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Evaluation of *in vitro* antioxidant activities of extracts of *Citrullus lanatus* seed

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ABSTRACT:

Background: *Citrullus lanatus* (watermelon), *a member of the Cucurbitaceae family, contains antioxidant molecules. It is a good source of carotenoids, vitamins, and minerals.*

Aim: *The present study investigated the in vitro antioxidant activities of aqueous and methanol extracts of C. lanatus seed.*

Methods: *Aqueous and methanol extracts of C. lanatus seed were prepared using standard method. The in vitro antioxidant activities of the extracts were also determined using standard methods.*

Results: *The diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity, reductive potential (RP), ferric reducing antioxidant power (FRAP), nitric oxide (NO) scavenging activity and total antioxidant capacity (TAC) of the methanol extract were significantly higher than those of aqueous extract ($p < 0.05$).*

Conclusion: *These results suggest that phenolics present in the extracts may play a role in free radical scavenging activities of the medicinal plant.*

Keywords: *Antioxidants, Citrullus lanatus, Diphenyl-2-picryl-hydrazyl, Free radical, Reducing power.*

INTRODUCTION

The use of medicinal plants for the treatment of diseases is as old as man [1]. Products from these plants are cheap and readily available [2, 3]. One of such plants is *Citrullus lanatus* (watermelon). Consumption of fruits is no longer a mere result of personal preference and taste, but a concern of health, since they contain important macronutrients and considerable amounts of micronutrients, such as minerals, fibers, vitamins and phytochemical compounds. Increasing evidence shows the importance of these micronutrients in human health [4, 5]. Specific phytochemicals isolated from fruit pulps are used as food supplements/additives and nutraceuticals [6].

Citrullus lanatus (watermelon), a member of the *Cucurbitaceae* family, has a deep green- or yellow-colored smooth thick exterior rind, with gray or light green vertical stripes. The fruit is pink, red or yellow inside with small black seeds embedded in the middle third of the flesh. Generally, *Citrullus lanatus* flesh (juice or pulp) is the main consumable portion; however, the outer rind is also consumed in some parts of the world [7]. *Citrullus lanatus* flesh contains antioxidant molecules such as carotenoids (lycopene and β-carotene), citrulline, minerals like potassium, and superoxide dismutase [8 - 10]. The rind contains alkaloids, saponins, cardiac glycosides, flavonoids, phenol, moisture, lipid, protein, fiber and carbohydrates [11]. *Citrullus lanatus* is related to cantaloupe, squash, pumpkin, as well as other plants that grow on vines on the ground [12]. Lycopene from this medicinal plant has been shown to protect against a growing list of cancers [13]. *Citrullus lanatus* seeds are rich sources of protein, B-group of vitamins, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper), as well as fat [14]. The seeds are used to prepare snacks, milled into flour and used for sauces. Oil from the seeds are used for cooking and production of cosmetics [15].

MATERIALS AND METHODS

Plant Sample Collection and Preparation

The plant seeds were obtained from New Benin Market, and identified at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. Preparation and extraction was carried out using standard method [16]. The aqueous and methanol extracts were concentrated using rotary evaporator and made into powder via lyophilisation.

DPPH Radical Scavenging Assay

The free radical scavenging capacity of the plant extracts against 1, 1–diphenyl–2–picrylhydrazyl (DPPH) free radical was determined by a slightly modified method of Brand-Williams *et al.* [17]. Briefly, 0.5 mL of 0.3 mM DPPH solution in methanol was added to 2 mL of various concentrations of the extracts (0.2 - 1.0 mg/mL). The test tubes were shaken and incubated in the dark for 15 min at room temperature, and the absorbance was read at 517 nm. All tests were performed in triplicate. Ascorbic acid (vitamin C) was used as control, with similar concentrations as the test samples. A blank containing 0.5 mL of 0.3 mM DPPH and 2 mL methanol was prepared and treated as the test samples. The radical scavenging activity was calculated as shown in Equation 1:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{Ao - A_1}{Ao} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Where Ao was the absorbance of DPPH radical + methanol; A_1 was the absorbance of DPPH radical + sample extract or standard.

Reducing Power Assay

The reducing power (RP) of extracts was determined according to the method described by Lai *et al.* [18]. Briefly, 1 mL of different concentrations of extracts (0.1- 1.0 mg/mL) in water was mixed with 2.5 mL of 0.2 M phosphate buffer, pH 6.6 and 2.5 mL of 1 % potassium ferricyanide. The mixture was incubated at 50 oC for 20 min. Thereafter, 2.5 mL of trichloroacetic acid (10 %) was added to the mixture to stop the reaction. Distilled water (2.5 mL) and 0.5 mL of 0.1 % FeCl3 were then added and the absorbance was read at 700 nm. Higher absorbance values indicated higher reducing power. Ascorbic acid served as the control.

