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Assessment Of the Effects of The Ethanol and Aqueous Extracts of *Clerodendrum Volubile* Leaves on Liver Mitochondrial Membrane

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Abstract- ***Cost effectiveness, efficacy, and availability have made many people especially in Sub-sahara Africa turned to herbal medicine. Natural products including plant extracts have been explored for their potential health effects. The study employed several techniques to determine the in vitro antioxidant and free radical scavenging activities of ethanol and aquous extract of Clerodendrum volubile. Several models were used because the total antioxidant capacity of an antioxidant cannot be measured by using just single method. Among others, the results of this study justify the traditional uses of the Clerodendrum volubile leaves for therapeutic purposes. It has revealed its efficacy as a radical scavenger and its ability to enhance the reduction in the concentration of molecular oxygen which enhances a smooth transfer of electron in the electron transport system.***

Key Words: Ethanol And Aqueous Extract, Clerodendrum Volubile Leave, Radical Scavenger And Molecular Oxygen

I. INTRODUCTION

The liver plays a vital role in maintaining overall health, and mitochondrial dysfunction has been implicated in various liver diseases. Natural products, such as plant extracts, have been explored for their potential hepatoprotective effects. Clerodendrum volubile, a plant species, has been traditionally used in medicine, and its extracts have shown antioxidant and protective properties in various studies.

The demand for herbal medicines for disease treatment is on the increase due to their efficacy, availability and affordability Augustine et al, 2023. Herbal medicines account for close to 80 percent of the health care needs of the world's population, especially for a large number of people in many places of developing countries WHO 2013. In sub-Sahara Africa, medicinal plants have are been used for the treatment of different ailments and diseases without scientific investigation of their therapeutic potentials. Recently, researchers have examined numerous medicinal plants for their bioactive and pharmacological properties Augustine et al, 2023

1.1 Statement of Research Problem

As reported by Gokhan et al., 2011, synthetic antioxidants are found to be carcinogenic in biological system natural antioxidants of herbal sources. He opinioned further that it may also be employed to supplement the role of glutathione, thus, leading to the rising interest towards natural antioxidants of herbal sources. Furthermore, because of affordability or cheapness of using synthetic antioxidants Ascobic acid, Tocopherol and others like it, people do many times abused it by indiscriminate use, not only that, it is not even valued has expected.

1.2 Significance of the Study

This research has the potential to contribute to the development of novel therapeutic strategies for liver diseases, utilizing natural products with hepatoprotective properties. The search for natural antioxidant has been intensified because of their capacity to reduce the impact of free radical reactions thereby giving protection from diseases Terao and Piskula, 1997. The findings may also provide insights into the traditional use of Clerodendrum volubile in medicine and its potential applications in modern healthcare.

1.3 Aim and Objectives
This study aims to assess the effects of ethanol and aqueous extracts of Clerodendrum volubile leaves on liver mitochondrial membranes, exploring their potential benefits in maintaining liver health and preventing liver damage. Investigating the antioxidant and protective effects of Clerodendrum volubile extracts on liver mitochondrial membranes.

1.4 Literature review
Apoptosis plays an essential role in the survival of organisms and is considered to be an imperative component of various processes including normal cell turnover, proper development and functioning of the immune system, multiplication of mutated chromosomes, hormone-dependent atrophy, and normal embryonic development, elimination of indisposed cells and maintenance of cell homeostasis.

Morphological hallmarks of apoptosis in the nucleus are chromatin condensation and nuclear fragmentation, which are accompanied by rounding up of the cell, reduction in cellular volume (pyknosis) and retraction of pseudopodes (Kroemer et al., 2005). Chromatin condensation starts at the periphery of the nuclear membrane, forming a crescent or ring-like structure. The chromatin further condenses until it breaks up inside a cell with an intact membrane, a feature described as karyorrhexis. The plasma membrane is intact throughout the total process. At the later stage of apoptosis some of the morphological features include membrane blebbing, ultrastructural modification of cytoplasmic organelles and a loss of membrane integrity (Kroemer et al., 2005).

The genus Clerodendrum is widely distributed in the tropics and sub-tropics. It comprises of small trees, shrubs and herbs (Shrivastava and Patel, 2007, Erukainure et al., 2014). Clerodendrum is known to be a very large and diverse genus with over 580 identified species which are widely distributed in Asia, Australia and America, and among many species of Clerodendrum genus is C. volubile (Erukainure et al., 2014).

Clerodendrum volubile (figure 1.1) (popularly called the magic leaf or white butterfly leaf) has the local name “Marugbo” in southern part of Nigeria. It is known for its food and potent medicinal values, though scientific database for these claims have just began to grow (Erukainure, 2014). C. volubile is a climbing shrub of about 3 m, and glabrous except the inflorescences. It is found in the deciduous forests (Burkill, 1985).

