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Full Length Research Paper

Studies on *in-vitro* antioxidant and free radical scavenging potential and phytochemical screening of leaves of *Ziziphus mauritiana* L. and *Ziziphus spina-christi* L. compared with Ascorbic acid

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In order to determine the *in vitro* antioxidant and free radical scavenging potential and phytochemical properties of leaves of *Ziziphus mauritiana* L. and *Ziziphus spina-christi* L. in comparism with Ascorbic Acid. The present study was undertaken using *in vitro* antioxidant methods like hydroxyl radical, DPPH radical, lipid peroxidation and superoxide radical standardization methods. The Ethanol and Hexane extracts of *Z. mauritiana* L. and *Z. spina-christi* L. leaves were subjected to the methods listed above. The results of antioxidant activity revealed that, the ethanol extract has lower EC_{50} values than the hexane extract of *Z. mauritiana* L. and *Z. spina-christi* L. The lower EC_{50} value indicates the higher free radical scavenging ability of the plants. So, the ethanol extract has a better antioxidant activity than hexane extract. These results were compared with the standard ascorbic acid solution. The phytochemicals such as cardiac glycosides, polyphenols, saponins and tannins were identified, among others. It is suspected that these active constituents acting singly or in synergy may be responsible for the observed antioxidant activity of these plant species.

Key words: *Ziziphus mauritiana* L., *Ziziphus spina-christi* L., free radical, phytochemical screening, antioxidant activity.

INTRODUCTION

Plants are natural reservoir of antimicrobial agents of medicinal values. These agents are thought to be almost free from the side effects usually associated with synthetic antimicrobials. So many of the present day drugs are known to have been isolated from natural sources and their isolations were based on the information about the uses of the agents in folklore medicine. Ziziphus is a genus of about 40 species of spiny shrubs and small trees in the buckthorn family, Rhamnaceae, distributed in the warm-temperate and subtropical regions throughout the world. According to the Sunset Western Garden Book, (1995), the leaves are alternate, entire, with three prominent basal veins, and 2 to 7 cm (0.79 to 2.8 in) long; some species are deciduous, others evergreen. The flowers are small, inconspicuous yellow-green. The fruit is an edible drupe, yellow-brown, red, or black, globose or oblong, 1 to 5 cm (0.39 to 2.0 in) long, often very sweet and sugary, reminiscent of a date in texture and flavour. The best known species is *Z. zizyphus* (Jujube). Other species include *Z. spina-christi* from southwestern Asia, *Z. lotus* from the Mediterranean region, and Ber (*Z. mauritiana*), which is found from western Africa to India. *Z. joazeiro* grows in the Caatinga of Brazil.

Some species, like *Z. mauritiana* Lam. and *Z. spina-christi* (L.) wild occur on nearly every continent. *Z. mauritiana* and *Z. spina-christi* have very nutritious fruits and are usually eaten fresh. The fruits are applied on cuts and ulcers. They are also used to treat pulmonary ailments and fevers and to promote the healing of fresh

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wounds, for dysentery (Adzu et al., 2001). The leaves are applied locally to sores, and the roots are used to cure and prevent skin diseases (Adzu et al., 2001). The seeds are sedative and are taken sometime with buttermilk to halt nausea, vomiting and abdominal pains associated with pregnancy (Kaaria, 1998). The leaves are applied as poultices and are helpful in liver troubles, asthma and fever (Michel, 2002). Plant materials are cheap and significantly contribute to the improvement of human health in terms of cure and prevention of diseases Okoko and Oruambo, (2008). Plants have been useful as food and medicine and a few have been studied especially African medicinal plants (Lee, et al., 2003; Ogle, et al., 2003; Adebooye and Opabode, 2004; Ayodele, 2005). They contain vitamins needed by human body for healthy living (Szeto, et al., 2002; Jimoh, et al., 2008).

The present studies were performed to assess the *in vitro* antioxidant activity by using methods such as superoxide radical, hydroxyl radical, lipid peroxidation and DPPH radical scavenging analysis.