Determination of Ferric Reducing Antioxidant Power (FRAP)

A modified method of Benzie and Strain [19] was used for the FRAP assay. The principle behind this assay is the ability of the sample to reduce ferric tripyridyltriazine (Fe (III)- TPTZ) complex to ferrous tripyridyltriazine (Fe (II) - TPTZ), which at low pH produces an intense blue colour that can be read at 593 nm. Briefly, 1.5 mL of freshly prepared FRAP solution (25 mL of 300 mM acetate buffer pH 3.6, 2.5 mL of 10 mM 2,4,6-tripyridylstriazine (TPTZ) in 40 mM HCl, and 2.5 mL of 20 mM ferric chloride (FeCl3 · 6H2O) solution) was mixed with 1 mL of varied concentrations of the extracts (0.2 - 1.0 mg/mL). The reaction mixtures were incubated at 37 oC for 30 min and the absorbance was read at 593 nm. Ascorbic acid served as the control, while

$FeSO_4$ was used for calibration and values expressed as mmol $FeSO_4$ equivalents per gram of sample.

Nitric Oxide Radical Scavenging Capacity Assay

The method described by Makhija *et al.* [20] was used. Briefly, 1 mL of 10 mM sodium nitroprusside was mixed with 1 mL of extract prepared in phosphate buffer. The mixture was incubated at 25 °C for 150 min. To 1 mL of the incubated solution, 1 mL of Griess' reagent was added. Then, the absorbance was read at 546 nm. The % inhibition of nitric oxide radical was calculated as shown in Equation 2:

$$\text{Nitric oxide scavenging activity (\%)} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100 \quad \text{………………………… (2)}$$

Evaluation of Total Antioxidant Capacity

The TAC of the extracts was evaluated using the phosphomolybdenum method based on the procedure described by Prieto *et al*. [21]. The assay is based on the reduction of Mo (+6) to Mo (+5) by the extracts and subsequent formation of green phosphate Mo (+5) complex at acidic pH. Briefly, 0.3 mL of graded concentrations of extracts was mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against a blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract was used as the blank. The TAC was expressed as milligram equivalents of ascorbic acid and calculated as shown in Equation 3:

$$\text{TAC (mg AAE/g extract)} = \frac{C \times V}{m} \quad \text{…………………………………………………………. (3)}$$

Where c = concentration of ascorbic acid in mg/mL extrapolated from the standard calibration curve; V = volume of extract in mL; and m = weight of crude plant extract in grams.

STATISTICAL ANALYSIS

Data are presented as mean ± SEM. Statistical analysis was performed using SPSS (21.0). Statistical significance was assumed at $p < 0.05$.

RESULTS

In Vitro Antioxidant Activities of Extracts of *C. lanatus* seed

The DPPH radical scavenging activity, RP, FRAP, NO scavenging activity and TAC of the methanol extract of *C. lanatus* seed were significantly higher than those of the aqueous extract ($p < 0.05$; Figures 1 - 5).

