage disease. *Archives of Medical Science,* **5,** 86-90. http://www.termedia.pl/Glutathoine-peroxidase-superoxide-dismutase-and-catalse-activites-in-hepatic-tisssue-from-children-with-glycogen-storage-disease,19,12292,1,1,html.

[41] James RKW, Achilles Katamba, Elli Leontsini, Fred Nuwaha. Hypertension awareness, treatment and control in Africa: A systematic review. BMC Cardiovasular Disorders; 2013.

[42] Jananie R, Priya V, Vijayalakshmi K. Determination of bioactive components of Cynodondactylon by GC-MS analysis. NY Sci J. 2011;4:16-20.

[43] Jegadeeswari N, Muthukumarasamya, Mohan. GC-MS analysis of bioactive components of aristolochiakrysagathra (aristolochiaceae). Journal of current chemical and Pharmaceutical sciences, 2014;2(4): 226-232.

[44] Khatoon N, Gupta RK. Probiotics beverages of sweet lime an sugarcane juices and its physiochemical, microbiological and shelf-life studies. Journal of Pharmacognosy and Phytochemistry. 2015; 4(3):25-34.

[45] Kwan C-Y, et. Al. Endothelium-dependent vasorelaxant effects of the aqueous extracts of the EucommiaulmoidesOliv. Leaf and bark; implications on their antihypertensive action. Vascular Pharmacology. 2003;40(5):229-235.

[46] Lammerhofer MI, Lindner WP. Direct high performance liquid chromatographic enantiomer separation of anti-inflammatory planar chiral [2:2] paracyclophane-4-acetic acid. Chromatographia. 2004;60(1):S13S17.

[47] Lawal OA, et al. Chemical composition and antibacterial activity of essential oil from the leaves of Aframomummelegueta (Roscoe) K. Schum from Nigeria. Journal of Essential Oil Bearing Plants. 2015; 18(1): 222-229.

[48] Liu J-C, et al. Antihypertensive effects of tannins isolated from traditional Chinese herbs as non-specific inhibitors of angiontensin converting enzyme. Life Sciences. 2003;73(12):1543-1555.

[49] McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutr. 2009;12(4):444-54.

[50] Ming Q, Codling K, Yuqin Y, Zupei C. China: improving USI to ensure optimal iodine nutrition for all. IDD Newsletter.

[51] Muhilal, Murdiana A, Azis I, Saidin S, Jahari AB, Karyadi D. Vitamin A-fortified monosodium glutamate and vitamin A status: a controlled field trial. Am J. Clin Nutr. 1988;48(5): 1265-70.

[52] Muthayya S, Rah JH, Sugimoto JD, Roos FF, Kraemer K, Black RE. The Global Hidden Hunger Indices and Maps: An Advocacy Tool for Action. PLOS ONE. 2013; Published online. Nutr. 2015;6 (1): 124-31.

[53] Ogu GI, et al. Antimicrobial and phytochemical evaluation of the leaf, stem bark and root extracts of Cyathulaprostrata (L) Blume against some human Ojekale et al.; EJMP, 14(2): 1-10, 2016; Article no. EJMP. 25007 9 pathogens. Journal of Intercultural Ethnopharmacology. 2012;1(1):35-43.

[54] Okwu J. Evaluation of the chemical composition of two Nigeria Medicinal plants. African Journal of Biotechnology. 2006;5:357-361. Ojekale et al.; EJMP, 14(2): 1-10, 2016; Article No. EJMP. 25007 10.

[55] Olawale HOCOUEEA. Novel anti-oxidant activity of Cyathula prostrate (L.) blume, in bulletin of environment, pharmacology and life sciences. 2013;91-98.

[56] Olayeriju OS, et al. Ethylacetate extract of red onion (Allium cepa L.) tunic affects hemodynamic parameters in rats. Food Science and Human Wellness. 2015;4(3): 115-122.

[57] Organization WH. Clinical guidelines for the management of hypertension; 2005.

[58] Perry, L.M., 1980. Medicinal plants of East and Southeast Asia. Attributed properties and uses. MIT Press, Cambridge, Massachusetts, United States & London, United Kingdom. P. 11.

[59] Priya KKSS. Toxicity assessment of the medicinal plant Cyathula prostrate. Journal of Applied Biosciences. 2009;13:681-687.

