Covenant Journal of Physical & Life Sciences (CJPL) Vol. 9 No. 2, Dec 2021
ISSN: *print 2354 – 3574; electronics 2354-3485 DOI:*

An Open Access Journal Available Online

Assessment of Free Radical Scavenging Potency and In-vitro Antioxidant Analysis of *Ximenia caffra* (Sour Plum) Leaf

Jacob A.D. *[1], Dauda J.A.[1], Daniel O.A.[1], Nayo R.O.[1], Maji D.C.[1], Umar I.[1] and Wada H.A.[1]

Department of Pure and Industrial Chemistry, Kogi State University, Anyigba, Kogi State, Nigeria.

***Corresponding author: E-mail: jacob.a@ksu.edu.ng**
Telephone: +2348062750505, +2349054333270

Received: 06.05.2021; Accepted: 22.11.2021
Date of Publication: December, 2021

Abstract: This study is focused on the determination of the free radical scavenging potency and in-vitro antioxidant analysis of extracts of *Ximenia Caffra* (sour plum) leaf, (using methanol, chloroform and n-hexane as solvent. About 50.0g of the powdered samples was weighed into a beaker and methanol (300 mL) was added into it. The same process was repeated for chloroform and n-hexane extract. The DPPH free radical scavenging assay were carried out using ascorbic acid as the positive control. The results of the analysis at 50, 100, 200, 400 and 800 µg/mL shows the following % inhibition: (40, 50, 65, 80, and 91), for the methanolic extract, (16, 18, 26, 29, and 33), for the chloroform extract and (13, 17, 21, 32, and 55), for the n-hexane extract. The IC_{50} (µg/mL) values revealed 71.83 for the methanol extract, 1500 for the chloroform extract, and 715.05 for the n-hexane extract. From the result, it can be concluded that sour plum leaves can be used in the treatment of certain diseases such as diabetes, hypercholesterolemia, insomnia, and liver problems in which the participation of reactive oxygen species have been implicated.
Keywords: *Ximenia caffra*, free radical, Scavenging, Potency, Antioxidant.

1.0 Introduction:

The oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species (ROS). However, ROS may also be very damaging, they can attack the lipids of cell membranes and DNA. The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases [1].

ROS are continuously produced during normal physiologic events and are removed by antioxidant defense mechanisms [2]. It is well known that ROS are closely involved in various human diseases such as Alzheimer's disease, aging, cancer, inflammation, rheumatoid arthritis and atherosclerosis [3]. It is commonly recognized that antioxidants can neutralize potentially harmful reactive

free radicals in body cells before they cause lipid and protein oxidation and may reduce potential mutation and therefore, help prevent cancer or heart diseases [4].

In living cells, ROS are continuously produced in numerous processes such as mitochondrial respiration, metabolism of Xenobiotics by cytochromes P450, inflammation, and phagocytosis. Exposure to UV and gamma radiation also generates ROS. ROS damage cellular macromolecules (lipids, proteins, nucleic acids) leading to oxidative stress [5].

It has been demonstrated that oxidative stress is involved in many diseases such as cardiovascular diseases, rheumatoid arthritis, neurodegenerative diseases, alcoholic and non-alcoholic steatohepatitis, diabetes mellitus, and cancer. As the incidence of these diseases is constantly increasing the research in the field of natural and synthetic antioxidants is still of high interest [6].

Numerous in vitro and in vivo studies have reported that polyphenolic compounds protect against oxidative stress. Some of these medicinal plants used in ethno medicine for the treatment and management of many of these diseases have been investigated for their antioxidative properties. Many of the metabolites from these medicinal plants especially flavonoids exhibited potent antioxidant activity in vitro and in vivo [7]. Oyedemi and Afolanya [8] investigated the in vivo and in vitro antioxidant activities of the aqueous leaves extract of *Leonotis leonurus* (L.) R.Br and found out that the leaf is a potential source of antioxidant and thus could prevent many radical related disease. Jamuna et al [9] analyzed the qualitative and quantitative phytochemicals and evaluated the in vitro antioxidant properties of various alcoholic and aqueous extracts of leaf and root parts of *Hypochaeris radicats*, and found that the in vitro antioxidant activities of the species clearly demonstrated that both the leaf and the root parts have prominent antioxidant properties. Islam et al [10] evaluated the in vivo and in vitro, pharmacological activities of *Ardisia solanacea* leaf extract and found out that different fractions of *A. solanacea* showed significant pharmacological potentiality in different in vitro and in vivo study model; Hence this study is keen on investigating the free radical scavenging ability and in vitro antioxidant analysis of sour plum (*Ximenia caffra*) leave extract.