MATERIAL AND METHODS

Chemicals

All the chemicals used were of analytical grade (ANALAR) British Drug House (BDH), Poole, England.

Plant material

Leaves of the two plants (*Z. mauritiana* Lam. and *Z. spina-christi* L.) were collected in polythene bags from in and around Gbako local government area of Niger State, Nigeria and transported to Federal Polytechnic, Bida and air dried for two weeks in the Microbiology Laboratory. The dried leaf material was then grounded into fine powder using blender (Monlinex 530, 240V) and packed in polythene bags for further use.

Extraction of active compounds using ethanol as solvent for extraction

10 g of the ground leaf samples were separately soaked in 200 ml of ethanol and allowed to stand for about 72 h for extraction. After 72 h, it was then filtered using No.1 Whatman filter paper. The filtered samples were sterilized by passing through Millipore filter and later evaporated to dryness (Mann et al., 2008, Abalaka et al., 2009).

In vitro anti oxidant study

The ethanol extracts and hexane extracts of *Z. spina-christi* and *Z. mauritaniana* leaves were tested for their free radical scavenging properties using different *in vitro* techniques as follows.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by studying the interactions between deoxyribose and test extracts for hydroxyl radicals which were otained by Fenton's reaction. The damage on deoxyribose due to the free radicals was determined calorimetrically by measuring the thiobarbituric acid reactive substances (TBARS) at optical density of 532 nm (Chaminda et al., 2001). Percentage of inhibition was also calculated and recorded.

DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method of Braca et al. (2001). An aliquot of 3ml of 0.004% DPPH alcohol solution and 0.1ml of plant extract at various concentrations were mixed and incubated at 37°C for 30 min at an absorbance of 517 nm. The percentage of inhibition of DPPH radical was calculated by comparing the results of the test with those of the control using the formula of Luximon-Ramma et al. (2002) as indicated below:

Percentage of inhibition = $[(Ao – A_1) / Ao] \times 100$

Where Ao = Absorbance of the control
A_1 = Absorbance of the plant extract/ standard

Lipid peroxidation inhibition activity

The inhibition of lipid peroxidation was performed in line with the method described by Gupta et al. (2000). Determination of the extent of lipid peroxidation was carried out using rat liver homogenate as the source of polyunsaturated fatty acids. The absorbance was measured at 532 nm. Percentage of inhibition was calculated using the formula of Raju et al. (2005).

Superoxide radical scavenging activity

Superoxide radical scavenging activity of each plant extract was measured according to the method of Luximon-Ramma et al. (2002). This works based on light induced superoxide generation by riboflavin and the subsequent reduction of nitroblue tetrazolium. All the solutions were prepared in phosphate buffer of about pH 7.8. Five hundred and sixty nanometer (560 nm) optical density was measured and the percentage inhibition was calculated and recorded accordingly.

Phytochemical analysis of plant extracts for active components

Phytochemical screening of the extracts was carried out according to the methods described by Trease and Evans (1989) for the detection of active components like saponins, tannins, alkaloids, phlobatanins, glycosides etc.

a.) Alkaloids- 1 ml of 1% HCl was added to 3 ml of the extract in a test tube. The mixture was then heated for 20 min, cooled and filtered about 2 drops of Mayer's reagent to1 ml of the extract. A creamy precipitate was an indication of the presence of alkaloids.
b.) Tannins- 1 ml of freshly prepared 10% KOH was added to 1 ml of the extract. A dirty white precipitate showed the presence of tannins.
c.) Glycosides- 10 ml of 50% H_2SO_4 was added to 1 ml of the extract and the mixture heated in boiling water for about 15 min. 10ml of Fehling's solution was then added and the mixture boiled. A brick-red precipitate was confirmatory for the presence of glycosides.
d.) Saponins- Frothing test: 2 ml of the extract was vigorously shaken in the test tube for 2 min. No frothing was observed.
e.) Flavonoids- 1 ml of 10% NaOH was added to 3 ml of the extract.

Table 1. Percentage inhibition and EC_{50} values of hydroxyl radical scavenging activity *in vitro* by ethanol and hexane extracts from leaves of *Ziziphus mauritiana* L. and *Ziziphus spina-christi* L.

