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Antioxidant Activity and Pharmacognostic Evaluation of the Methanol Leaf Extract of *Margaritaria discoidea* (Baill.) G.L Webster (*Phyllanthaceae*)

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Abstract: Free radicals have been proved to be a major factor that leads to many known diseases. Antioxidants in plants have been established to be useful in the fight against diseases known to man. This study was therefore, conducted to evaluate the antioxidant activity of the methanol leaf extract of *Margaritaria discoidea*. *In vitro* studies were employed in this evaluation, which includes DPPH Scavenging activity, Ferric radical antioxidant power and Total antioxidant capacity assays. The plant extracts used were similar in all assays employed (15.63µg/ml-1000µg/ml). Ascorbic acid and Gallic acid were used as standards. The results obtained showed a significant antioxidant potential of the plant, proving that the methanol leaf extract of *Margaritaria discoidea* is potentially useful as an antioxidant. In addition, standardization, chemo-microscopy, Stomatal and Epidermal cell analysis and Transverse section of the leaf of the plant were done. Similarly, phytochemical analysis was carried out. The results obtained showed high flavonoid and total phenolic content which lent credence to the justification of the high potential antioxidant capacity of *Margaritaria discoidea*.

Key words: Antioxidant · Redox-Reaction · Free Radical · Phytochemical · Chemo-Microscopy · *Margaritaria discoidea*

INTRODUCTION

When a compound or atom is atomized, it loses electrons; when it is reduced, it gains electrons. Thus, oxidation is broadly the addition of oxygen or removal of hydrogen. It is also significant that oxidation and reduction reactions occur in pairs; if one specie is oxidized, then another must be reduced at the same time – hence the coinage of the term 'Redox reaction' [1]. The oxidation and reduction of organic molecules form very important part of many organic reactions and synthesis.

A free radical is an atom or group of atoms that has at least one unpaired electron and is therefore unstable and highly reactive. Free radicals can damage cells and are believed to accelerate the progression of disease condition. Free radicals are generated in biological systems in the form of reactive oxygen species that are harmful [2].

Reactive Oxygen Species (ROS) is a term that encompassed all highly reactive, oxygen containing molecules including free radical. Types of ROS include the hydroxyl radical, hydrogen peroxide, superoxide anion radical, nitric oxide radical, singlet oxygen, hypochlorite radical and various lipid peroxides. These can react with cell membrane lipids, nucleic acid, proteins, enzymes and other small molecules [3].

Internally, free radicals are produced as a normal part of metabolism within the mitochondria, through xanthine oxidase, peroxisomes, inflammation processes, phagocytosis, arachidonate pathways, ischemia and physical exercise. External factors that help to promote the production of free radicals are smoking, environmental pollutants, radiation, drugs, pesticides, industrial solvents and ozone. It is ironic that these elements, essential to life (Especially oxygen) have deleterious effects on the human body through these reactive species [4].

The balance between the production and neutralization of ROS by antioxidants is very delicate and if this balance tends to the overproduction of ROS, the cells start to suffer the consequences of oxidative stress.

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Summarily, oxidative damage caused by ROS leads to DNA lesions, loss of functions of enzymes, increased cell permeability, disturbed signaling over the cell and eventual necrotic cell death or apoptosis [5].

Antioxidants are group of substances which when present at low concentrations, in relation to oxidizable substances, significantly inhibit or delay oxidative processes, while often being oxidized themselves.

In recent years there has been an increased interest in application of antioxidants in medicine as information is constantly gathered linking the development of human diseases to oxidative stress [6].

Models for Assessing Antioxidant Activity of plants: Both *in vitro* and *in vivo* assay methods are employed.

***In vitro* Assays:** In ethno-pharmacological and nutraceutical investigations, *in vitro* antioxidant activity assessment methods are often used to screen and confer antioxidant potential to plants or their phytochemicals [7]. In the case of medicinal plants, these assays are used to confer free radical scavenging activity to plants, which in turn has great importance in understanding the role of plants in minimizing the oxidative stress linked to pathophysiology of diseases.

Other commonly used antioxidant activity assessing methods such as ferric reducing antioxidant power (FRAP) and copper reduction assay involve SET mechanisms [8]. However, some methods, such as 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and {2, 2'–azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid)} (ABTS), involve both HAT and SET mechanisms [9].

***In vivo* Assays:** Within *in vivo* assays, plant antioxidants are generally assessed for their effects on the activity of endogenous antioxidant enzymes or oxidative damage biomarkers before and after induction of oxidative stress in experimental animals.

The formation of specific end products resulting from interaction of ROS with biologically important macromolecules such as DNA, protein and lipids is measured by quantifying oxidative damage biomarker methods. DNA damage is determined by measuring the 8-hydroxydeoxyguanosine content. Carbonyl and aldehyde (e.g. malondialdehyde) contents are measured as markers of protein and lipid oxidation, respectively [10].