Currently, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) are widely used in the food industry. However, restriction on the synthetic antioxidants is being imposed because of their toxicity to liver and carcinogenicity [11]. The phenolic constituents of plants are of interest as potential chemo preventive agents and plants may be an attractive alternative to currently available commercial antioxidants because they are biodegradable to non-toxic products [12]. Therefore, the development and utilization of more effective antioxidants of natural origins are desired. [13].

The sour plum tree grows at altitudes up to about 200 m where rainfall exceeds 500 mm per year on many soil types and that are often poor and dry. The flowers are unisexual and male and female flowers occur on different plants. The branches are either smooth or covered with flattened hairs and armed with spines at their bases. The bark of the tree is grayish-brown to black in color, and is longitudinally fissured. The leaves are simple, alternate, and elliptic in shape. When the tree flowers, the flowers are greenish to creamy white in color although they have been seen to be sometimes tinged pink or red. The roots are

used to treat abscesses, stomach aches, colic, malaria, coughs, and bilharzias. They can also be pounded, turned into porridge and eaten to reportedly prevent sterility in women [14]. It is thought that powdered roots can also be added to beer to act as an aphrodisiac. The tree's bark is used as a remedy for syphilis, hookworm, chest pains, and body pain. The seeds are generally roasted and then pounded for their oils to be used for wounds as an ointment. The leaves can be used to soothe inflamed eyes and has been reported to cure tonsillitis [15], the leaf extracts also show antimicrobial activities [16], and are rich in phytochemicals [17]. Most of these claims do not appear to have been scientifically validated, and further research is required. In view of these, this study is aimed at evaluating the free radical scavenging ability and in vitro antioxidant analysis of sour plum leaf extract.

2.0 Methods

2.1 Sample preparation and handling

The sour plum leaf samples were collected and washed with water to remove dirt; it was further air dried in the laboratory for 2 weeks under a minimum temperature of 40°c. The dried samples of leaves were later pulverized using electric blender to obtain a fine powder.

2.2 Extraction method

The method of extraction was adopted from Jacob et al [17]. About 50.0 g of the powdered samples was weighed into a beaker and methanol (300ml) was added into it. The same process was repeated for chloroform and n-hexane extract the beaker was then covered tightly with aluminum foil to avoid evaporation of the solvent. After 72 hours, they were then filtered using a filter paper and the filtrate was allowed to evaporate to dryness using a water bath at 45°C.

Percentage Yield for Methanol

$$= Mass\ of\ Sample + Mass\ of\ Beaker - Mass\ of\ Sample\ [Beaker\ (102g) + Extracted\ Sample\ (25g)]$$

2.3. DPPH radical scavenging activity

The DPPH radical scavenging assay was modified according to a method reported by Adesegun et al. [18]. The DPPH (0.1mM) working solution diluted with ethanol (150µL) was added to the sample (50µL). After 30 minutes in the dark, change in absorbance (decrease) at 515 nm was measured using a spectrophotometer. The DPPH radical scavenging effect was calculated using the following equation.

DPPH radical scavenging activity

$$(\%) = \frac{(Ac - As)}{Ac} \times 100 \quad (3.1)$$

Where: A_c is the absorbance of the control

A_s is the absorbance of the sample.

2.4. Qualitative Determination of Free Radical Scavenging Compounds Using DPPH

The presence of free radicals scavenging compound of *Ximenia caffra* was observed in n-hexane, chloroform of methanol [18]. Different concentration of each plant extract (5, 10, 15, 20 mg/ml) was spotted on a TLC plate and allowed to dry and the TLC plate was immersed in a freshly prepared DPPH solution (5.91mg DPPH in 50ml methanol).

2.5. Calculation of IC50

The IC50 values of all the extracts were determined using graphical methods [19]. In this approach, the inhibition (%) was plotted on the Y-axis directly against extract concentration on the X-axis. The IC50 values were gotten from the graph by extrapolation.

3.0 RESULTS AND DISCUSSION

3.1. Results

Table 1: DPPH assay for methanolic extract

Concentration of test sample (µg/ml)	% Inhibition	IC_{50}
50	40	71.83
100	50	
200	65	
400	80	
800	91	

Table 2: DPPH assay for chloroform extract

Concentration of test sample (µg/ml)	% Inhibition	IC_{50}
50	16	1500
100	18	
200	26	
400	29	
800	33	

Table 3: DPPH assay for n-hexane extract

Concentration of test sample (µg/ml)	% Inhibition	IC_{50}
50	13	715.05
100	17	
200	21	
400	32	
800	55	

Table 4: DPPH assay for Vitamin C

Concentration of test sample (µg/ml)	% Inhibition	IC_{50}
50	45	16.38
100	55	
200	68	
400	81	
800	98	