Folklore has it that Clerodendrum volubileisused traditionally in the treatment of arthritis, rheumatism, dropsy, swelling, oedema, gout; applied forgeneral healingas well as in pregnancy aspain killers, antiabortifacients, sedatives, among other uses.

(Burkill, 1985; et al., 2011). The leaves are often dried and used locally as spices in cooking, and sometimes the fresh leaves are blended and used for soup concoction. Its use in the treatment and in management of several ailments made it to be referred to as the magic leaf (Burkill, 1985).

Figure 1.1 Mature Clerodendrum volubile leaves and its flowers (Erukainure et. al. 2011).

Uses of Clerodendrum volubile
The existence of ethnomedicinal claims for Clerodendrum species in folk medicines and diverse traditional system of medicines, across the Asian and African continents for the management of several life-threatening ailments like jaundice, syphilis, typhoid, asthma, cataract, malaria, pyreticosis, hypertension as well as diseases of the skin, blood and lung cancer has drawn attention of researchers to these plants (Neeta and Tejas, 2007). This plant is an important medicinal treasure especially to people living in the south-south Nigeria (Neeta and Tejas, 2007; Fred-Jaiyesimi and Adekoya, 2012). A wide variety of claims have been reportedly made for the medicinal properties of Clerodendrum volubile in the

treatment of many ailment and clinical conditions, such as edema, rheumatism, dropsy, gout and arthritis. The leaf of Clerodendrum volubile has often been termed as magic leaf due to its high efficacy when used for management and treatment of numerous ailments e.g. ulcer and diabetes and other common diseases (Burkill, 1985). Nutritional value of this plant has also been reported (Ogunwa et. al., 2015).

Biological Membranes

Danielli and Dawson proposed in 1935 the so-called 'sandwich' (bread-and-butter) model of biological membranes' composition (figure 1.3.) that had been used in membranology, though with some small variations, for almost forty years. According to this model, proteins are dispersed in membranes on the surface of phospholipid layer. Each membrane type contains a specific set of proteins - receptors and enzymes but the base of every membrane is a bimolecular layer of lipids (lipid bilayer) that performs in each membrane two principal functions:

(1) Barrier for ions and molecules, and

(2) Structural base (matrix) for functioning of receptors and enzymes.

These are adapted and/or specialized for carrying out one or more vital functions. Eukaryotic cells in addition are compartmentalized by intracellular membranes that form the boundaries and internal structures of their various organelles. These sub-cellular organelles include, mitochondria, nuclei, chloroplasts, Golgi apparatus which are all intracellular organelles and the endoplasmic reticulum having intracellular surface.

Membranes function to organize biological processes by compartmentalizing them. The outer mitochondrial membrane, is (composed of about half lipid and half protein) freely permeable to small molecules and ions having molecular weights as high as 10,000 because it contains many copies of porin, a transmembrane protein with a large pore. The inner mitochondrial membrane allows only certain molecules (such as water, carbon-IV-oxide and oxygen) to pass through it and is much more selective than the outer membrane (Frey and Mannella, 2000). It is about 20 percent lipids and 80 percent protein – a higher proportion than occurs in other cellular membranes.

The high permeability of the outer membrane is mediated by Voltage Dependenct Anion Channel (VDAC), which is the most common protein in the outer membrane and is permeable to molecules of up to 5000 Daltons in its open configuration. In the contemporary basic model for membrane structure, extrinsic proteins are loosely adsorbed to the lipid bilayer, while the intrinsic proteins are loosely embedded in the bilayer and can transverse the whole bilayer.

The inner membrane also contains import channels as well as the adenine nucleotide trranslocator (ANT) that exchanges ADP and ATP between the mitochondrial matrix and the intermembrane space (Frey and Mannella, 2000)

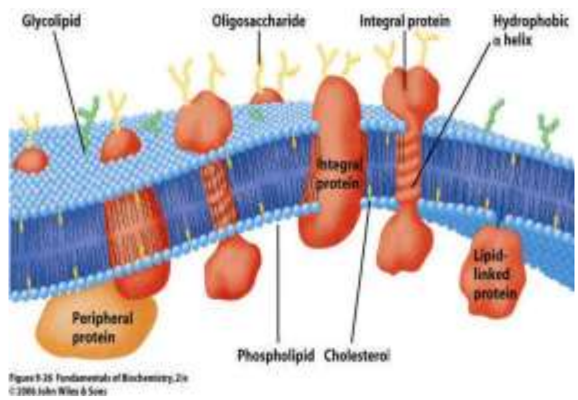

Figure 1.2: The Structure of Biological Membrane (Frey and Mannella 2000)

1.5 Materials and Methods

Chemicals and reagents:

Folin-Ciocalteus's reagent, HCl, methanol, gallic acid, H2SO4, Na2CO3, Aluminium chloride, Potassium acetate, Potassium persulphate, Sodium nitroprusside, hydrogen peroxide, sulfanilic acid, glacial acetic acid, naphthyl ethylenediamine dichloride, NADH were all purchased from Merck, USA. DPPH (1,1-diphenyl-1,2-picryl hydra Zyl), TPTZ (2,4,6, -tripyridyl-s-triazine), Ferrozine, Deoxyribose were purchased from Sigma Chemical Co. Ltd USA. Trichloroacetic acid (TCA), L-Ascorbic acid, and all other chemicals and reagents were of analytical grade and of highest purity (≥ 99.0 %).