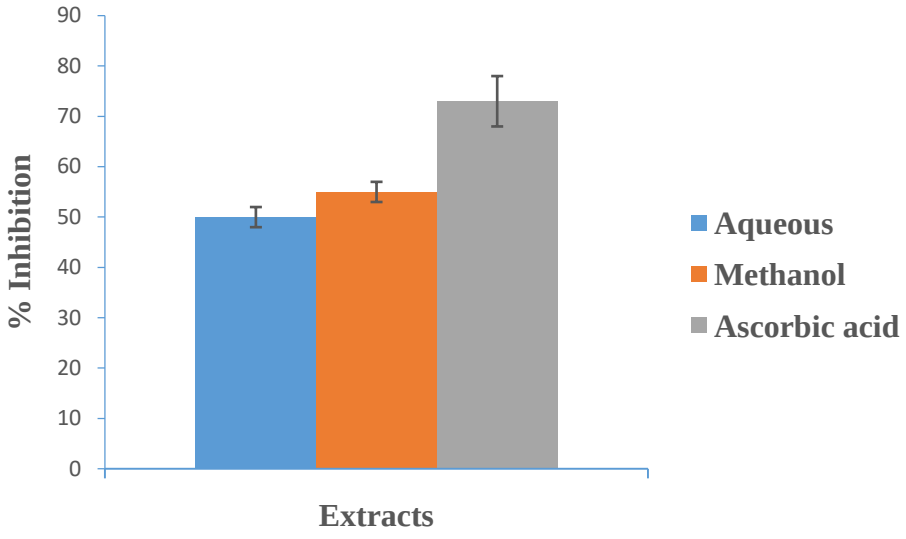

Figure 1: DPPH Scavenging Activity of Extracts of *C. lanatus* Seed

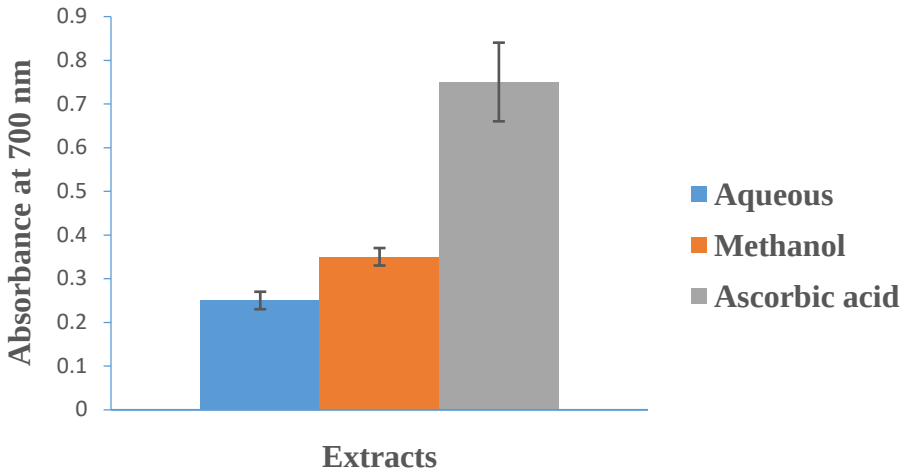

Figure 2: Reducing Power of Extracts of *C. lanatus* Seed

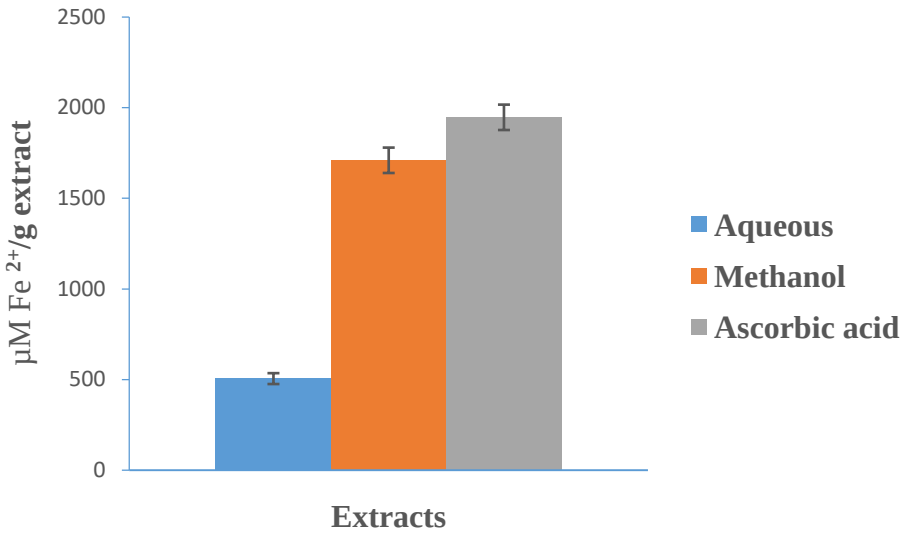

Figure 3: FRAP of Extracts of *C. lanatus* Seed

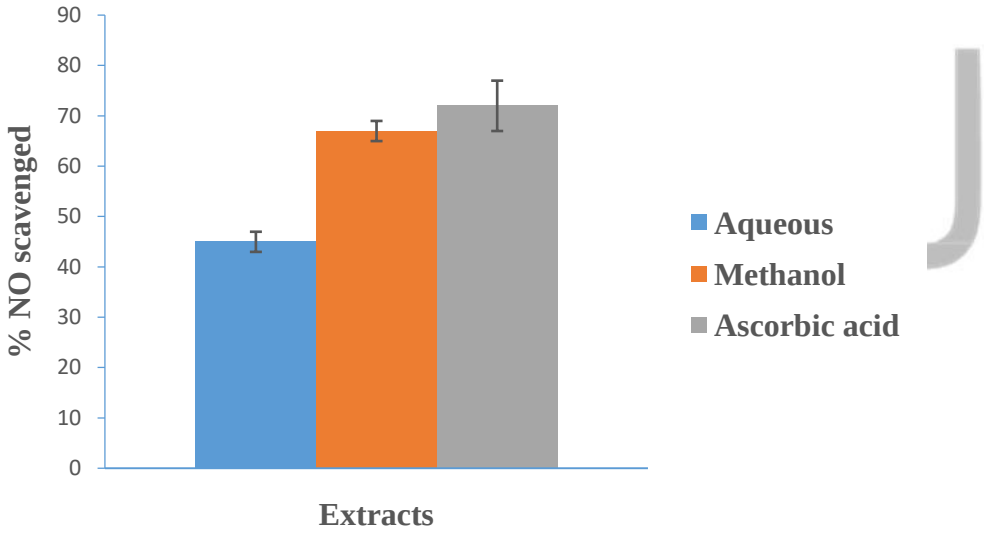

Figure 4: Nitric Oxide Scavenging Activity of Extracts of *C. lanatus* Seed

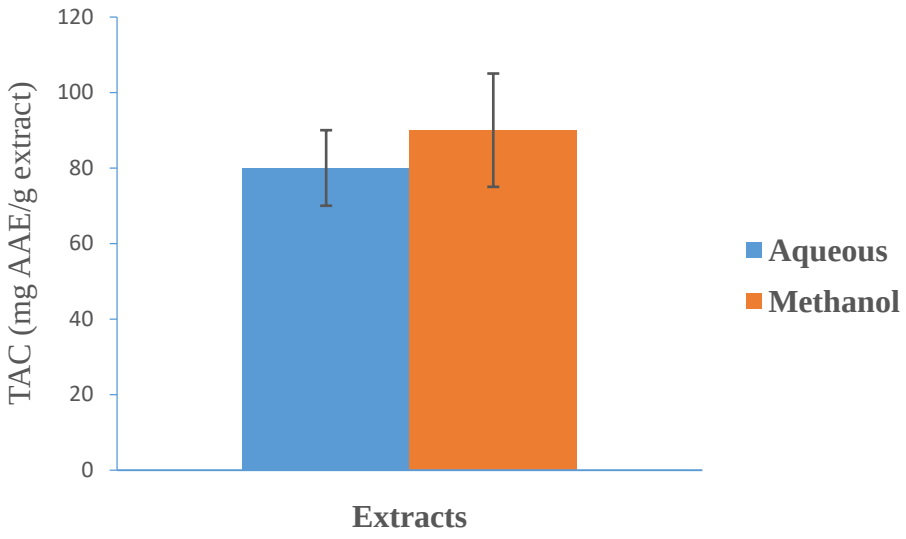

Figure 5: Total Antioxidant Capacity of Extracts of *C. lanatus* Seed

DISCUSSION

Free radicals are constantly formed in living cells and removed by antioxidant defenses. Antioxidant enzymes are the main line of defense against free radicals in animal and plant cells. When cells are exposed to oxidative stress a defense system ensures the expression and regulation of antioxidant enzymes as a defense mechanism to protect them from the damaging effect of free radicals. Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components [22]. They act by reducing the energy of the free radical or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt the oxidizing chain reaction to minimize the damage caused by free radicals. It has been reported that a substantial link exist between free radicals and more than sixty different health conditions, including aging, cancer, diabetes mellitus, Alzheimer's disease, strokes, heart attacks and atherosclerosis. By reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements, the body's potential to reducing the risk of free radical-related health problems is made more palpable [23]. The present study investigated the *in vitro* antioxidant activities of aqueous and methanol extracts of *C. lanatus* seed.