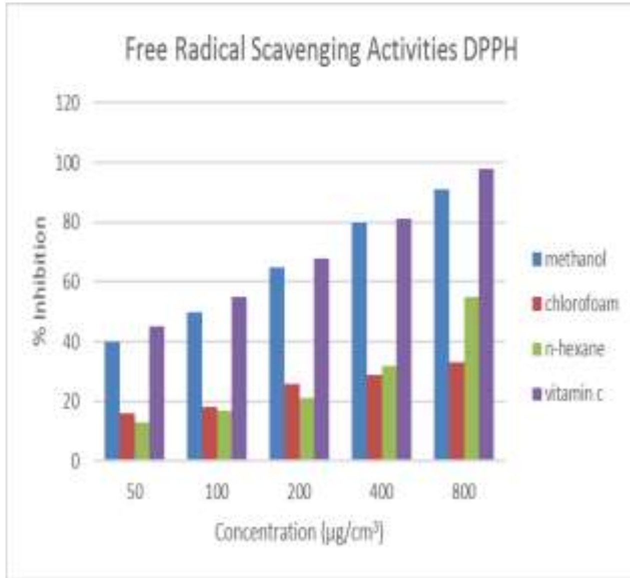
Table 5: Free Radical Scavenging Activity of the Samples Using DPPH

S/N	Concentration (µg/cm^3)	% Inhibition of Methanolic extract	% Inhibition of chlorofoam extract	% Inhibition of n-hexane extract	% Inhibition of vitamin C
1	50	40	16	13	45
2	100	50	18	17	55
3	200	65	26	21	68
4	400	80	29	32	81
5	800	91	33	55	98

Table 6: IC_{50} values of the extracts

Extracts	IC50(µg/mL)
Methanolic extract	71.83
Chlorofoam extract	1500
n-hexane extract	715.05
Vitamin C	16.38

Figure 1: Showing the Free Radical Scavenging activity of the extracts



Qualitative Determination of Free Radical Scavenging Compounds Using DPPH

Plate 1: Showing the Qualitative Analysis of Methanol Extract

Plate 2: Showing the Qualitative Analysis of Chloroform Extract

Plate 3: Showing the Qualitative Analysis of n-hexane Extract

3.2. Discussion

The presence of free radical scavenging compounds was detected in all three extracts. However, that the methanolic extract has a very high percentage (%) inhibition at a concentration of 800µg/ml.

The in vitro free radical scavenging activity of the formulation (Table 5 and Figure 1), revealed that, the free radical scavenging potential of the extracts are concentration dependent. The methanolic extract demonstrated the highest free radical scavenging potential (at all concentrations) when compared with the standard (Ascorbic acid). Also, the n-hexane extracts show high potency (at 800 µg/mL) when compared with the standard. The IC50 values (Table 6) revealed the methanolic extract to be more potent than the chloroform and n-hexane extracts. Methanolic extract had IC50 value of 71.83 µg/mL. The lower the IC50 value the more potent is the extract.

Plates 1, 2 and 3, show the qualitative analysis of the methanolic, chloroform and n-hexane extracts, respectively, and it is observed that the extracts showed yellow color on the TLC plate, and according to Halilu et al. [20], free radical scavenging compounds show yellow or white spot on the TLC plate, hence the chloroform and methanolic extract show high potency as revealed.

4.0. Conclusion

The in vitro free radical scavenging activity of the plant extracts from methanol, n-hexane and chloroform, revealed that, the free radical scavenging potential of the extracts are concentration dependent. The methanolic extract demonstrated the highest free radical scavenging potential (at 50, 100, 200, 400 and 800 µg/ml respectively) when compared with the standard (Ascorbic acid). The IC_{50} values revealed that the methanolic extract is more potent than the chloroform and n-hexane extract. The methanolic extract had IC_{50} value of 71.83 µg/ml. The lower the IC_{50} value the more potent is the extract.

From the result it is justified that sour plum leaves can be used in the treatment of certain diseases such as diabetes, hypercholesterolemia, insomnia, and liver problems in which the participation of reactive oxygen species have been implicated. This is as a result of the antioxidant and free radical scavenging ability of sour plum leaves.

References

[1]. Gulcin, I., Oktay, M., Kirecci, E. and Kufrevioglu, O. I. (2003). Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L.) seed extracts. *Food Chemistry*, **83**,371–382.

[2]. Gulcin, I., Oktay, M., KufrevioÛlu, O. I. and Aslan, A. (2002). Determination of antioxidant activity of lichen Cetraria islandica (L) Ach. *Journal of Ethnopharmacology*, **79**,325-329.