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Collection of plant material and preparation of extracts:
Fresh samples of Clerodendrum volubibe leaves were collected in Osogbo, Osun state, Nigeria. The plant was identified at the Botany Unit, Department of Biological Sciences, Osun State University, Kajola, Ilesa road, Nigeria. The sample was pressed and dried. The samples were air dried for over 60 days and pulverized into powder using an electrical blender. The powdered leaf was cold-macerated with (1:4 w/v) volumes of 90 % ethanol for 72 hours and aqueous extract for 72 hours. Crude extract was obtained by filtration followed by evaporation of the solvent in a rotatory evaporator. The paste was weighed and used to prepare the stock solution for studies.

1.6 Phytochemical analysis
The freshly prepared extract fractions (ethanol and hot water) were qualitatively analyzed for the presence of alkaloids, resins, tannins, saponins, flavonoids, glycosides, phenols, anthraquinones, cardiac glycosides, steroids, phlobatannins, reducing sugars according to method described by (Monjur-Al-Hossain et al., 2013).

Evaluation of alkaloids: One milliliter of the extract fractions was shaken with diluted HCl for 2minutes. The filtrates are treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow precipitate indicated the presence of alkaloids (Monjur-Al-Hossain et al., 2013).

Evaluation of steroid (Salkowski Test): One milliliter of extract fractions was mixed with concentrated H_2SO_4. Formation of wine red colour indicated the presence of sterols (Monjur-Al-Hossain et al., 2013).

Evaluation of glycosides: A small amount of the extract fractions was suspended in 1ml of water and a few drops of aqueous sodium hydroxide were added. A yellow color was considered as an indication for the presence of glycosides (Monjur-Al-Hossain et al., 2013).
Evaluation of saponins (Froth Test): Fifteen milliliters of distilled water were added to 100 mg of each of the crude extract fractions and filtered into test tube. The mixture was shaken for 10minutes until the formation of stable persistent froth. Formation of stables 5-minute persistent froth indicated the presence of saponins (Djaafar and Ridha, 2014).

Evaluation of tannins (Ferric Chloride Test): One hundred milligrams of each of the crude fractions were dissolved in 10 mg ethanol, and the mixture was filtered. 2 ml of each of the filtrate was transferred into the test tube and few drops of 0.1%-$FeCl_3$ were added. The test group was compared with the control group, to which the extracts were not added. A brownish green coloration was interpreted as the presence of tannins (Mirunalimni et al., 2010).

Evaluation of phenolic compounds (Ferric Chloride Test): One hundred milligrams of each of the crude fractions were dissolved in 10 mg ethanol, and the solution was filtered. 2 ml of the filtrate was pipetted into the test tubes, followed by the addition of 5 ml distilled water. 4 drops of 5 % $FeCl_3$were added into the filtrate. The formation of dark green precipitate showed the presence of phenolic compounds (Raaman, 2006).

Evaluation of reducing sugars
Two hundred micrograms of the extract fractions were shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for two minutes. An orange precipitate on boiling with the Fehling's solution indicated the presence of reducing sugars (Monjur-Al-Hossain et al., 2013).

Evaluation of terpenoids (chloroform test): The crude extract of 100 mg was dissolved in the chloroform 5mL and filtered into the test tube. The mixture was added carefully with 3ml of H_2SO_4 along the side of the test tube. The reddish-brown color at the interface of the two phases characterized the presence of terpenoids (Ajiboye et al., 2013).

Quantitative assays
The quantitative analysis of the bioactive compounds present in the sample was determined spectrophotometry using tannic acid, catechin, and garlic acid as standards.

Determination of total phenolic content
The number of total phenolics in the extract factions were determined with Folin– Ciocalteu reagent with

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slight modification described by Ajayi et al., 2016. Briefly, 1.0 ml of extract solution (5 mg/ml) was added in a 100 ml volumetric flask that contained about 60 ml distilled water. Then, 5.0 ml of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 1 - 8 min, 15.0 ml Na_2CO_3 (20 %) was added and the volume was made up to 100 ml using distilled water. The mixture was allowed to stand for 2 hr with intermittent shaking. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, UK).