Diphenyl-2-Picrylhydrazyl (DPPH) Scavenging Assay: The molecule 1, 1-diphenyl-2-picrylhydrazyl (DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet colour characterized by an absorption band in ethanol solution centered at about 517nm. When a solution of DPPH is mixed with that of a substance (MX) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour.

In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. The percentage of the DPPH radical scavenging is calculated using the equation as given below;

% inhibition of DPPH radical = $([A_{br}- A_{ar}]/A_{br})$ X 100. When A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place [11].

Total Antioxidant Capacity: Total antioxidant capacity assay is spectroscopic method of the quantitative determination of antioxidant capacity, through reduction of Mo (VI) to Mo(V) by the sample analyse and subsequent formation of a green phosphate Mo(V) complex at acidic PH. Total antioxidant capacity can be calculated by the method described by Jan *et al*. [12].

Ferric Reducing antioxidant Power (FRAP) Assay: The reducing power of the crude extract fractions are usually evaluated as described by Jones *et al*. [13]. This ferric reducing antioxidant power of extract is expressed as gallic acid equivalent.

Ethno-Medicinal and Pharmacological Uses: It is commonly used in West Africa as a purgative and as an anthelminthic in Central Africa [14]. When dried and powdered, it is rubbed on the body in febrile conditions and in Central Africa a bark-preparation is rubbed into scarifications as a stimulant and tonic. In Malawi, powdered bark extract is applied to swellings and inflammation.

MATERIALS AND METHODS

Collection, Identification and Preparation of Plant Material: The leaves of *Margaritaria discoidea* were collected from Nsukka in Enugu State in May 2016 and authenticated by Mr. Nwafor Felix, a taxonomist in the Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka. The herbarium sample (PCG/UNN/0306) is deposited in the herbarium.

Sand particles were removed and the leaves were dried under shade for two weeks. The leaves were pulverized into fine powder using a milling machine and stored in an airtight container.

Extraction: The methanol extract was obtained by macerating 400g of the leaf powder with 1.5 liters of absolute methanol for 3 days and filtered using 12.5mm No. 1 Whatman filter paper. The crude methanol extract was concentrated by pouring into a beaker and allowing the volatile methanol to evaporate to dryness for 10 days at a room temperature in a cool airy place. The extract was scraped out of the beaker and stored in an airtight amber container and stored in a cool dry place.

Preparation of Different Concentration of Plant Extract: The stock solution for the antioxidant models were prepared by re-constituting 100mg of the extract in 10ml of absolute methanol as the solvent system to obtain a concentration of 10mg/ml of the extract. The extract was further diluted by a ten-fold serial dilution to obtain a diluted concentration of 1mg/ml. (1000µg/ml), using distilled water. The different extract concentrations required were obtained.

Preparation of 0.2M Phosphate Buffer (pH 6.6): The phosphate buffer of the concentration and pH above was prepared according to the method of Mubo *et al*. [15]

Antioxidant Tests on Plant Extract: 1, 1-diphenyl -2-picrylhydrazyl (DPPH) Radical Scavenging Asssay.

The DPPH free radical scavenging activity of methanol leaf extract of *M. discoidea* was determined according to the method reported by Mukherjee [16]

Ferric Reducing Antioxidant Power (FRAP) Assay: Ferric reducing antioxidant potential (FRAP) of the methanol leaf extracts of *M. discoidea* was measured according to the method proposed by Benzie and Strain [7]. FRAP reagent was prepared by mixing in 25 mL acetate buffer (30 mM; pH 3.6), 2.5 mL TPTZ solution (10 mM) and 2.5 mL ferric chloride solution (20 mM).

Total Antioxidant Capacity (TAC) Assay: To determine total antioxidant capacity (TAC) of *M. discoidea* methanol leaf extract as per phosphomolybdate assay, the procedure described by Nain *et al*. [17] was adopted.

Standardization Parameters

Microscopical Analysis: Foliar epidermis of the adaxial (Upper surface) and abaxial (Lower surface) surfaces of the leaves were prepared by clearing method. The leaf samples were cleared by soaking in commercial bleach "Hypo" containing 3.5% sodium hypochlorite for 18 hrs. Then, the epidermal strips of the leaf samples were scrapped gently with the aid of a pair of forceps and placed on a clean slide and then stained with Safranin solution and covered with a cover slip. The slides were viewed under a light Olympus Tokyo (Japan No.271961) microscope at x40, x100 and x400 magnifications and photomicrographs were taken with a Motican Camera 2.0. The following parameters were observed and assessed: Epidermal cells; the type and number of epidermal cells were counted and recorded, Stomata type: the stomata complex types were observed and recorded, Stomata size (Length and width): the stomata length and width were measured using Motic microscope software a total of 10 fields of views for each sample, Stomata frequency; the stomata density was determined as the number of stomata per square millimeter, Stomata index; the stomata index was determined as follows:

$$SI = \frac{N}{N+E} X100$$

where: N = Number of stomata in a field of view, E = Number of epidermal cells in the same field of view.

