IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 11, Issue 1 Ver. I (January. 2018), PP 13-18
www.iosrjournals.org

Comparative Antioxidative and Cytotoxic Activity of Extracts of Moringa Oleifera and its Mistletoe

Abdullahi Garba Usman[1], H.M Ammani[2], Shamsu.S Bala[3]
([1,2]*Near East University, Cyprus)*
([3]Bayero University kano, Nigeria)
Corresponding Author: Abdullahi Garba Usman

Abstract:*This work is aimed to compare the cytotoxic activity of Moringa oleifera and viscum cruciatum (Mistletoe) plants using brine shrimps utility technique. it also compares the anti-oxidative activity of the plants using DPPH radical scavenging assay. 100g of powdered samples were percolated with 96% ethanol. The extracts were macerated with petroleum ether, ethyl acetate and methanol respectively. The petroleum ether fraction of Viscum cruciatum and that of Moringa oleifera showed higher activity to brine shrimp lethality test wit LC50 values of 99.459 and145.684 respectively, while that of methanol fraction of Viscum cruciatum and that of Moringa oleifera showed fair activity to brine shrimp lethality test with LC50 values of 471.220 and 573.893. While, the methanol extract of Viscum cruciatum and that of Moringa oleifera showed higher activity in DPPH radical scavenging assay with IC50 values of 22.482 and 10.857 respectively, while that of crude ethanol extract of Viscum cruciatum and ethyl acetate fraction of Moringa oleifera showed moderate and fair activity to DPPH radical scavenging assay with IC50 values of 263.77 and 582.023 respectively.*

Keywords: *Anti-oxidative activities, Brine shrimp lethality Bioassay, Cytotoxic activity, DPPH, Moringa Oleifera, Mistletoe.*

Date of Submission: 30-12-2017 Date of acceptance: 16-01-2018

I. Introduction

In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries, because herbal medicines have been reported safe without any adverse side effect especially when compared with synthetic drugs. Thus, searches for a new drug with better and cheaper substituents from plant origin are a natural choice. The medicinal value of those plants lies in some chemical substances that produce a definite physiological action on human body [1].

In some countries, herbal medicines are still a central part of the medicinal system [2-4]. Medicinal plants have been used in Africa before the introduction of antibiotics and other modern drugs [5]. *Moringa oleifera* (Moringaceae) is one of the 14 species of family *Moringaceae*, native to India, Africa, Arabia, Southeast Asia, South America, and the Pacific and Caribbean Islands [6]. Literature revealed that the Moringa tree was introduced to Africa from India at the turn of the twentieth century where it was to be used as a health supplement [7]. The Moringa plant has been consumed by humans throughout the century in diverse culinary ways [8]. Almost all parts of the plant are used culturally for its nutritional value, purported medicinal properties and for taste and flavor as a vegetable and seed. The leaves of *M. oleifera* can be eaten fresh, cooked, or stored as a dried powder for many months reportedly without any major loss of its nutritional value [9]. Moringa leaf has been purported to be a good source of nutrition and a naturally organic health supplement that can be used in many therapeutic ways [10]. The leaves are a very rich source of nutrients and contain the essential vitamins A, C and E. Though not proven, it is has been considered by many to contain as much vitamin A as a carrot, vitamin C as an orange and vitamin E as a pomegranate. Leaves rich in biologically active carotenoids, tocopherols and vitamin C have health promoting potential in maintaining a balanced diet and preventing free-radical damage that can initiate many illnesses [11]. While the provitamins cannot be identified in the leaves, they can be monitored after conversion to their respective vitamins within the body. The edible Moringa leaves contain essential provitamins, including ascorbic acid, carotenoids [12] and tocopherols. Epidemiological studies have indicated that *M. oleifera.* While its *mistletoe (Viscum Cruciatum)* commonly called the red-berry mistletoe is specie of mistletoe in the family santalaceae. The plant has small leaves. The flowers have four petals. The barriers are red containing 1 seed. It ranges through south west Spain, southern Portugal, North Africa, Australia and Asia. All parts of the plants are poisonous if eaten. Its fruits are harmless to birds which disperse the seeds. It is also used as Christmas decoration [13]. *Viscum cruciatum* has been used to treat cardiovascular diseases (heart diseases). Also crude vascum cruciatum herb is used to make tea to treat hypertension at a dosage of 10g/day. It is also used to treat cancer [14].