The total phenolic content was determined as milligrams of gallic acid equivalent (GAE) using an equation obtained from the standard gallic acid calibration graph

$$\text{Total Phenolic Content} = \frac{\text{Absorbance reading for test} \times \text{Standard concentration}}{\text{Absorbance reading for standard}}$$

Determination of total flavonoid

The total flavonoid content of the extracts was measured by $AlCl_3$ colorimetric assay (Ordon et al., 2006). Briefly, to 0.5 ml of sample, 0.5 ml of 2 % $AlCl_3$ ethanol solution was added. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 1mg/ml. All determinations were carried out in duplicates. The number of flavonoids in plant extract was determined in catechin equivalent (CE) using an equation obtained from the standard catechin calibration graph (Appendix II).

$$\text{Total Flavonoids Content} = \frac{\text{Absorbance reading for test} \times \text{Standard concentration}}{\text{Absorbance reading for standard}}$$

In vitro antioxidant assays. Determination of reducing power

The reducing power of the extracts was measured according to the method of Oyaizu (1986), based on Fe (III) to Fe (II) transformation. The Fe (II) was monitored by measuring the formation of Perl's Prussian blue at 700 nm. The extract or standard (100 µg/ml) was mixed with phosphate buffer (pH 6.6) and potassium ferricyanide. The mixture was incubated at 50 °C for 20 mins. Trichloroacetic acid (10 %, 2.5ml) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1 %, 0.5ml) and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated reductive potential of the extract.

Determination of scavenging of hydrogen peroxide power

The ability of extract to scavenge hydrogen peroxide was determined according to the method of Ilhami et al (2005). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of the plant extract were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min. against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage inhibition of hydrogen peroxide of extracts and standard compounds was calculated using the following formula:

$$\% \text{ inhibition } [H_2O_2] = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample of extract and standards.

Determination of hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the extracts was determined by the method of Klein et al. (1981) with a slight modification. 0.5 ml of extract/standard at different concentration was taken in test tubes. 1 ml of Fe-EDTA solution (0.13 % ferrous ammonium sulphate and 0.26 % EDTA), 0.5 ml of 0.018 % EDTA solution, 1 ml of 0.85 % DMSO solution and 0.5 ml of 22 % ascorbic acid were added into the test tubes. The test tubes were capped tightly and warm at 85 °C for 15 minutes into the water bath. After incubation, the test tubes were uncapped and 0.5 ml ice cold TCA (17.5 %) was added to each of test tubes immediately. 3 ml of Nash's reagent (7.5 g of ammonium acetate, 300 µl glacial acetic acid and 200 µl acetyl acetone were mixed and made up to 100 ml) was added to all the tubes and incubated at room temperature for 15 minutes. Absorbance was taken in UV-spectrophotometer at 412 nm wave length.

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Percentage hydroxyl radical scavenging (% HRSA) activity was calculated using the following equation:
% HRSA ={(A0-A1)/A0} × 100

Where A0 is the absorbance of the control, and A1 is the absorbance of the extracts/standard.

Determination of DPPH – radical scavenging activity

The radical scavenging activity of plant extracts were measured as described by Mensor et al., (2001) The stable 2, 2 diphenyl-1-picryldydrazyl (DPPH) radical was used for the determination of DPPH radical scavenging activities of the extracts. A portion (1 ml) each of the different concentrations (40-2000 µg ml-1) of the extracts or standard (catechin) in test tubes was added to 1 ml of 1 mM DPPH in methanol. The mixtures were vortexed and then incubated in a dark chamber for 30 minutes after which the absorbances were measured at 517 nm against a DPPH control containing only 1 ml of methanol in place of the extract. All calculations were carried out in triplicates. The inhibition of DPPH was calculated as a percentage using the expression:

1.7 Results

Phytochemical screening of extracts of Clerodendrum volubile

Table 1.1 shows the qualitative analysis of phytochemicals in aqueous leaf extract of Clerodendrum volubile. There is presence of alkanoids, steroids, glycosides, saponins, tannins, flavonoids and phenolic compounds in the extract.

Table 1.1: Qualitative phytochemical analysis of aqueous extract of Clerodendrum volubile

S/N	Phytochemicals	Specific test	Observation
1	Alkaloids	Mayer's test	+
2	Steroid	Sulphuric acid test Salkowski test	+ +
3	Flavonoids	Sulphuric acid test	+
4	Saponins	General test	+
5	Glycosides	General test	+
6	Phenol/Tannin	Ferric chloride test	+
7	Reducing Sugar	Benedict test	+
8	Terpenoids	Chloroform test	-

Table 1.2 shows the qualitative analysis of phytochemicals in ethanol leaf extract of Clerodendrum volubile. The result confirms the presence of alkanoids, steroids, saponins saponins, tannins, flavonoids and phenolic compounds in the extract.