Trichome parameters; the trichome types, size, density and index were determined following the same procedures as the stomata above, Vein islet number and veinlet termination number.

All parameters were observed on both the adaxial and abaxial surfaces of the leaves [18].

Transverse section (TS) of the leaf was made using a Reichert sledge microtome. The sections were microtome at 10-15 microns and were picked with the aid of a camel hair brush from the tip of the microtome knife into separate Petri dishes containing 70% absolute alcohol and labeled appropriately. Safranin and Fast green served as biological stains in differentiating lignified tissues.

Physicochemical Analysis: Analysis of physicochemical constants of the leaf and stem bark powdered drug were determined to evaluate the quality and purity of the drug [19]. Parameters such as total ash, water-soluble ash and acid-insoluble ash values were calculated as per WHO guidelines. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components. The moisture content and pH were also determined.

Chemo-Microscopic Studies: The leaves were dried under shade and pulverized with local mortar and pestle. Chemo-microscopy was conducted on the powders to determine the presence of starch, calcium oxalate crystals and lignified vessels. A judicious quantity of the sample was dropped on a glass slide. One drop of chloral hydrate was dropped and passed over a Bunsen burner repeatedly until bubbles formed. This signified the successful clearing of the tissues. Thereafter, the tests for lignin, calcium oxalate crystals, starch grains and oil body were carried out.

Phytochemical Evaluation: The procedures for the analysis of the phytoconstituents were in accordance with the standard methods [20, 21].

RESULTS

Results of Percentage Yield
Weight of powder = 400g
Weight of extract = 30.35g
Percentage Yield = 7.6%

Results of DPPH Radical Scavenging Assay is presented in Fig. 1.

Similarly, the result of the DPPH radical scavenging assay by Ascorbic acid is presented in Fig. 2.

The result of the Ferric reducing power of the extract of the *M. discoidea* is shown in Fig. 3.

The result of the Ferric reducing Antioxidant power of Gallic acid is shown in Fig. 4.

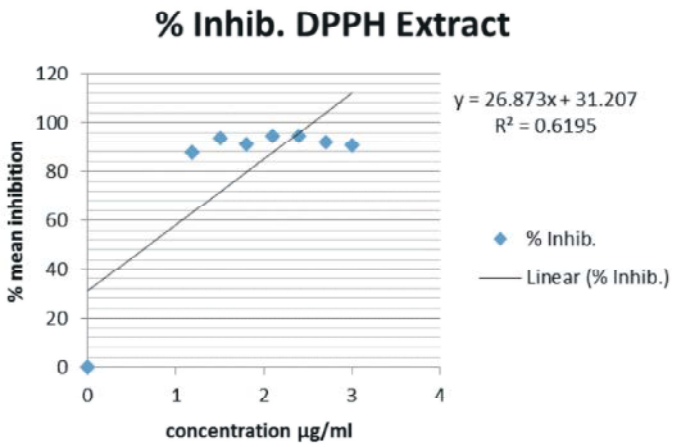


Fig. 1: DPPH radical Scavenging Assay of the Extract of M. *discoidea*
A Plot % means inhibition of DPPH against concentrations of extract
From the Scatter Plot above, the EC50 of the sample is 0.700µg/ml
Note: The lower the EC50, the more potent the sample.

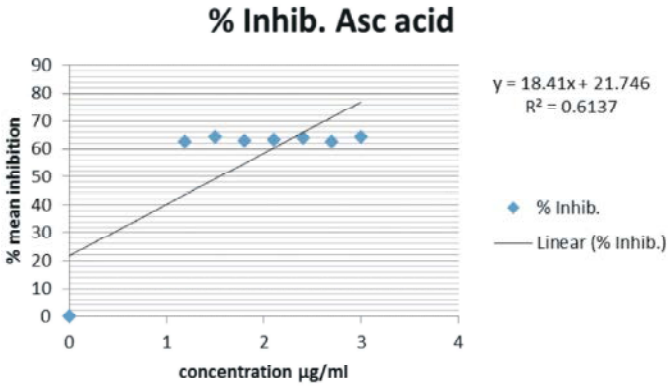


Fig. 2: DPPH Radical Scavenging Assay by Ascorbic acid
A plot of % means inhibition of DPPH against concentrations of Ascorbic acid
From the scatter plot above, the EC50 of Ascorbic acid is 1.54µg/ml.
Note: The lower the EC50, the more the antioxidant potency.