Plant/extract	Quantity in micrograms (µg)					
	10	50	100	200	300	EC_{50} values
AA	3.56±1.01	14.28±2.93	24.37±2.26	55.24±2.13	76.21±1.27	219.31
EEZS	2.45±1.25	12.14±2.36	21.24±1.57	38.67±1.46	56.08±2.4	357.23
HEZS	4.13±2.79	12.15±1.05	21.68±2.90	37.32±2.14	62.45±0.29	253.71
EEZM	3.12±2.43	14.23±0.19	19.52±0.46	36.23±1.22	69.22±1.23	198.34
HEZM	5.48±1.43	15.43±2.54	36.29±1.67	44.67±0.90	78.27±2.32	234.11

AA – Ascorbic acid, EEZS-Ethanol extract of *Z. spina-christi,* HEZS- Hexane extract of *Z. spina-christi,* EEZM- Ethanol extract of *Z. mauritiana*, HEZM- Hexane extract of *Z. mauritiana.*

Table 2. Percentage inhibition and EC_{50} values of DPPH radical scavenging activity *in vitro* by ethanol and hexane extracts from leaves of *Ziziphus mauritiana* L. and *Ziziphus spina-christi* L.

Extract/ plant	Quantity in micrograms (µg)					
	10	50	100	200	300	EC_{50} values
AA	28.33±1.49	44.50±0.93	61.26±2.32	77.07±2.38	79.24±1.20	78.12
EEZS	20.81±0.25	42.14±1.23	51.01±0.34	64.21±3.06	68.20±2.14	18.13
HEZS	26.21±0.53	45.09±1.04	59.07±2.13	74.10±0.46	80.22±0.11	93.83
EEZM	28.02±1.20	48.12±0.17	61.24±1.43	76.13±1.13	79.11±1.36	101.02
HEZM	29.54±3.03	35.16±2.19	43.09±1.05	58.13±0.21	83.13±2.56	124.21

AA – Ascorbic acid, EEZS-Ethanol extract of *Z. spina-christi,* HEZS- Hexane extract of *Z. spina-christi,* EEZM- Ethanol extract of *Z. mauritiana*, HEZM- Hexane extract of *Z. mauritiana.*

There was no yellow colouration which is indicative of the absence of flavonoids.

f. Steroids- Salkowski test: 5 drops of concentrated H_2SO_4 was added to 1 ml of the extract in a test tube. Red colouration was observed which is indicative for the presence of steroids.

g. Phlobatanins- 1 ml of the extract was added to 1% HCl. No red precipitate observed which means negative result.

h. Triterpenes- 1 ml of the extract was added to 5 drops of Acetic anhydride and a drop of concentrated H_2SO_4 added. The mixture was then steamed for 1 h and neutralized with NaOH followed by the addition of chloroform. Absence of blue-green colour indicates the absence of triterpenes.

RESULTS

In this study results are given in Tables 1 to 5. Table 1 shows the percentage inhibition and EC_{50} values of hydroxyl radical scavenging activity *in vitro* by ethanol and hexane extracts from leaves of *Z. mauritiana* L. and *Z. spina-christi* L. Table 2 shows percentage inhibition and EC_{50} values of DPPH radical scavenging activity *in vitro* by ethanol and hexane extracts from leaves of *Z. mauritiana* L. and *Z. spina-christi* L. Table 3 shows percentage inhibition and EC_{50} values of Lipid peroxidation *in vitro* by ethanol and hexane extracts from leaves of *Z. mauritiana* L. and *Z. spina-christi* L. Table 4 shows percentage inhibition and EC_{50} values of superoxide radical scavenging activity *in vitro* by ethanol and hexane extracts from leaves of *Z. mauritiana* L. and *Z. spina-christi* L. Table 5 showed the presence of five different constituents such as cardiac glycosides, polyphenols, resins, saponins and tannins in *Z. mauritiana* but *Z. spinachristi* contains only three of the chemical constituents which include polyphenols, saponins and tannins.