II. Materials And Methods

2.1 Chemicals

Distilled water, ethanol, petroleum ether, ethyl acetate, methanol, dimethyl sulphoxide (DMSO), sea water,brine shrimp eggs,1,1 diphenyl-2 picrylhydrazyl (DPPH), Butylated hydroxytoluene (BHT) and Ascobic acid (Vit. C) were obtained from Bayero University Kano, Nigeria.

2.2 Collection of plant sample

Plant samples of *Moringa oleifera* and its mistletoe (*viscum cruciatum*) were collected from D/kudu of Kano state, Nigeria. After complete cleaning and rinsing with water, leafs were air dried for fourteen days. The dried leafs were then grinded in coarse powder, which was then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

2.3 Extraction procedure

The powdered plants materials were successively extracted using 200ml of distilled Ethanol which was followed by petroleum ether, ethyl acetate, and methanol. All extracts were filtered individually through filter paper and poured on petri dishes to evaporate the liquid solvents from the extract to get dry extracts. After drying crude extracts were weighed and stored in stock vials and kept for further use.

2.4 DPPH Free Radical Scavenging Assay

The free radical scavenging capacity of the extracts was determined using DPPH radical scavenging activity bioassay [15]. Freshly prepared DPPH solution was taken in test tubes and extracts were added followed by serial dilutions (500µg/ml to 10µg/ml) and were incubated in dark with 200µM solution of DPPH in methanol and after 30 min, the absorbance was read at 517 nm using a spectrophotometer. Ascorbic acid and Butylated hydroxy toluene (BHT) was used as standard. Control sample was prepared containing the same volume without any extract and standard and the absorbance was read at 517 nm using a spectrophotometer. Methanol was served as blank.Three drop of DMSO and sea water (4ml) were added to each 1000ug/ml, 100ug/ml, and 10ug/ml concentrations respectively, each dosage was tested in triplicate. Using dropper, 10 larvae of *artemia salina* were introduced into each of the sample vials containing the mixture above. More sea water was added to each sample vials in order to make the volume of its content exactly 5ml. the larvae were allowed to stay in this sample vials for about 24 hours after which the survivals were recorded [16].

2.5 Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds [17]. The brine shrimp, *Artemia salina*, was used as a convenient monitor for the screening. The eggs of the brine shrimp, *A. salina*, were collected from an aquarium shop (Lagos, Nigeria) and hatched in seawater for 48hrs to mature shrimp. The cytotoxicity assay was performed on brine shrimp using Meyer method. The test samples (extract) were prepared by dissolving in Three drop of DMSO and sea water (4ml) were added to each 1000ug/ml, 100ug/ml, and 10ug/ml concentrations respectively, each dosage was tested in triplicate. Using dropper, 10 larvae of *artemia salina* were introduced into each of the sample vials containing the mixture above. More sea water was added to each sample vials in order to make the volume of its content exactly 5ml. the larvae were allowed to stay in this sample vials for about 24 hours after which the survivals were recorded [18].

III. Results And Discussions

The free radical scavenging activity of different extracts of *Moringa Oliefera* leaf was studied by its ability to reduce the DPPH, a stable free radical and any molecule that can donate an electron or hydrogen to DPPH, can react with it and thereby bleach the DPPH absorption. DPPH is a purple colour dye having absorption maxima of 517 nm and upon reaction with a hydrogen donor the purple colour fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance. The petroleum ether, ethyl acetate and methanol extracts showed activity, whereas ascorbic acid and BHT at the same concentration exhibited 96.66% and 92.59 % inhibition respectively. Five extracts exhibited considerable DPPH free radical scavenging activity as indicated by their IC50 values and this has been showed in (Table 3.1). While table 3.2 shows IC50 values of different extracts of *Viscum cruciatum* in DPPH scavenging.