Antioxidants of nutritional origin play key roles in complementing *in vivo* antioxidant enzymes and molecules in the fight against free radicals. The DPPH radical can accept an electron or hydrogen ion to become a stable molecule [24]. Scavenging of DPPH radical is a widely used

method for evaluating the free radical scavenging ability of plant or chemical materials [25]. Phenols and flavonoids represent phytochemicals whose relative abundance in plant extracts has been linked to antioxidant effect [26, 27]. Reactive nitrogen species (RNS) are free radicals derived from the interaction of NO with oxygen or reactive oxygen species (ROS) [28]. Nitric oxide is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals such as superoxide anion [29]. Nitric oxide (NO) is generated from amino acid L-arginine by the enzymes in the vascular endothelial cells, certain neuronal cells, and phagocytes [30]. Low concentrations of NO are sufficient in most cases to effect the physiological functions of the radical. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation, and antimicrobial and antitumor activities [31]. Chronic exposure to NO radical is associated with various carcinomas and inflammatory conditions such as juvenile diabetes, multiple sclerosis, arthritis, and ulcerative colitis. The toxicity of NO increases greatly when it reacts with superoxide radical, forming the highly reactive peroxynitrite anion ($ONOO^-$) [29].

Phenolic compounds are antioxidant agents which act as free radical terminators. Nitric oxide has been shown to be directly scavenged by flavonoids [32].

Half maximal inhibitory concentration (IC_{50}) is a measure of the potency of a substance in inhibiting a specific biological or biochemical function. It is inversely proportional to antioxidant potential and hence a lower IC_{50} corresponds to higher antioxidant potential [33]. The results obtained in this study indicate that aqueous and methanol extracts of *C. lanatus* seed show great promise as important antioxidant molecules.

CONCLUSION

The results of this study suggest that phenolics present in the extracts of *C. lanatus* seed may play a role in free radical scavenging activities of the medicinal plant.

REFERENCES

1. Grabley, T. and Akuodor, C.W. (2010). Medicinal Plants and Traditional Medicine. Watermelon Crop Information. *Cucurbit breeding horticultural science.*

2. Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. *2nd Edition. Spectrum Books Ltd.*, Ibadan, Nigeria. Pp. 289.

3. Akah, P.A. and Nwabie, A.I. (1994). Evaluation of Nigerian traditional medicinal plants used for rheumatic inflammatory disorders. *Journal of Ethnopharmarcology.* 42: 179 - 182.

4. Obon, J.M., Diaz-Garcia, M.C. and Castellar, M R. (2011). Red fruit juice quality and authenticity control by HPLC. *Journal of Food Composition and Analysis*. 6: 760 – 771.

5. Rufino, M.S.M., Alves, R.E., de Brito, E.S., Perez-Jimenez, J., Saura-Calixto, F. and Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*. 121: 996 – 1002.

6. Ayala-Zavala, J.F, Vega-Vega, V., Rosas-Dominguez, C., Palafox-Carlos, H., Villa-Rodriguez, J.A., Siddiqui, M.W., Dávila-Aviña, J.E. and González-Aguilar, G.A. (2011). Agro-industrial potential of exotic fruit byproducts as a source of food additives. *Food Research International*. 44: 1866 – 1874.

7. Touhami, M., Laroubi, A., Elhabazi, K., Loubna, F., Zrara, I., Eljahiri, Y., Oussama, A., Grases, F., and Chait, A. (2007). Lemon juice has protective activity in a rat *Urolithiasis* model. *BMC Urol*ogy. 7 (1): 18.

8. Rimando, A.M. and Perkins-Veazie, P.M. (2005). Determination of citrulline in water melon rind. *Journal of Chromatography.* 1078 (1 - 2): 196 - 200.

9. Perkins, P.V., Collins, J.K., Angela, R.D. and Warren, R. (2006). Carotenoid content of 50 water melon cultivars. *Journal of Agricultural and Food Chemistry*. 54 (7): 2593 - 2597.

10. Bueno, P., Varela, J., Gime´nez-Gallego, G. and del Rıo, L.A. (1995). Peroxisomal copper,zinc superoxide dismutase: characterization of the isoenzyme from watermelon cotyledons. *Plant Physiology.* 108: 1151 – 1160.

11. Ercal, N., Gurer-Orhan, H. and Aykin-Burns, N. (2001). Toxic metal and oxidative stress part 1: mechanism involved in metal-induced oxidative damage. *Current Topics in Medicinal Chemistry.* 1 (6): 529 - 539.

12. Bawa, A.S and Bains G.S. (1977). Integrated processing of watermelons for juice and seed. *Indian Food Packer* 31 (6): 12 - 15.

13. Cho, E., Seddon, J.M., Roser, B., Willet, E.C. and Hankinson, S.E. (2004). Prospective study of intake of fruits, vegetables, vitamins and carotenoids and risk of age. *Maculopathy. 6:* 883 - 892.

14. Braide, W., Odiong, I.J. and Oranusi S. (2012). Phytochemical and Antibacterial properties of the seed of watermelon (*Citrullus lanatus*). *Prime Journal of Microbiology Research*. 2 (3): 99 - 104.