DISCUSSION

The EC_{50} (50% effective concentration) values for hydroxyl radical with ethanol extract and hexane extract of *Z. mauratiana* were found to be 357.23 and 253.71 µg, *Z. spina-christi* 198.34 and 234.11 µg respectively while that of ascorbic acid was found to be 219.31 µg. From these results the ethanol extract of *Z. spina-christi* leaves was found to have better hydroxyl radical scavenging activity when compared to ethanol and hexane extracts of *Z. mauritiana as* shown in Table 1, Figure 1. It has been discovered that a single hydroxyl radical can result in formation of many molecules of lipid hydroperoxides in the cell membrane, which may severely disrupt its function and lead to cell death. DPPH (2,2-diphenyl-1-picrylhydrazyl**)** is a well-known radical and a trap ("scavenger") for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. Because of a strong absorption band centered at about

Table 3. Percentage inhibition and EC_{50} values of Lipid peroxidation *in vitro* by ethanol and hexane extracts from leaves of *Ziziphus mauritiana* L. and *Ziziphus spina-christi* L.

Extract/ plant	Quantity in micrograms (µg)					
	10	50	100	200	300	EC_{50} values
AA	8.36±1.45	24.20±2.93	41.37±3.24	57.17±2.19	75.15±0.19	191.42
EEZS	2.98±2.45	14.84±2.31	28.10±1.67	40.06±2.37	51.12±1.04	382.02
HEZS	1.89±1.57	11.29±0.14	25.98±1.09	36.36±2.54	49.32±1.34	356.78
EEZM	2.24±1.23	15.45±1.29	22.21±1.23	43.43±2.02	71.21±0.43	298.65
HEZM	6.23±2.13	25.26±1.23	40.67±2.30	54.04±1.06	80.14±1.34	376.25

AA – Ascorbic acid, EEZS-Ethanol extract of *Z. spina-christi,* HEZS- Hexane extract of *Z. spina-christi,* EEZM- Ethanol extract of *Z. mauritiana*, HEZM- Hexane extract of *Z. mauritiana.*

Table 4. Percentage inhibition and EC_{50} values of superoxide radical scavenging activity *in vitro* by ethanol and hexane extracts from leaves of *Ziziphus mauritiana* L. and *Ziziphus spina-christi* L.

Extract/ plant	Quantity in micrograms (µg)					
	10	50	100	200	300	EC_{50} values
AA	13.06±2.01	34.23±1.90	54.23±1.25	59.04±1.03	66.01±2.18	138.26
EEZS	8.05±2.05	13.03±1.76	22.16±2.77	42.33±2.07	67.08±2.3	282.01
HEZS	11.03±2.61	20.22±2.35	37.08±1.90	57.20±1.04	78.55±1.87	203.70
EEZM	10.10±1.34	24.02±2.17	31.22±1.23	46.12±2.11	66.34±0.12	156.45
HEZM	9.89±2.12	12.21±3.78	32.02±2.89	39.32±1.0	56.12±9.2	265.22

AA – Ascorbic acid, EEZS-Ethanol extract of *Z. spina-christi,* HEZS- Hexane extract of *Z. spina-christi,* EEZM- Ethanol extract of *Z. mauritiana*, HEZM- Hexane extract of *Z. mauritiana.*

Table 5. Results of the phytochemical screening of ethanolic extracts of *Z. mauritiana* and *Z. spinachristi*

Organic compounds	*Z. mauritiana*	*Z. spinachristi*
Alkaloids	-	-
Anthraquinones	-	-
Cardiac glycosides	+	-
Phlobatannins	-	-
Polyphenols	+	+
Resins	+	-
Saponins	+	+
Tannin	+	+

Key: + = present, - = absent.

520 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520 nm or in the EPR signal of the DPPH (Alger, 1997).