Table 3.1: %Scavenging activity of *Moringa oleifera* extracts

Concentration (µg/mol)	Crude	Methanol	Ethyl acetate	Pet. Ether
500	93.87	94.17	54.37	90.50
250	92.56	95.24	39.31	65.55
100	75.85	90.987	36.56	47.15
50	63.667	87.50	31.57	34.46

25	51.34	60.70	29.83	24.38
10	37.98	45.65	25.81	18.62

Table 3.2: %Scavenging activity of *Viscum cruciatum* extracts

Concentration (µg/mol)	Crude	Methanol	Ethyl acetate	Pet. Ether
500	43.56	92.75	93.65	83.53
250	26.16	91.42	92.06	71.65
100	20.72	71.74	90.83	47.70
50	16.53918	64.60	64.64	25.73
25	14.33	94.59	50.78	20.82
10	14.52	38.03	26.27	25.13

Table 3.3 Results values of IC50 OF *Moringa OLEIFERA*

Fractions	IC50 (µg/ml)
F-CE	21.57
F-MEOH	10.857
F-EA	582.023
F-PE	87.719

Table3.4 Results values of IC50 OF *Viscum cruciatum*

Fractions	IC50 (µg/ml)
F-CE	263.770
F-MEOH	22.482
F-EA	24.352
F-PE	94.397

COMPARISON CHART FOR THE RESULTS OF ANTIOXIDANT ASSAY

NOTE: The higher the IC50 value the lower the activity

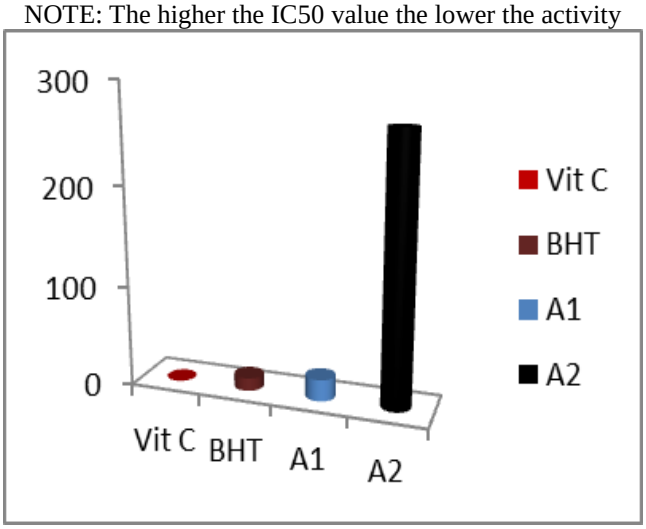

Figure 3.1

KEY: A1= Ethanolic extract of *M.Oleifera* A2= Ethanolic extract of *V. Cruciatum*