[3]. Funda, S. P. and Hakan, G. (2008). The role of free radicals in ethiopathogenesis of diseases. *Advances in Molecular Biology*, **1**: 1-9.

[4]. Valento, P., Fernandes, E., Carvalho, F., Andrade, P. B., Seabra, R. M. and Bastos, M. L. (2002). Antioxidant activity of Hypericum androsaemum Infusion: Scavenging Activity against Superoxide Radical, Hydroxyl Radical and Hypochlorous Acid. *Biological Pharmaceutical Bulletin*, **25**,1320-1323.

[5]. Krishna, B., Prema, G., Jaideep, M., Jayamathi, K. M., Cherian, C. and Ram, C. (2013). Antioxidant and free radical scavenging activity of curcumin determined by using different in vitro and ex vivo models. *Journal of Medicinal Plants*, **17**(3),2-5.

[6] Powell, C. L., Swenberg, J. A. and Rusyn, I. (2005). Expression of base excision DNA repair genes as a biomarker

of oxidative DNA damage. *Cancer Letter*, **229**,1–11.

[7]. Sofidiya, M. O., Odukoya, O. A., Familoni, O. B. and Inya-Agha, S. T. (2006). Free radicals scavenging activity of some Nigerian medicinal plant extracts. *Pakistan Journal of Biology and Science*, **9**,1438-1441.

[8] Oyedemi, S. O. and Afolayan, A. J. (2011). In vitro and in vivo antioxidant activity of aqueous leaves extract of Leonotis leonurus (L.) R.Br. *International Journal of Pharmacology*, **7**(2),248-256.

[9] Jamuna, S., Subramaniam, P. and Krishnamoorthy K. (2014) Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. *Asian Pacific Journal of Tropical Biomedicine*, **4**(1),359-367.

[10] Islam, M. R., Naima, J., Proma, N. M., Hussain, M., Uddin, S. M. and Hossain, M. K. (2019). In-vivo and in-vitro evaluation of pharmacological activities of Ardisia solanacea leaf extract. *Clinical Phytoscience*, **5**(1),1-11.

[11]. Lim, S. N., Cheung, P. C. K., Ooi, V. E. C. and Ang, P. O. (2002). Evaluation of antioxidative activity of extracts from a brown seaweed, Sargassum siliquastrum. *Journal of Agriculture and Food Chemistry,* **50**,3862-3866.

[12]. Kayano, S., Kikuzaki, H., Fukutsuka, N., Mitani, T. and Nakatani, N. (2002). Antioxidant activity of prune (Prunus domestica L.) constituents and a new synergist. *Journal of Agriculture and Food Chemistry*, **50**,3708-3712.

[13]. Luo, X. D., Basile, M. J. and Kennelly, E. J. (2002). Polyphenolic antioxidants from the fruit of Chrysophyllum cainito L. (star apple). *Journal of Agriculture and Food Chemistry*, **50**,1379–1382.

[14]. Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Simons, A. (2009). Agroforestree Database: a tree reference and selection guide version. **4**.

[15]. Baloyi, J. K., and Yvonne R. (2004). "*Ximenia Caffra*." National Biodiversity Institute.

[16] Jacob, A. D., Ogaji, O. D., Okonkwo, V. I., Abdullahi, A. S., Ocheme, G. W., Ejim, C. C. and Idris, B. A. (2021). Assessment of the Antimicrobial Activities of Ximenia caffra (sour plum) leaf. *International Journal of Science for Global Sustainability*, **7**(2),128-133.

[17] Jacob, A. D., Dauda, J. A., Idowu, O. L., Adaji, M. U., Akeji, S. A., Ejim, C. C. and Idowu, O. M. A. (2021). Comparative Assessment of the Phytochemical Composition of Ximenia caffra (sour plum) leaf. *Arabian Journal of Chemical and Environmental Research*, **8**(2),418-435

[18] Adesegun, S. A., Elechi, N. A. and Coker, H. A. (2008). Antioxidant activities of methanolic extract of Sapium ellipticum. *Pakistan Journal of Biological Sciences*, **11**(3),453-457.

[19] Bulus, T., Ahmed, A. B., Aboi, T. Y. and Danbaki, D. A. (2016). Determination of IC50 and IC90 values of ethanolic extracts of some medicinal plants against Trypanosoma brucei brucei. *Archives of Clinical Microbiology*, **7**(3),1-5.

[20] Halilu, M. E., Akpulu, I. K., Agunu, A., Ahmed, A. and Abdurahman, E. M. (2008). Phytochemical and antibacterial evaluation of Parinari curatetellifolia planch ex benth (chrysobalanaceae). *Nigerian Journal of Basic and Applied Sciences*, **16**(2),281-285.