Table 1.2: Qualitative phytochemical analysis of ethanol extract of Clerodendrum volubile

S/N	Phytochemicals	Specific test	Observation
1	Alkaloids	Mayer's test	+
2	Steroid	Sulphuric acid test Salkowski test	+ +
3	Flavonoids	Sulphuric acid test	+
4	Saponins	General test	+
5	Glycosides	General test	_
6	Phenol/Tannin	Ferric Chloride test	+
7	Reducing Sugar	Benedict test	_
8	Terpenoids	Chloroform test	_

Quantitative analysis of phenolic and flavonoid contentspresent in aqueous and ethanol extracts ofClerodendrum volubile

Figure: 1.4: Total Phenol content in aqueous and ethanol extracts from Clerodendrum volubile

The total phenol content in both ethanol and aqueous extract of Clerodendrum volubile, each valve represent a mean ± SD (n=2). CL – EE: Clerodendrum volubile ethanolic extract, CL- I: Clerodendrum volubile Aqueous Extract.

Results in Figure 1.1 shows that ethanol extract of Clerodendrum volubile contained higher amount of phenol compare to aqueous leaves extract.

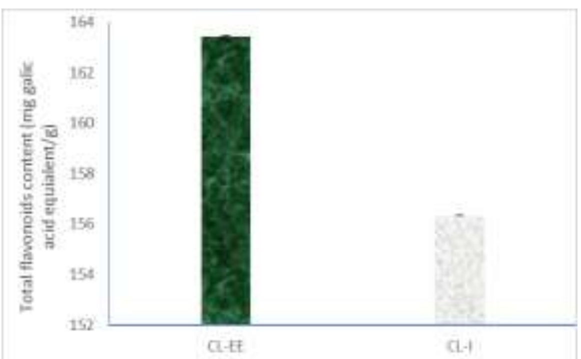

Figure:1.5: Total Flavonoids content in aqueous and ethanol extracts from Clerodendrum volubile

The total flavonoids of ethanol and aqueous of Clerodendrum volubile, each valve represent a mean ± SD (n = 2). CL – EE: Clerodendrum volubile ethanol extract, CL- I: Clerodendrum volubile Aqeous Extract. Total Flavonoids content in Clerodendrum volubile ethanol extract (CL-EE) was higher than Clerodendrum volubile aqeous extract (CL-I).

Antioxidant and free radical scanvenging properties in aqueous and ethanol extracts of Clerodendrum volubile

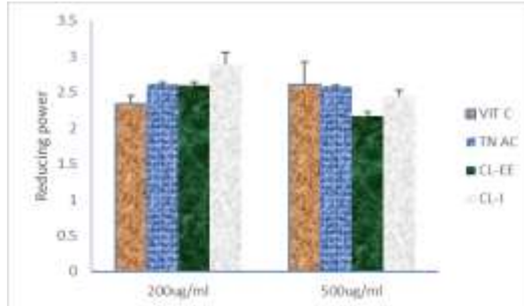

Figure 1.6: The free radical reducing power of different concentrations of ethanol and aqueous leaf extracts of Clerodendrum volubile as compared to antioxidant standards (ascorbic and tannic acids). Data represents Means of triplicates of different concentrations analyzed.

Figure 3.3.1 revealed the reducing potential of Clerodendrum volubile. The reducing properties are generally associated with the presence of reductones which have been shown to exert antioxidantaction by breaking the free radical chain or by donating a hydrogen atom.

The aqueous leaf extracts of Clerodendrum volubile used in the study shows significant reducing power when compared with ascorbic acid and tannic acids at 200 ug/ml.

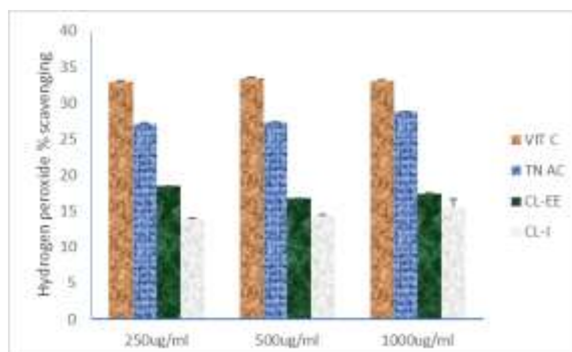

Figure 1.7: The hydrogen peroxide scavenging activities of different concentrations of ethanol and aqueous leaf extracts of Clerodendrum volubile as compared to antioxidant standards (ascorbic and tannic acids). Data represents Means of triplicates of different concentrations analyzed.

The result shows that both ethanol and aqueous leaves extract of Clerodendrum volubile promotes hydrogen peroxide decompositionin a concentration dependent manner. Scavenging of H2O2 by Clerodendrum volubile leaf extract could be because of its phenolic compounds which, through donation of electron, reduce H2O2 to water (Banerjee and Bonde, 2011). This activity of the plant extract is biologically advantageous since cells must prevent accumulation of H2O2

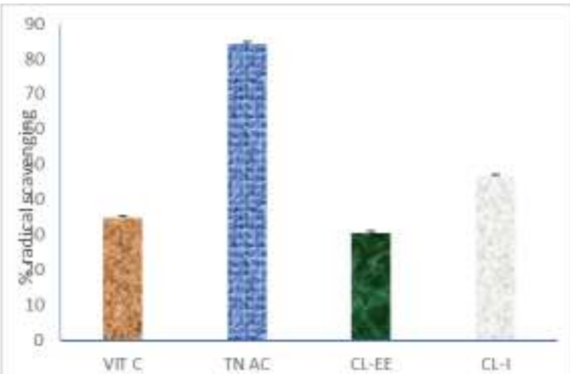

Figure 1.8: The hydroxyl radical scavenging activities of different concentrations of ethanol and aqueous leaf extracts of Clerodendrum volubile as compared to antioxidant standards (ascorbic and tannic acids). Data represents Means of triplicates of different concentrations analyzed.