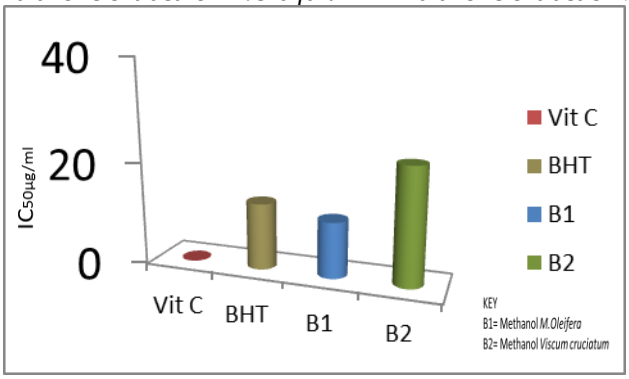

FIGURE 3.2

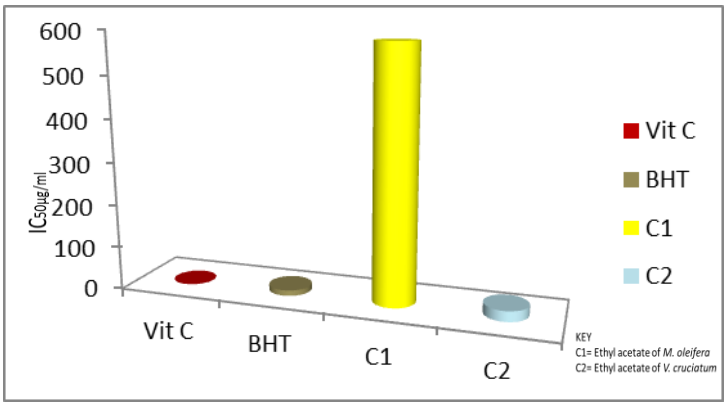

FIGURE 3.3

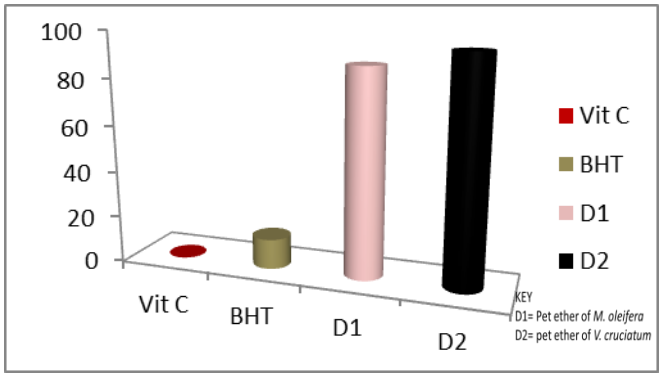

FIGURE 3.4

From the comparison chart between *Moringa oleifera* and *Viscum cruciatum* with the standards (Vit C. and BHT) showing their respected IC50 values. For the crude extracts fractions in comparison, the Vit C. showed higher activity, followed by BHT and then fraction of crude extract of *M. oleifera* in which they all showed excellent activity, having IC50 values of 0.02, 12.95 and 21.571 respectively while crude extract fraction of *V. cruciatum* showed moderate activity with IC50 value of 263.77. For the methanol fractions in comparison, Vit. C has the highest activity followed by methanol fraction of *M. oleifera*, followed by BHT and then methanol fraction of *V. cruciatum* with IC50 values of 0.02, 10.857, 12.95 and 22.482 respectively in which they all showed excellent activity. For the ethyl acetate fractions in comparison, vit. C. has the highest activity followed by BHT and then ethyl acetate fraction of *V. cruciatum* with IC50 values of 0.02, 12.95, and 24.352 respectively, while ethyl acetate fraction of *M. oleifera* has moderate activity with IC50 value of 582.023.. Also, for the petroleum ether fractions in comparison, Vit. C. has the the highest activity, followed by BHT, followed by petroleum ether fraction of *M. oleifera* and then *V. cruciatum* in which they all have excellent activity with IC50 values of 0.02, 12.95, 87.719 and 94.397 respectively.

Table 3.5: Results values of LC50 of Moringa oliefera

Samples (µg/ml)	No. of survived shrimp after 24 hours	No. of dead shrimp after 24 hours	LC50(µg/ml)
F-PE 1000 100 10	 3 2 2 5 6 6 8 8 9	 23 13 5	145.684
F-CE 1000 100 10	 4 5 3 7 6 6 8 9 8	 17 11 5	468.070
F-EA 1000 100 10	 12 4 7 6 7 9 6 9	 22 10 6	203.152
F- MEOH 1000 100 10	 6 3 3 7 8 8 9 8 9	 18 7 4	573.893

Table 3.5: Results values of LC50 of Viscum *cruciatum*

Samples (µg/ml)	No. of survived shrimp after 24 hours	No. of dead shrimp after 24 hours	LC50(µg/ml)
F-PE 1000 100 10	 3 4 1 5 7 4 6 8 7	 22 14 9	 99.459
F-CE 1000 100 10	 2 3 6 8 5 6 8 7 9	 21 11 6	 212.615
F-EA 1000 100 10	 5 0 3 5 7 5 7 8 9	 22 12 6	 166.809
F- MEOH 1000 100 10	 2 4 6 7 7 7 9 8 9	 18 9 4	 471.220

KEY
F- Fraction of PE- Petroleum Ether; CE-Crude Extract; EA-Ethyl acetate;
MEOH-Methanol

The bioassay of the extracts of *Moringa oleifera* (Table 3) of the various fractions showed that all the fractions; petroleum ether, crude extract and ethyl acetate with LC50 values of 145.684, 468.070 and 203.1522 respectively are moderately active. While methanol with LC50 value 573.893 has fair activity. Also the bioassay of the extract of *V. cruciatum* (Table 4) of the various fractions showed that petroleum ether with LC50 values of 99.459 is very active, while crude extract, ethyl acetate and methanol with LC50 values 212.615, 166.809 and 471.220 respectively are moderately active. Finally, all the two plants showed activity to brine shrimp lethality assay.