[60] Quisumbing, E., 1978. Medicinal plants of the Philippines. Katha Publishing Co., Quezon City, the Philippines. P.272.

[61] Ramadoss SKKBKJNAMR. Cardioprotective Effect of Cyathula prostrate Linn on Isoproterenol Induced Myocardial Infarction in Rat. 2012;3.

[62] Ramadoss SKKBKJNSMR. Hepatoprotective effects of Cyathula Prostrata Linn. In paracetamol induced liver injury in rats. Journal of Pharmaceutical Research and Opinion, 2011;1(7): 178-180.

[63] Sarumathy KMSDRTV, Jayakanthi J. Evaluation of photoconstituents, nephroprotective and antioxidant activities of Clitoriaternatea. Journal of Applied Pharmaceutical Science. 2011;1(5): 164172.

[64] Savithramma LRAS. Screening of medicinal plants for secondary metabolites. Middle-East Journal of Scientific Research. 2011;8(3):579-584.

[65] Sight & Life. The #Future fortified global summit on food fortification Events proceedings and recommendations for food fortification programs.; 2016.

[66] Sofowora A. Medicinal plants and traditional medicine in Africa. John Wiley and sons LTD; 1982.

[67] Solon FS, Klemm RD, Sanchex L, Darnton-Hill I, Craft NE, Christian P, et al. efficacy of a vitamin A-fortified wheat-flour bun on the vitamin A status of Filipino school children. Am J Clin Nutr. 2000;72(3):738-44.

[68] Solon FS, Solon MS, Mehansho H, West KP, Jr., Sarol J, Perfecto C, et al. evaluation of the effect of Vitamin A-fortified margarine on the vitamin A status of preschool Filipino children. Eur J Clin Nutr. 1996;50(11):720-3.

[69] Sonibare MA, Olatubosun OV. Pharmacognostic and free radical scavenging evaluation of Cyathulaprostrata (Blume) L. Pharmacogn J. 2015;7(2): 107-116.

[70] Sparg SGMELJVS. Biological activities and distribution of plant saponins. Journal of Ethnopharmacology. 2004;94:219-243.

[71] Takemoto, T., Ogawa, S., Nishimoto, N., Hirayama, H. & Taniguchi, S., 1968. Constituents of Achyranthes and Cyathula genera. YakugakuZasshi 88: 1293-1297. (in Japanese).

[72] Taylor CL, Bailey RL, Carriquiry AL. use of Folate-Based and Other Fortification Scenarios Illustrates Different Shifts for Tails of the Distribution of Serum 25-Hydroxyvitamin D Concentrations. J. Nutr. 2015;145(7):1623-9.

[73] Thurnham DI. Nutrition of adolescent girls in low and middle-income countries. Sight and Life. 2013;27:26-37.

[74] UNICEF Regional Office for Central and Eastern Europe and the Commonwealth of Independent State (CEE/CIS). The "Day of Three Laws" in Kyrgyzstan 2009 [Available from: http://www.unicef.org/ceecis/media_11364.html.

[75] UNICEF. The State of the World's Children 2015. Reimagine the future. New York: United Nations Children's Fund; 2015.

[76] Vijayakumar MPKKSANA. Anticancer activity of CYathulaprostrata (Linn) Blume against Dalton's lymphomae in mice model. 2014;27:1911-1917.

[77] WHO/FAO. Guidelines on Food Fortification with Micronutrients. Geneva: World Health Organization/Food and Agriculture randomized controlled trials. Am J Clin Nutr. 2012;96(2):309-24.

[78] Wirth JP, Laillou A, Rohner F, Northrop-Clewes CA, Macdonald B, Moench-Pfanner R. Lessons learned from national food status in populations. Cochrane Database Syst Rev: John Wiley & Sons, Ltd; 2014.

[79] Wu Y-BW, Jian-Guo Yi, Jun Chen, TiQuiang Lin, Xiong-Hao Wu, Jin-Zhong. Osteoblastic activity of ethanolic extract and volatile compounds from Er-Zhi-Wan, a famour traditional Chinese herbal formula. African Journal of Pharmacy and Pharmacology. 2012;6(24):1733-1740.

[80] Yang Z, Huffman SL. Review of fortified food and beverage products for pregnant and lactating women and their impact on nutritional status. Matern Child Nutr. 2011;7 Suppl 3:18-43.