In the present analysis the ethanol and hexane extracts of *Z. mauritaniana* leaves showed strong scavenging activity with DPPH than those of *Z. spina-christi*. The EC_{50} values for the two plants extracts were ethanol 18.13, and hexane 93.83, for *Z. mauritiana* and 101.02 and 124.21, for *Z. spina-christi.* The above results compare favourably with that of standard ascorbic acid which had the EC_{50} value of 78.12. These activities indicate that extracts from these plants are good antioxidants and strong antimicrobial Table 2, Figure 2. *In vitro* lipid peroxidation was induced in rat liver by using ammonium ferrous sulphate and ascorbic acid. The EC_{50} values for lipid peroxidation with ethanol extract and hexane extract of *Z. mauratiana* were 382.02 and 356.78 µg, for *Z. spina-christi* 298.65 and 376.35 µg respectively while that of ascorbic acid was 191.42 µg. The ethanol extract of *Z. mauritiana* leaves have higher lipid peroxidation inhibition than hexane extract of the plant

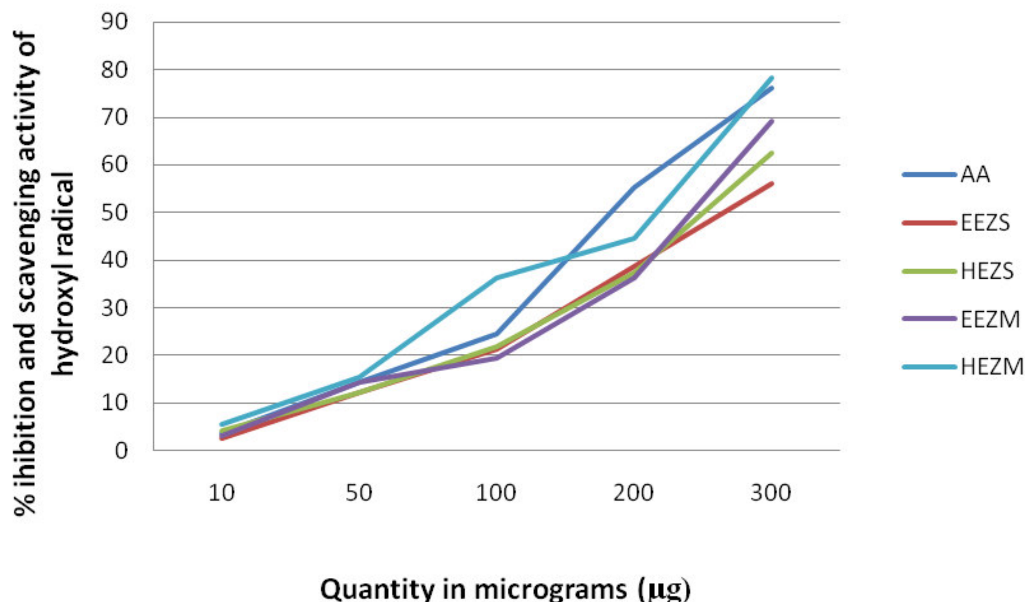

Figure 1. In *vitro* concentration dependent percentage inhibition of hydroxyl radical scavenging activity by ethyl alcohol extract and hexane extract of *Z. mauritiana* and *Z. spina-christi* leaves compared with ascorbic acid.