Hydroxyl radical is one of the potent reactive oxygen species generated in the human body under physiological conditions, where it interacts with polyunsaturated fatty acid components, especially phospholipids of cell membrane leading to cell damage. They have the capacity to abstract hydrogen atoms from membrane lipids (Yen and Duh, 1994), attack proteins as well as effect breaks in DNA via oxidation. Tannic acis and aqueous leaf extract of Clerodendrum volubile used in the study shows significant hydroxyl radical scavenging properties when compared with ascorbic acid.

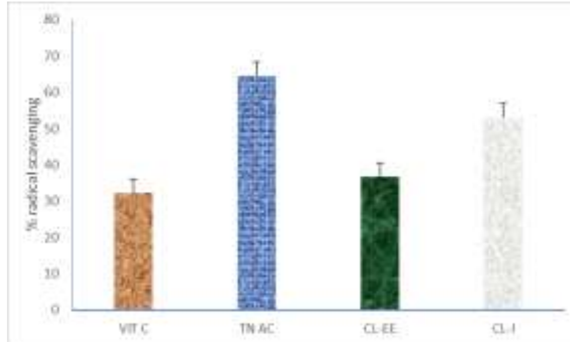

Figure 1.9: The DPPH radical scavenging activities of different concentrations of ethanol and aqueous leaf extracts of Clerodendrum volubile as compared to antioxidant standards (ascorbic and tannic acids). Data represents Means of triplicates of different concentrations analyzed.

The results showed that both extracts of Clerodendrum volubile exhibited inhibition of DPPH radical. The extracts showed higher DPPH radical scavenging ability compared to vitamin C (standard). This result revealed the electron donating potential of the plant extract.

The results for various antioxidant models used in this study. It became pertinent to use several models because the total antioxidant capacity of an antioxidant cannot be measured by using just single method. Several assays has to be use to evaluate the overall antioxidant potential or reducing capacity, as a measure of an organism total ability to withstand free radical stress (Gulcin et al., 2010).

Evaluation of Mitochondrial Membrane Permeability Transition Pore

Evaluation of the in vitro effects of the varying concentrations of the ethanolic leaf extract of Clerodendrum volubile on rat liver mitochondrial membrane permeability transition pore in the absence of Ca2+

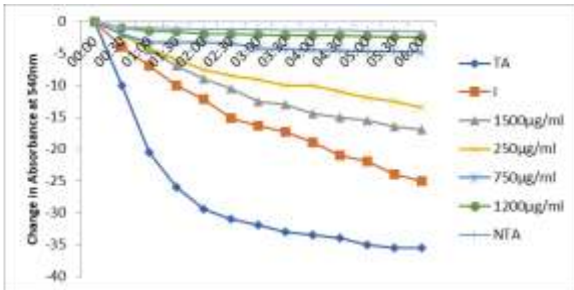

Figure 1.10: Effect of varying concentrations of ethanol leave extract of Clerodendrum volubile on diabetic rat liver mitochondrial memebrane permeability transition pore in the absence of Calcium

NTA: Non triggering agent TA: triggering Agent Spermine: as inhibitor.

The varying concentrations of the extract inhibited the MMPT in the liver of diabetic rats in the absence of calcium.

Evaluation of the in vitro effects of the varying concentrations of the ethanolic leaves extract of Clerodendrum volubile on rat liver mitochondrial membrane permeability transition pore in the presence of Ca2+

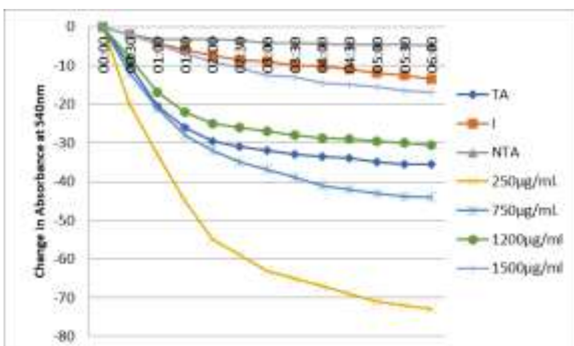

Figure 1.11: Effect of varying concentration of ethanol leaf extract of Clerodendrum volubile on diabetic rat liver mitochondrial memebrane permeability transition pore in the presence of Calcium

NTA: Non triggering agent TA: triggering Agent Spermine: as inhibitor

When 750, 250, 1500, and 1200 µg/ml extract were incubated with exogenous calcium in the diabetic group, they had high inhibitory effects in a time-dependent manner, while 1500ug/ml had highest inhibitory effect, compared to Spermine (an inhibitor) in a concentration-dependent manner as shown in figure 4.8, 4.9, 4.10 & 4.11, but there were some variations.

