Journal of Cell Biology and Biochemistry Research Vol. 4(1), pp. 01 3-019, February, 2020
Available online at http://www.apexjournal.org
ISSN 2315-8870© 2020 Apex Journal International

Full Length Research

Free radical and reactive oxygen species scavenging potentials of *Luffa cylindrica* leaf extracts

***Oluremi A. Saliu[1], Adewumi M. Akanji[2], Oluwafemi A. Idowu[3], and Biodun N. Saliu[4]**

[1]Department of Environmental Health Science, Faculty of Health Sciences, National Open University of Nigeria, Abuja, Nigeria
[2] Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria
[3]Department of Biochemistry, College of Natural and Applied Sciences, Oduduwa University, Ile-Ife, Osun State, Nigeria
[4]National Universities Commission, Abuja, Nigeria

Accepted 17 February, 2020; Published 20 February, 2020

Free radical and reactive oxygen species scavenging potentials of four solvent extracts of *Luffa cylindrica* leaf was evaluated *in vitro*. Leaves of *L. cylindrica* were extracted with distilled water (aqueous), methanol, ethylacetate, and hexane. The aqueous, methanolic and hexane extracts effectively scavenged 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in dose dependent manner with the methanolic extract producing the highest DPPH scavenging effect of 96% at a concentration of 50 mg/ml. Also aqueous and methanolic extracts of *L. cylindrica* leaf showed a dose dependent 2, 2-azino-**bis (3-ethylbenzthiazone-6-sulphonic (ABTS) scavenging activity producing 98% and 99% scavenging effect at 50 mg/ml respectively when compared with the synthetic antioxidant, butylated hydroxytoluene (BHT). The four extracts (aqueous, methanolic, ethylacetate and hexane) expressed significant activity ($p < 0.05$) for** total antioxidant **capacity, ferric ion reducing power and as well scavenged hydroxyl ion radical and superoxide anion reactive oxygen species when compared with ascorbic acid. These results establish Luffa cylindrica as an effective natural antioxidant agent and therefore accounts for the wide use of the plant in treating different ailments.**

Key words: Reactive oxygen species, Antioxidant, Ascorbic acid, Phenolics, *Luffa cylindrica*.

Introduction

Oxidative stress results from an imbalance between reactive oxygen species (ROS) and endogenous antioxidant system. ROS are molecules with unpaired oxygen electrons such as superoxide anion (O_2^-), hydroxyl radical (OH^-), singlet oxygen ($^1O_2$), Ozone (O_3) etc that induce physiological stress in the form of oxidative stress resulting into aging and in the damage of cells, tissues, DNA as well as the biological membrane. Reactive oxygen species are generated endogenously (in the living system) e.g. oxidative phosphorylation that takes place in the mitochondrion during normal body metabolism and exogenously from pollutants, drugs, xenobiotics, radiation etc. ROS when generated interact with other molecules in the body to gain stability by forming a radical chain which induces oxidative stress and thus subsequently causes the damage of vital cells and DNA in the body. ROS have been implicated in the pathogenesis of several diseases such as cancer (Kinnula and Crapo, 2004), diabetes (Pietta *et al*., 1998), cardiovascular diseases (Singh and Jialal, 2006), immune deficiency disease (Middleton *et al*., 2000) and other related diseases. Antioxidants however, are molecules capable of preventing the deteriorative effects caused by the ROS. They act by donating electrons to ROS in order to terminate their radical chain reactions and are therefore important in the prevention of human diseases. Antioxidants may also be beneficial in improving the wellbeing of life and may have a potential

*Corresponding author email: remisaliu@yahoo.com

for substantial savings in the cost of health care delivery. There are natural and synthetic antioxidants, the former is naturally present in food while the latter are industrially produced and are mostly used as food additive. Synthetic antioxidants such as butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA) etc. are suspected to be carcinogenic and are reported to have side effect (Namiki,1990). Natural antioxidants like ascorbic acid (Vitamin C), Vitamin E (tocopherol), carotenoids, lycopene, carotene and phenolic compounds (e.g. flavonoids) present in leafy vegetables, fruits, leaves, seeds and all parts of plant are safer, possess anti-cancer, anti-tumor, anti-mutagenic and hepatoprotective properties (Ajila *et al*., 2007). In view of this, there is high interest and demand in the use of natural antioxidants especially those of plants origin due to their presumed safety, nutritional and therapeutic value (Ajila *et al*., 2007). Researchers have therefore grown interest in examining plant extracts as a source of cheaper and effective antioxidants thus *Luffa cylindrica*.