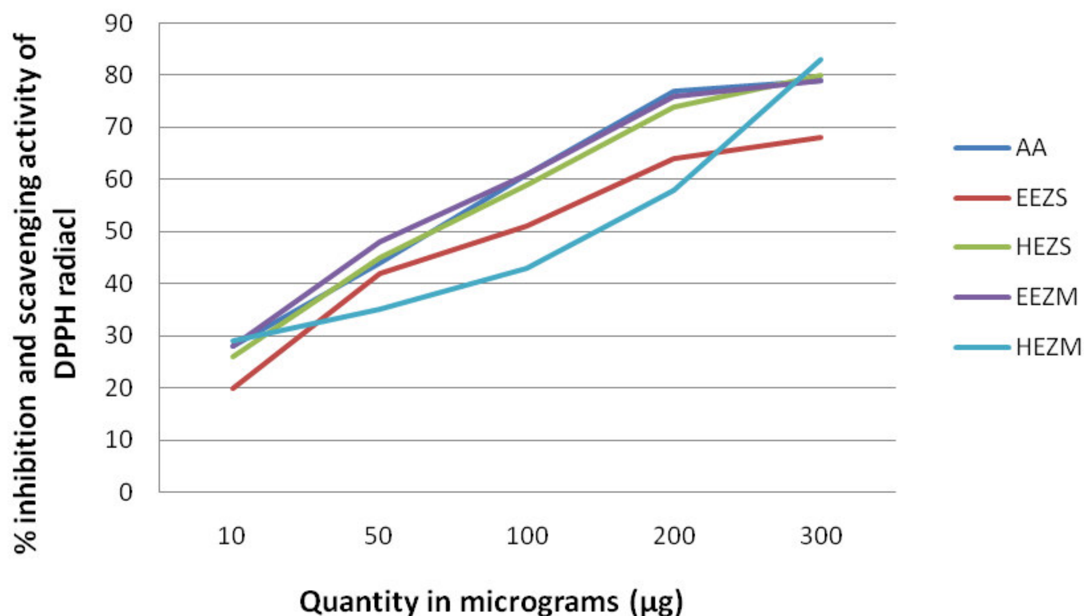

Figure 2. *In vitro* concentration dependent percentage inhibition of DPPH radical scavenging activity by ethyl alcohol extract and hexane extract of *Z. mauritiana* and *Z. spina-christi* leaves compared with ascorbic acid.

and ethanol and hexane extracts of *Z. spina-christi* Table 3, Figure 3. These results mean that the extracts showed concentration dependent prevention towards generation of lipid peroxides and could be strong antioxidants.

The EC_{50} values for superoxide radical scavenging with ethanol extract and hexane extract of *Z. mauratiana* were 282.01 and 203.70 µg, and for *Z. spina-christi* were156.45 and 265.22 µg respectively while that of ascorbic acid was 138.26 µg. The ethanol extract of *Z. mauritiana* leaves have higher superoxide scavenging

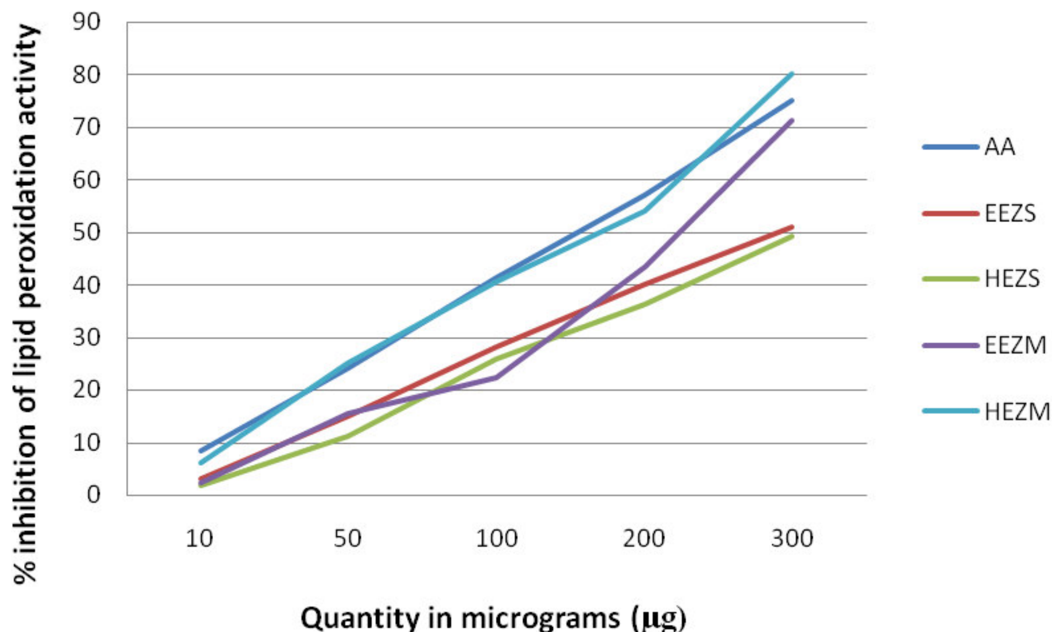

Figure 3. *In vitro* concentration dependent percentage inhibition of lipid peroxidation by ethyl alcohol extract and hexane extract of *Z.* mauritiana and *Z. spina-christi* leaves compared with ascorbic acid.