Ethanol extract of Clerodendrum volubile inhibited mitochondrial permeability pore opening in the absence of Calcium in a concentration-dependent manner while the extract reversed Calcium-induced opening of the pore in the presence of Calcium.

Evaluation of the in vitro effects of the varying concentrations of the aqueous leaves extract of Clerodendrum volubile on rat liver mitochondrial membrane permeability transition pore in the absence of Ca2+

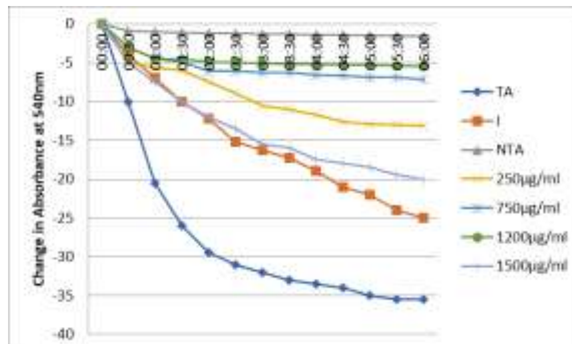

Figure: 1.12. Effect of varying concemntration of aqueousleaf extract of Clerodendrum volubile on diabetic rat liver mitochondrial memebrane permeability transition pore in the absence of Calcium.

a) Evaluation of the in vitro effects of the varying concentrations of the aqueous leaf extract of Clerodendrum volubile on rat liver mitochondrial membrane permeability transition pore in the presence of Ca2+

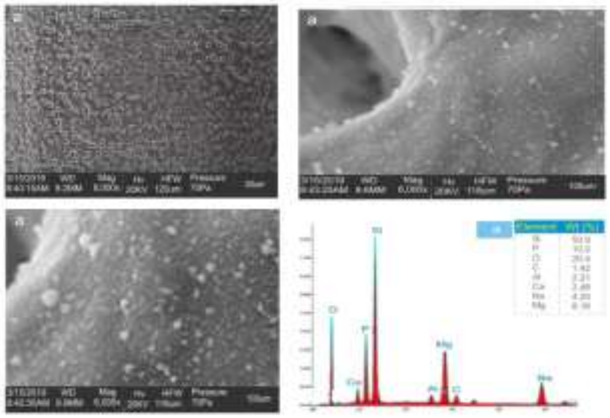

Figure 1.13: EDX and SEM of experimental rat tail collagen

Scan Electron Microscope Couple with Energy dispersion X-ray Spec for Elemental composition of sample of collagen. Silicon enhances collagen production. The results show the presence of the essential component of the collagen.

Summary, Conclusion and Recommendation

Antioxidant and free radical scavenging assays are one of the most widely used approaches to ascertain the pharmacological and biological activities of medicinal plant extracts and phytochemicals (Gulcin et al., 2010). This present study employed several techniques to determine the in vitro antioxidant and free radical scavenging activities of ethanol and aquous extract of Clerodendrum volubile.

The preliminary phytochemical analysis of the extracts used for this study revealed that the extracts contain bioactive compounds such as terpenoids, saponins, cardiac glycosides, flavonoids and tannins, alkaloid, phenolic compounds have long been recognized to possess anti-allergic, anti-inflammatory, antiviral and anti-proliferative, anticarcinogenic and antioxidant (Harbone, 1994; Frankel et al., 1998; Evan et al., 2001). Saponins have been reported to have beneficial effects on blood cholesterol levels. They bind with bile salt and cholesterol in the intestinal tract. Cardiac glycosides have been variously used from time immemorial as diuretics and heart tonics due to their beneficial effects on the heart. Cardiac steroids are widely used in the modern treatment of congestive heart failure and for treatment of atrial fibrillation.

Flavonoids have been documented to have great detoxification activities against most oxidizing molecules including singlet oxygen and other reactive oxygen species (Bravo, 2009) which have been implicated in pathogenesis of several diseases.

Reports have shown that there is an inverse relationship between the intake of flavonoids and the risk of coronary heart disease, stroke Keli et al., 1996, lung cancer and stomach cancer (Garcia et al., 1999).The results of total phenolic and flavonoids contents in Figure 3.2.1 and 3.2.2 suggested that they may be the major contributors to the in vitro antioxidant and free radical scavenging activities demonstrated by extracts used in the study.