IV. Conclusions and Recommendations

4.1 **Conclusion**

All the two plants extracts showed good activity in brine shrimp lethality test, except petroleum ether fraction of *Viscum cruciatum* which showed very good activity and methanol fraction of *Moringa oleifera* that showed fair activity.

Also, all the fractions of the two plants showed excellent activity to DPPH radical scavenging assay, except crude extract of *Viscum cruciatum* that showed moderate activity and ethyl acetate of *Moringa oleifera* that showed fair activity to DPPH radical scavenging assay.

As such, all the plants are recommended for medicinal as well as Pharmacological uses.

Further research need to be done in order to determine the metabolites responsible for the pharmacological response and effect of these plants.

Referrence

[1]. Aliero A.A, Wara S.H. validating the medicinal potential of viscum cruciatum. *Afr J. pharmacol 3 (2), 2009, 335-338.*
[2]. Aquino R, Peluso G, De Tommasi. New polyoxypregnane ester derivatives from viscum cruciatum. *J. Nat prod. 29, 1996, 555-564*
[3]. Bayala M.T, Swadogo L. anti-androgenic activity of Leptademia hastate (peers) Decene: competitive effects of the aqueous of the plants. *Biotec Agron Soc environ 15(3), 2011, 223-229.*
[4]. Talepo Po, Bassole IHM, Tamboura HN, Belemtougri R.G. Anti-spermatogenic activity of Moringa Oleifera, leaf steams aqueous extracts in male wistar rats. *J. Pharmacol Toxicol. 6, 2011, 1-9*
[5]. Adoum, N.T Dabo. A novel antioxidant assay of ferric reducing capacity measurement. *J. medicinal Chem. 4, 1997, 49-50.*
[6]. Freigber C.E, Vanderjagi DJ, Rivera C. Analysis of nutritional components of eight famine foods of the republic of Niger. *J. Plant foods Hum Nutr 52, 1998, 17-18.*
[7]. George E., M.K Jones. Medicinal plants of philliphines Khalha publishing. *Phytother Res 15, 2004, 131-134.*
[8]. Hakson. Anti-oxidative properties of Moringa Oleifera. *A journal of food chemistry 15, 2010, 295-302.*
[9]. Haward, Kumar. *Central institute of medicinal plants (CIMP), Uttar Pradesh, India. 2001.*
[10]. Jerry L. Mclaughing L. Rogers, M.S Khem. *Clinical evaluation of a portable lactate 28, 2013, 268-304*
[11]. Mathien G. Meissa D. Traditional leafy vegetables in Senegal: medicinal uses. *Aftr J. Tradith complement Altern med 10, 2007, 469-475*

[12]. Salina, A.W and K.B.J. Pharmacological biology and chemical sciences. *Plant foods Hum Nutr 53, 1971, 57-69*
[13]. J.k Amfi, T.K John. Toxicity of Moringa Oleifera extracts. *School of pharmacy and pharmaceutical sciences, Purdue University, West Lafayette, Indiana 2001.*
[14]. Tamboura HH, Bayala B, Lompo M, Gluisson IP. Morphological characteristics and acute toxicity of Moringa Oleifera used by veterinary healers in Burkina Faso. *Aftr j. Trad 2, 2005, 13-14.*
[15]. Fuller. Antioxidant activity and hepatoprotective property of leaf extracts of Moringa oleifera. *J.org chem. 2, 199, 295-302*
[16]. Krishna. *School of pharmacy and pharmaceutical science purdue in such heterogenous botanicals. University of Lafayette, 2003.*
[17]. Meyer. The use of biological assays to evaluate botanicals. *J. prod. 28 (2), 1997, 456-470*
[18]. Ortho H, G.M Ferrozine as the colour forming complemation reagent. *Journal of Chem. India, 2,2009, 191-195.*

A.G Usman" Comparative Antioxidative and Cytotoxicic Activity of Extracts of Moringa Oleifera and its Mistletoe." IOSR Journal of Applied Chemistry (IOSR-JAC) 11.1 (2018): 13-18.