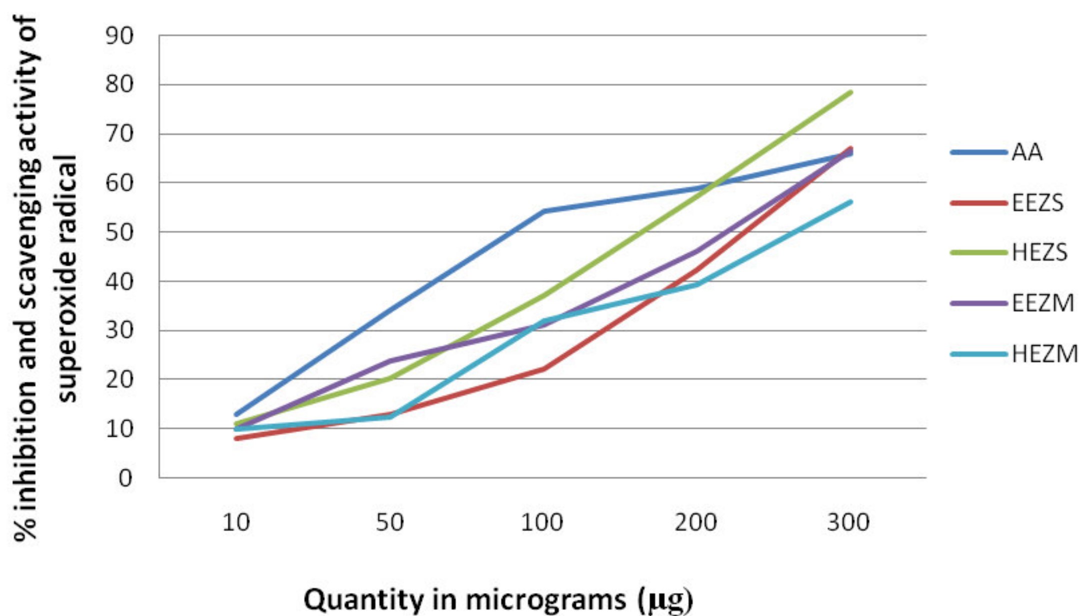

Figure 4. *In vitro* concentration dependent percentage inhibition of superoxide radical scavenging activity by ethyl alcohol extract and hexane extract of *Z.* mauritiana and *Z. spina-christi* leaves compared with ascorbic acid.

activity than hexane extract of the plant and ethanol and hexane extracts of *Z. spina-christi* Table 4, Figure 4. Superoxides are produced from molecular oxygen due to oxidative enzymes of body and by non enzymatic reactions like auto oxidation by catecholamines (Sainani et al., 1997). (Figure 5).

In this particular study ethanol and hexane extracts of the two plants were found to scavenge the superoxides generated by photo reduction of riboflavin. Phytochemical screening showed the presence of five different constituents which include cardiac glycosides, polyphenols, resins, saponins and tannins in *Z.*

Figure 5. *Ziziphus mauritiana* Kingdom: Plantae Division: mognoliophyta Class: Magnoliopsida Order: Rosales Family: RhamnaceaeTribe: Paliureae, Genus: *Ziziphus*

mauritiana but *Z. spinachristi* contains only three of the chemical constituents which include polyphenols, saponins and tannins. Sterols like β-sitosterol, terpenoid, phytosterols, triterpenoids, alkaloids, saponins, flavonoids, glycosides and tannins have been reported to have antioxidant activity (Dragland et al., 2003; Cai et al., 2004). The elevated DPPH radical scavenging ability of the leaf extracts of these plants might be due to the presence of high concentration of these organic compounds. Kashiwada et al. (1990) and Gupta et al. (2000) recorded similar findings with extracts from *Cassia fistula* hence, the observed antioxidant capacity of extracts of these plants could be as a result of the presence of these organic constituents.

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