Luffa cylindrica (L.) Roem commonly called sponge gourd, loofa, vegetable sponge, bath sponge or dish cloth gourd, is a member of *Cucurbitaceous* family well cultivated in Africa. In Nigeria, the local names of *L. cylindrica* are kan-kan oyinbo (Yoruba), Ahia mmala (Igbo), Ihion osa (Edo) and Sooso (Hausa). *L. cylindrica* has great medicinal values, with the leaves reported to have antiemetic and anti-inflammatory (Khan *et al*., 2013), analgelsic (Salman *et al*., 2013), hepatoprotective (Sharma *et al*., 2014), analgesic and antipyretic (Saliu *et al*., 2019) activities. In this study, the leaf of the plant was investigated for its free radical and reactive oxygen species scavenging potential.

Materials and Methods

Reagents

Butylated hydroxyl toluene (BHT), hydrogen peroxide, nitroblue tetrazolium (NBT) and α, α-diphenyl-β-picrylhydrazyl (DPPH) were product of Sigma Chemical Co., St. Loius, MO. All other reagents used were of analytical grade and commercially obtained.

Collection of Plant Sample

Fresh leaves of *Luffa cylindrica* were collected in Suleja, Niger State, Northern Nigeria. The authentication of the plant leaves was done at National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria with voucher number assigned and deposited in the herbarium of the institute.

Preparation of Leaf Extracts

The leaves of *L. cylindrica* were air-dried and pulverized with an electrical blender (Mazeda Mill, MT 4100, Japan). 250 g of the pulverized leaves was macerated in 2.5 liters of distilled water for 72 hours to give the aqueous extract. Another 250 g of the pulverized leaves was successively extracted with 2.5 liters each of hexane, ethyl acetate and methanol solvents. All the resulting extracts were filtered with Whatman No. 1 filter paper. The filtrates obtained were concentrated in a rotary evaporator (RE-300B model, China) at 65 – 70^{o}C and dried to a constant weight on a water bath to give a percentage yield of 23%, 13%, 10% and 4% for the aqueous, hexane, ethyl acetate and methanol extracts respectively.

In vitro Free Radical and Reactive Oxygen Scavenging Assay

1.1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

1.1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of the four extracts (aqueous, etylacetate, n-hexane and methanolic) were determined by the method described by Burits and Bucar (2000). Briefly, to 2 ml of different concentrations (10, 20, 30, 40 and 50 µg/ml) of the extract, 4 ml of 0.004% methanol solution of DPPH was added and the mixture was incubated for 30 minutes, the absorbance was read against a blank containing only methanol at a wavelength of 517 nm. Ascorbic acid was used as the reference antioxidant compound. The experiment was carried out in triplicates. DPPH radical scavenging activity was calculated as percentage inhibition (%I) using the expression:

$$\% I = (A_{control} - A_{sample} / A_{control}) \times 100$$

$A_{control}$ = absorbance of the control reaction (containing all reagents except the test compound).
A_{sample} = absorbance of the test compound, % I = Percentage Inhibition

Superoxide anion scavenging assay

The method described by Robak and Gryglewski (1980) was adopted to determine the superoxide anion scavenging activity of the four extracts. Briefly, superoxide anion radical was generated in a reaction mixture containing 0.5 ml of nitroblue tetrazolium (NBT) (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 500 µl (1 mg/ml) of the extract and 0.5 ml of Tris–HCl buffer (16 mM, pH 8.0). The reaction was initiated by adding 0.5 ml of phenazine methosulfate (PMS) solution (0.12 mM) to the mixture and incubated at 25^{o}C for 5 minutes. The absorbance was measured at 560 nm against a blank sample. L-ascorbic acid was used as the standard antioxidant compound. The percentage inhibition of superoxide generation was calculated from the

expression:

$\% I = (A_{control} - A_{sample} / A_{control}) \times 100$

$A_{control}$ = absorbance of the control reaction (containing all reagents except the test compound).
A_{sample} = absorbance of the test compound.
% I = Percentage Inhibition

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of the four solvent extracts was determined in a Fenton reaction (Fe^{3+}-Ascorbic acid- EDTA H_2O_2 system) adopting the method described by Kunchandy and Rao (1990). The reaction mixture (1.0 ml) consisted of 100 µl of 2-deoxyribose (28 mM in 20 mM KH_2PO_4-KOH buffer, pH 7.4), 500 µl of the different solvent extracts (1 mg/ml), 200 µl EDTA (1.04 mM) and 200 µl $FeCl_3$ (1:1 $^{v}/_{v}$), 100 µl of H_2O_2 (1.0 mM) and 100 µl ascorbic acid (1.0 mM) which was incubated at 37°C for 1 hour and then cooled. After cooling, 1 ml of thiobarbituric acid (1%) and 1.0 ml of trichloroacetic acid (2.8%) were added and further incubated for 20 minutes. The mixture was allowed to cool and absorbance was measured at 532 nm against a blank sample. Hydroxyl radical scavenging activity was calculated using the expression:

$\% I = (A_{control} - A_{sample} / A_{control}) \times 100$

$A_{control}$ = absorbance of the control reaction (containing all reagents except the test compound).
A_{sample} = absorbance of the test compound.
% I = Percentage Inhibition

2, 2-azino-bis (3-ethylbenzthiazone-6-sulphonic acid (ABTS) scavenging assay

The method described by Re *et al.* (1999) was used to determine the 2, 2-azino-bis (3-ethylbenzthiazone-6-sulphonic acid (ABTS) scavenging activity of the extracts. ABTS•+ was produced by adding 7 mM aqueous stock solution of ABTS+ to 2.4 mM potassium persulfate which was allowed to stand in the dark for 12-16 hours at 25°C (room temperature). The radical was stable in this form for more than two days when stored in the dark at room temperature. Prior to analysis, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to give an absorbance of 0.7000 ± 0.02 at 734 nm. Then, 2 ml of diluted ABTS•+ solution was added to 20 µl of the sample at varying concentrations (10, 20, 30, 40, 50 mg/ml) and incubated at room temperature. After 30 minutes of incubation, the absorbance was recorded at 734 nm and percentage of inhibition was calculated as stated in the previous section above. Butylated hydroxyl toluene was used as the reference antioxidant compound. The experiment was performed in triplicates.

Ferric reducing power assay

Ferric reducing antioxidant power of the extracts (aqueous, ethylacetate, n-hexane and methanolic) was determined by the method described by Oyaizu (1986). Briefly, 0.5 ml each of the extract and ascorbic acid (reference antioxidant compound) prepared at concentration of 5000 µg/ml were dissolved separately into 0.5 ml phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] in a test tube. The mixture was incubated at 50°C for 20 minutes. The reaction was terminated by the addition of 0.5 ml of trichloroacetic acid (TCA) and centrifuged at 3000 rpm (1500 x *g*) for 10 minutes. 0.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml ferric chloride. Absorbance was read at 700 nm. The experiment was carried out in triplicates. Increased absorbance of reaction mixture indicates increased reducing ferric power.

Statistical Analysis

Data were expressed as mean ± standard error of mean. Analysis of variance (ANOVA) was used to analyze data, followed by Dunnet's test for multiple comparisons. p-values < 0.05 were considered to be statistically significant.

Results

The phytochemical screening of the four extracts of *Luffa cylindrica* leaf is depicted in Table 1. The four extracts tested positive only to alkaloids while triterpenes, cardaic glycoside, flavonoids, phenolics, tannins and saponins were seen to be variably present among the extracts.