15. Jensen, B.D., Toure, F.M., Hamattal, M.A., Toure, F.A. and Nantoumé, D.A. (2011). Watermelons in the Sand of Sahara: Cultivation and use of indigenous landraces in the Tombouctou Region of Mali. *Ethnobotany Research and Applications.* 9: 151 - 162.

16. Abu, O.D., Imafidon, K.E. and Iribhogbe M.E. (2015). Biochemical effect of aqueous leaf extract of Icacina trichanta Oliv. On urea, creatinine and kidney oxidative status in CCl4-induced Wistar rats. *Nigerian Journal of Life Sciences*. 5 (1): 85 - 89.

17. Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*. 28 (1): 25 – 30.

18. Lai, L.S., Chou, S.T. and Chao, W.W. (2001). Studies on the antioxidant of Hsian-tsao (Mesona procumbems Heml) leaf gum. *Journal of Agricultural and Food Chemistry*. 49: 963 – 968.

19. Benzie, I.F.F. and Strain J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Analytical Biochemistry*. 239: 70 - 76.

20. Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry. 269 (2): 337 – 341.

21. Makhija, I., Chandrashekar, K.S., Lobo, R. and Jaykumar, B. (2011). Phytochemical and pharmacological profile of Leucas lavandulaefolia: A review. *Research Journal of Medicinal Plant*. 5 (1): 500 - 507.

22. Teixeira, H.D., Schumacher, R.I. and Meneghini, R. (1998). Lower intracellular hydrogen peroxide levels in cells overexpressing CuZn-superoxide dismutase. Proc Natl Acad Sci. 95: 7872 – 7875.

23. Grazioli, V., Schiavo, R., and Casari, E. (1998). Antioxidant enzymatic activities and lipid peroxidation in cultured human chondrocytes from vertebral plate cartilage. FEBS Lett. 431: 149 – 153.

24. Du, G., Li, M., Ma, F. and Liang, D. (2009). Antioxidant capacity and the relationship with polyphenol and vitamin C in Actinidia fruits. Food Chemistry. 113: 557 - 562.

25. Lee, S.E., Hwang, H.J., Ha, J.S., Jeong, H.S. and Kim, J.H. (2003). Screening of medicinal plant extracts for antioxidant activity. Life Science. 73: 167 - 179.

26. Ayoola, G. A., Folawewo, A. D., Adesegun, S. A., Abioro, O. O., Adepoju-Bello, A. A. and Coker, H. A. B. (2008). Phytochemical and antioxidant screening of some plants of Apocynaceae from South West Nigeria. African Journal of Plant Science. 2 (9): 124 – 128.

27. Padmanabhan, P. and Jangle, S.N. (2012). Evaluation of DPPH radical scavenging activity and reducing power of four selected medicinal plants and their combinations. International Journal of Pharmaceutical Sciences and Drug Research. 4 (2):143 – 146.

28. Tsai, P-J., Tsai, T-H., Yu, C-H. and Ho, S-C. (2007). Evaluation of NO-suppressing activity of several Mediterranean culinary spices. Food and Chemical Toxicology. 45 (3): 440 – 447.

29. Amaeze, O.U., Ayoola, G.A., Sofidiya, M.O., Adepoju-Bello, A.A., Adegoke, A.O. and Coker, H.A.B. (2011). Evaluation of antioxidant activity of Tetracarpidium conophorum (Müll. Arg) Hutch and Dalziel leaves. Oxidative Medicine and Cellular Longevity. 2011: 7.

30. Nagmoti, D.M., Khatri, D.K., Juvekar, P.R. and Juvekar, A.R. (2011). Antioxidant activity and free radical-scavenging potential of Pithecellobium dulce Benth seed extracts. Free Radical and Antioxidants. 2 (2): 37 – 43.

31. Bhaskar, V.H. and Balakrishnan, N. (2009). Analgesic, anti- inflammatory and anti- pyretic activities of Pergularia daemia and Carissa carandas. Daru Journal of Faculty of Pharmacy. 17 (3): 168 – 174.

32. Lakhanpal, P. and Rai, D.K. (2007). Quercetin: a versatile flavonoid. Internet Journal of Medical Update. 2 (2): 22 – 37.

33. Chanda, S., Dave, R. and Kaneria, M. (2011). In vitro antioxidant property of some Indian medicinal plants. Research Journal of Medicinal Plants. 5 (2):169 – 179.