The result in figure 3.2.2 reveal the reducing power of different concentrations of extracts of Clerodendrum volubile, compared with Vitamin C and Tannic acid. The yellow colouration of the testing mixture changes to green depending on the reducing power of the test specimen. The presence of the reducing agents in the sample solution causes the reduction of the Fe^{3+}/ Ferricyanide complex to the ferrous form. Thus, ferrous ion can be monitored by absorbance measurement at 700 nm. The reducing properties have been documented to exert antioxidant action by donating of a hydrogen atom to break the free radical chain (Gardon, 1990). Increasing absorbance at 700 nm indicates an increase in reducing ability. The extracts used in the study shows significant reducing power when compared with Vitamin C and Tannic acid used as standard antioxidants.

The carcinogenic and mutagenic ability of free radicals is due to the direct interactions of hydroxyl radicals with DNA molecules which play a critical role in carcinogenesis pathway (cancer formation). Hydroxyl radicals have the ability to cause damage to almost every biological cell and can be generated during biotransformation reaction in the body.

Superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which can subsequently produce extremely reactive hydroxyl radicals in the presence of divalent metal ions, such as iron and copper. Results in Figure 3.3.3 shows that the extracts used in the present study have significant hydroxyl radical scavenging abilities when compared with Vitamin C and Tannic acid. The hydroxyl radical detoxification may be used to the presence of polyphenols and flavonoids in the extracts.

Hydrogen peroxide scavenging activity of the various concentrations of extracts used in this study was presented in Figure 3.3.2 when compared with the standard antioxidants: ascorbic acid and tannic acid. Hydrogen peroxide is a very weak oxidant but can become very toxic when it is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (•OH) that can initiate lipid peroxidation and cause DNA damage (Sahreen et al., 2011). Hydrogen peroxide can also inhibit the activities of some enzymes by oxidizing their essentialthiol (-SH) groups. The extracts used in this study efficiently scavenged hydrogen peroxide whichmay be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide thereby neutralizing it into water.

Hydrogen donating ability is one of the indexes to ascertain the antioxidant properties of phytochemical. Radical scavenging activities are very important to prevent the damaging role of free radical in different diseases. DPPH is known to abstractlabile hydrogen and the ability to scavenge the DPPH radical is related to the inhibition of lipid peroxidation. The result in figure shows that the extracts used in this study has a significant free radical scavenging activities when compared with standard antioxidants: tannic acid and ascorbic acid. Thus, the extracts show higher scavenging ability over vitamin C. This indicates that they could have a better free radical scavenging ability and inhibit lipid peroxidation more efficiently than Vitamin C.

In the presence of Calcium, the extracts significantly inhibited mitochondrial membrane permeability transition pore opening (MPT) in the selected organ of diabetic rats as compared to results obtained in the absence of Ca2+. This is almost the same with the report of Ajayi et.al., 2019 which indicates that the extract was able to inhibit large amplitude swelling caused by exogenous Calcium alone. It equally corresponds to the work of Banerjee et. al., 2016. It is clear that apoptosis occurs under both physiological and pathological conditions, such as diabetes Merkle, 2009.

The results confirmed that the extract inhibited MMPT pore opening in the presence of Ca2+. The inhibition of MPT by the extract invariably may led to significant decreases in the activity of caspase 3, which is a downstream factor necessary for apoptotic cell death to occur. Thus, the ethanol extract of Clerodendrum volubile may be able to protect liver against damage, resulting from Ca2+ overload that may trigger cell death, and as such it may be useful in the management of diseases related to tissue wastage such as cardiomyopathy and nephropathy which are associated with Type 2 diabetes mellitus. The results of this study justify the traditional uses of the Clerodendrum volubile leaves for therapeutic purposes. It has also revealed its efficacy as a radical scavenger and its ability to enhance the reduction in the concentration of molecular oxygen which enhances a smooth transfer of electron in the electron transport system.

The findings could also be of commercial interest to both pharmaceutical companies and research institutes towards the production of new drugs.On Fourier Transform Infrared Spectroscopy (FTIR) results OH dominated another functional group present and as such the most active functional group.

Clerodendrum volubile leaves extract was not able to regenerate injured rat skinfibroblast at a concentration of 1200 µg/ml and 1500 µg/ml respectively over 48 hours but not beyond 72 hours post-treatment compared to control.

Results in this study revealed in vitro free radical scavenging activities of the extracts on hydrogen peroxide, hydroxyl radicals, and DPPH. The high reducing powers of the extracts were also corroborated with the flavonoids and phenolic contents. This work demonstrated that the extracts possessed significant antioxidant activities over well characterized standard antioxidants, thus, suggesting the extracts as sources of natural antioxidants that could have great therapeutic relevance in preventing the progression of diseases associated with oxidative stress. The inhibition of MMPT pore by the ethanol and aqueous extract will invariably be led to significant decrease in the activity of caspase 3 which is necessary for apoptotic cell death to occur. Thus, both extracts of Clerodendrum volubile may be useful in the management of cell wasting diseases which is associated with diabetes mellitus.

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