The four solvent extracts showed varying 1, 1-diphenyl-2-picryhydrazyl (DPPH) inhibition. The DPPH scavenging activities of ascorbic acid used as the reference antioxidant compound and the extracts (aqueous, methanolic and ethylacetate) were dose dependent with the aqueous, methanolic and hexane extracts exhibiting higher DPPH scavenging activity than ascorbic acid. The result showed that the hexane extract of *L. cylindtica* leaf exhibited the strongest inhibitory activity against DPPH at all concentrations (10, 20, 30, 40 and 50 mg/ml) while the ethylacetate extract showed the least inhibitory activity against DPPH radical at these concentrations (Table 2).

Data are mean determinant of three replicates ± SEM. (Values with different alphabets down the column are significantly different).

The aqueous and methanolic extracts among other extracts investigated scavenged 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical in dose-dependent manner. The two extracts (aqueous and methanolic) expressed the strongest tendency to

Table 1: Phytoconstituents present in solvent extracts of *Luffa cylindrica* leaf

Secondary Metabolites	Aqueous Extract	Mathanolic Extract Inference	Ethylacetate Extract	Hexane Extract
Saponins	+	+	-	-
Tannins	-	+	-	-
Anthraquinones	-	-	-	-
Triterpenes	-	+	+	+
Phenolics	+	+	-	-
Flavonoids	-	+	+	-
Alkaloids	+	+	+	+
Phlobatannins	-	-	-	-
Cardaic Glycosides	+	+	-	+

Keys: + = Detected, - = Not detected

Table 2: DPPH scavenging activity of solvent extracts of *Luffa cylindrica* leaf % Inhibition

Extracts 10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml
Aqueous	73.69 ± 1.02[a]	77.12 ± 1.04[a]	78.96 ± 1.05[a]	79.92 ± 1.02[a]	79.92 ± 1.02[a]
Methanolic	57.56 ± 1.01[b]	67.96 ± 1.02[b]	80.51 ± 1.01[a]	87.80 ± 1.01[b]	96.18 ± 1.01[b]
Ethylacetate	14.05 ± 1.01[c]	16.19 ± 1.03[c]	26.00 ± 1.02[b]	37.89 ± 1.02[c]	65.38 ± 1.04[c]
Hexane	89.60 ± 1.01[d]	96.36 ± 1.03[d]	97.37 ± 1.03[c]	98.80 ± 1.01[d]	89.24 ± 1.03[d]
Ascorbic acid	49.51 ± 1.01[e]	57.26 ± 1.02[e]	63.01 ± 1.01[d]	65.33 ± 1.01[e]	68.75 ± 0.04[e]

DPPH = 1, 1 diphenyl-2-picryhydrazyl

scavenge ABTS at the concentrations of 10, 20 and 60 mg/ml than butylated hydroxyl toluene (BHT). The strongest inhibition of ABTS radical was expressed by the methanolic extract of *L. cylindrica* leaf. On a contrary, the ethylacetate and hexane extracts expressed no scavenging activity against ABTS radical (Table 3).

Data are mean determinates of three replicates ± SEM. (Values with different alphabets down the column are significantly different).

The aqueous, methanolic and ethylacetate extracts exhibited a significant ($p < 0.05$) higher total antioxidant capacity (TAC) than the reference antioxidant compound (ascorbic acid). Among these three extracts, the methanolic extract had the highest TAC followed by the aqueous extract which also showed a promising capacity while the hexane extract showed the least total antioxidant capacity (Table 4). The four extracts (aqueous, methanolic, ethylacetate and hexane) exhibited scavenging activity against ferric ion radical. The ferric reducing antioxidant power was significantly ($p < 0.05$) higher in all the four extracts than as observed with ascorbic acid. The aqueous extract expressed the highest ferric reducing antioxidant power while the methanolic extract showed the least (Table 4). Aqueous and methanolic extracts showed a significantly ($p < 0.05$) lower hydroxyl radical scavenging activity compared with ascorbic acid. On the other hand, ethylacetate and hexane extracts showed a significantly ($p < 0.05$) higher hydroxyl radical scavenging activity when compared with ascorbic acid. The ethylacetate extract expressed the strongest hydroxyl radical scavenging activity (Table 4). The ability of the extracts to scavenge superoxide ion was significantly ($p < 0.05$) higher in methanolic, ethylacetate and hexane extracts when compared with the reference antioxidant compound (ascorbic acid). The aqueous extract showed the least superoxide ion radical scavenging activity than ascorbic acid and the other three extracts (methanolic, ethylacetate and hexane) (Table 4).

Data are mean determinates of three replicates ± SEM. (Values with different alphabets down the column are significantly different).

Table 3: ABTS scavenging activity of leaf extracts of *Luffa cylindrical* % Inhibition

Extracts	10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml
Aqueous	93.73 ± 1.02[a]	94.68 ± 1.01[a]	95.04 ± 1.01[a]	97.31 ± 1.01[a]	98.86 ± 1.01[a]
Methanolic	96.30 ± 1.01[b]	97.31 ± 1.03[b]	98.87 ± 1.01[b]	99.44 ± 1.02[b]	99.70 ± 1.02[a]
Ethylacetate	-	-	-	-	-
Hexane	-	-	-	-	
BHT	61.50 ± 1.02[c]	76.87 ± 1.02[c]	90.19 ± 1.03[c]	96.64 ± 1.01[a]	98.15 ± 1.01[a]

BHT = Butylated hydroxyltoluene, **ABTS =** 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid

Table 4: *In vitro* antioxidant activity of leaf extracts of *Luffa cylindrica*

Extracts	TAC (mg/100g)	FRAP (µg/100g)	OH^- activity (mg/100g)	O_2^- activity (µg/100g)
Aqueous	143.73±1.41[a]	498.97±1.27[a]	4.34±1.02[a]	19.14±1.28[a]
Methanolic	166.46±1.29[b]	376.43±1.30[b]	22.30±1.10[b]	120.88±1.26[b]
Ethylacetate	127.53±1.26[c]	490.84±1.09[c]	57.58±1.02[c]	90.90±1.06[c]
Hexane	79.01±1.11[d]	401.88±1.05[d]	39.85±1.01[d]	119.31±1.23[b]
Ascorbic acid	123.73±0.31[e]	318.27±1.04[e]	35.54±1.10[d]	84.75±1.28[d]

TAC = Total antioxidant capacity, **FRAP** = Ferric reducing antioxidant power, **OH^-** = Hydroxyl radical, **O_2^-** = Superoxide radical

Discussion

The variation observed in the secondary metabolites among the four extracts may be due to the polarity nature of the solvents used for extraction. The secondary metabolites reported in this study for the extracts of *Luffa cynidrica* are known to be free radical scavenging compounds. Phenolic compounds for example flavonoids and phenolics belong to a class of antioxidants that act as free radical terminators. The antioxidant activity of phenolic compounds is attributed to their redox property which makes them reducing agents, hydrogen donors and singlet oxygen quenchers (Hassan *et al*., 2009). Flavonoids have also been reported in several literatures to possess antioxidant activity (Farounbi *et al*., 2002) due to their metal ion chelating ability (Savita et al., 2011). Therefore their presence in plant or as food additive could help in preventing diseases caused by the free radicals. DPPH radical assay is a fast and widely used assay for assessing the antioxidant property of a compound. It is a stable radical characterized with a violet or purple colour which decolorizes in the presence of an antioxidant and the decrease in absorbance of an antioxidant agent under investigation for DPPH inhibition indicates high DPPH radical scavenging activity of the agent (Krishnaiah *et al*., 2012). The DPPH inhibition expressed by the extracts indicate their ability to act as reducing agents which could have been facilitated by the secondary metabolites present in the extracts particularly phenolics and flavonoids acting by donating hydrogen atom to the radical DPPH (1, 1-diphenyl-1, 2-picryhydrazyl) and converting it to ($DPPH\text{-}H^+$) α, α-diphenylhydrazyl, a non-radical form. Flavonoids and phenolics are well documented in literature to be responsible for the antioxidant activity expressed by most plants Visioli et al., 1998; Lamson and Brignall, 2005; Torres *et al*., 2006; Visioli *et al*., 1998). In ABTS radical scavenging assay, a blue/green $ABTS^+$ chromophore is generated as a radical from the reaction of ABTS with potassium persulfate and its reduction is measured spectrophotometrically at 745 nm in the presence of hydrogen–donating antioxidants. The ABTS radical inhibition exhibited by the methanolic and aqueous extracts may be due to the hydroxyl groups present

specifically in phenolics rather than flavonoids since the ethylacetate also contain flavonoids but did not express any scavenging activity towards ABTS. The use of butylated hydroxyl toluene (BHT) as a synthetic antioxidant reference compound in this study is to investigate if *L. cylindrica* has the potential to serve as a better substitutes to this synthetic antioxidant purported to be carcinogenic in food additives. Mechanism such as prevention of chain initiation, binding of transitional metal ion or decomposition of peroxide attributed to phenolics and flavonoids may be responsible for the total antioxidant capacity exhibited by the extracts (El-Mastas *et al*., 2006). Ferric ion take part in formation of hydroxyl radical in the Fenton reaction and can also lead to oxidative damage. Therefore preventing the oxidation process of ferric ion can help prevent further reactions that can induce oxidative damage. The reducing power exhibited by the extracts may be in conjunction with the phytoconstituents particularly phenolics and flavonoids present in the methanolic and aqueous extracts which act as reductants, reducing ferric ion from its radical form i.e. Fe^{3+} to a non radical form i.e. ferrous ion (Fe^{2+}). This observation is in line with Gordon, (1990) who reported that compound having reducing properties exert antioxidant action by donating hydrogen atom in order to break the free radical chain formed by reactive oxygen species. The hydroxyl radical scavenging activity exhibited by the extracts implies that the extracts could prevent damage associated with hydroxyl radical on cells and biological membranes. Hydroxyl radical is a powerful reactive oxygen species in the biological system that can penetrate into membrane and interact with the lipid moieties of the membrane in induce damage (Halliwell and Gutteridge, 1981; Khan *et al*., 2012). Superioxide anion (O^{2-}) radical serve as the major source of other free radicals like singlet oxygen (O^{-}), hydroxyl radical (OH^{-}) and hydrogen peroxide (H_2O_2) that induce oxidative stress in the biological system (Pietta 2000). The superoxide ion scavenging activity exhibited by the extracts is an indication that *Luffa cylindrica* leaf is capable of terminating radical chain reaction that can further result to production of more deadly reactive oxygen species that damage lipids, protein and DNA.

Conclusion

This study establishes Luffa cylindrica as an effective natural antioxidant agent which may accounts for the wide use of the plant in Africa for treating different ailments. This antioxidant activity exhibited by L. cylindrica leaf might be attributed to its phytochemical constituents particularly the phenolic compounds.

Conflict of interest

The authors declare that there are no conflicts of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Ajila, C.M., Naidu, K.A., Bhat, U.J.S., Rao, P. (2007). Bioactive compounds and antioxidant potential of mango peel extract. *Food. Chem.,* 105: 982-988.

Burits, M., Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res*. 14: 323-28.

El-Mastas M., Gulah, I., Isildak, O.O., Kufreviogu, I., Ibaoglu, K., Aboulenien, H.Y. (2006). Radical scavenging activity and antioxidant capacity of bay leaf extracts*. J. Iranian Chem Soc*. 3:258-266.

Farounbi, E.O., Akanni, O.O., Emerole, G.O. (2002). Antioxidant and scavenging activities of kolaviron *in vitro Pharm Bio.* 40: 107-116.

Gordon, M.H. (1990). The mechanism of antioxidant action *in vitro*. In Food antioxidants. Edited by Hudson BJ. London: *Elsevier J. App. Sci.,* pp.1-18.

Halliwell, B., Gutteridge, J.M.C. (1981). Formation of thiobarbituric acid reactive substances from deoxyribose in the presence of iron salts*:* the role of superoxide and hydroxyl radicals. *FEBS Lett*. 128: 347–352.

Hassan, S.M.R., Mokarram., H.M.D., Akter, R., Jamila, M., Ehsanul, H.M., Rahman, S. (2009). DPPH free radical scavenging activity of some Bangladesh medicinal plants. *J. Med. Plants. Res.,* 31(11): 875-879.

Khan, K.W., Ahmed, S.W., Ahmed, S., Hassan, M.M. (2013). Antiemetic, and anti-inflammatory activity of leaves and flower extracts of *Luffa cylindrica* (L). Roem. *J. Ethnobio and Trad Med. Photon.,* 118: 258-263.

Khan, R.A., Khan, M.R., Sahreen, S., and Ahmed, M. (2012). Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper (L.) Hill. Chem. Central. J.,* 6:12.

Kinnula, V.L., Crapo, J.D. (2004). Superoxide dismutases in malignant cells and human tumors. *Free Radic. Biol. Med*., 36: 718-744.

Krishnaiah, D., Sarbatly, R., and Nithyanandam, R.R. (2012). A review of the antioxidant potential of medicinal plant species. *Food. Bioprod. Process.,* 89: 217–233.

Kunchandy, E., and Rao, M.N.A. (1990). Oxygen radical scavenging activity of *curcumin*. *Int. J. Pharm*., 58: 237–240.

Lamson, D.W., Brignall, M.S. (2000). Antioxidants and Cancer III: Quercetin, *Altr. Med. Rev*., 5: 196-208.

Middleton, E., Kandaswamy, C., Theoharides, T.C. (2000). The effects of plants flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. *Pharmacol Rev*., 52: 673-751.

Namiki, M. (1990). Antioxidants/antimutagens in foods. *CRC Crit Rev Food. Sci. Nutr.,* 29: 273-300.

Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucose amine *CRC Crit. Rev. Food. Sci. Nutr.,* 44: 307–315.

Pietta, P., Simonetti, P. P. and Mauri, P. (1998). Antioxidant activity of selected medicinal plants. *J. Agric. Food. Chem.,* 46: 4487-4490.

Pietta, P.G. (2000). Flavonoids as antioxidants. *J. Nat. Prod*., 63(7): 1035-1042.

Re, R., Pellegrini, N. N., Proteggente, A., and Pannala, A.,Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolourisation assay. *Free Rad. Biol. Med.,* 26: 1231–1237.

Robak, J., Gryglewski, R.J. (1988). Flavonoids are scavengers of superoxide anions. *J. Biochem. Pharmaco.,* 37: 837–841.

Saliu, O.A., Akanji, M.A., Idowu, O.A. Saliu, N.B. (2019). Pharmacological evidence favouring the ethnomedicinal use of *Luffa cylindrica* leaf in the relief of pain and fever. *D. Res. J. Health. Pharmaco.,* 7(4): 38-46.

Salman, A., Kanwal, W.K., Syed, W.A. (2013). Analgelsic activity of leaves, flowers and fruits peel of *Luffa cylindrica* (L.) Roem. *Int. J. Adv. Pharma. Sci*., 4(6): 1401-1408.

Savita, S., Rao, D.V., and Sharma, R.A. (2011). *In vitro* and *in vivo* antioxidant activity and total phenolic content of *Pongamia pinnata (L) Pierre:* An important medicinal plant. *Int. J. Biotech.,* 4(6): 568-574.

Sharma, N.K., Keshari, P., Jha, K., Singh, H.K., and Shrivastava, A.K. (2014). Hepatoprotective activity of *Luffa cylindrica* (L) M.J. roem leaf extracts in paracetamol intoxicated rats. *Ind. J. Nat. Prod. Res.,* 5(2): 143-148.

Singh, U., and Jialal, I. (2006). Oxidative stress and atherosclerosis. *Pathophysiol.,* 13: 129-142.

Torres, R., Faini, F., Modak, B.., Urbina, F., Labbe, C., and Guerrero, J. (2006). Antioxidant activity of coumarins and flavonols from the resinous exudate of *Haplopappus multifolius*. *J. Phytochem.,* 67: 984-98.

Visioli, F., Bellomo, G., and Galli, C. (1998). Free radical-scavenging properties of olive oil polyphenols. *Biochem. Biophys. Res. Comm.,* 247: 60–64.