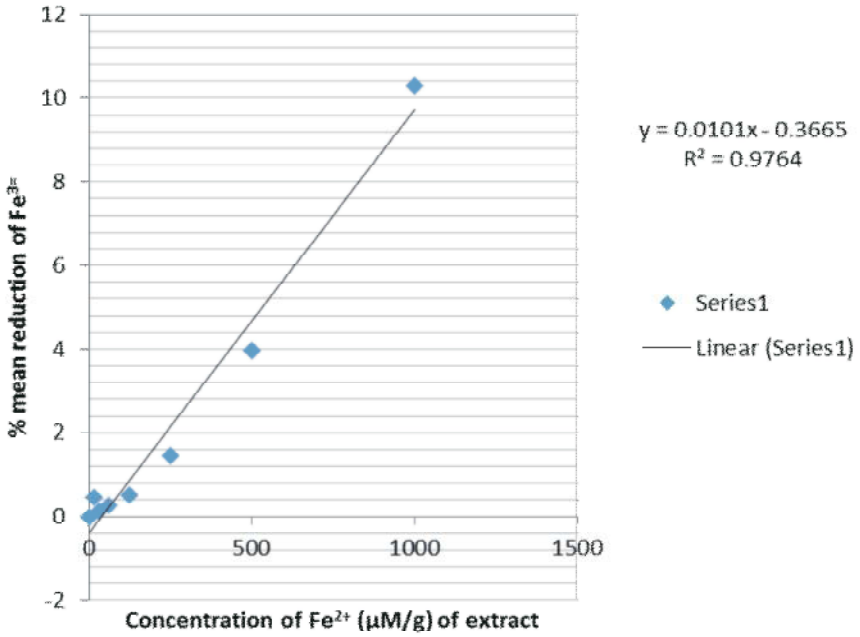


Fig. 3: Ferric reducing power of the extract of *M. discoidea*
From the scatter plot above, the EC50 of the Extract is
Fe^{2+} (µM/531.34g) of Extract of *Margaritaria discoidea*

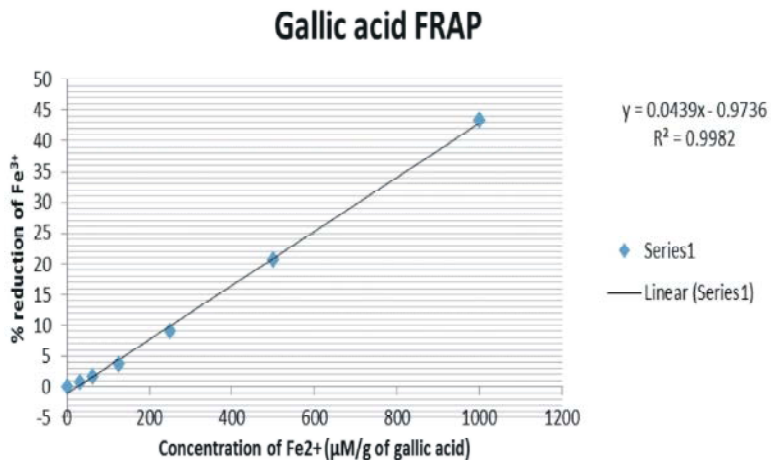


Fig. 4: Ferric reducing Antioxidant power of Gallic acid
From the scatter plot above, the EC50 of Gallic acid is
Fe^{2+} (µM/136.07g) of Gallic Acid
Result of the Total Antioxidant Capacity Assay

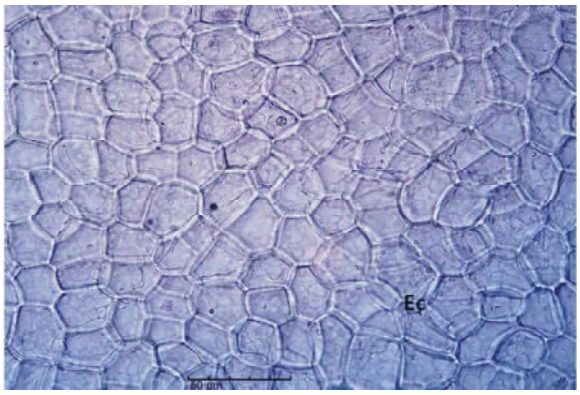
Table 1: Total Antioxidant Capacity Assay of the leaf extract of *Margaritaria discoidea*

Conc. µg/ml	Absorbance	Ascorbic acid equivalent
15.63	0.354	13.261± 0.461
31.25	0.381	7.138 ± 0.417
62.5	0.410	3.844 ± 0.061
125	0.443	2.076 ± 0.095
250	0.478	1.120 ± 0.103
500	0.514	0.177 ± 0.019
1000	0.302	0.602 ± 0.023

Values are expressed as Mean ± S.E.M
n=3

Results of the Chemo-Microscopy of the leaf of *Margaritaria discoidea*

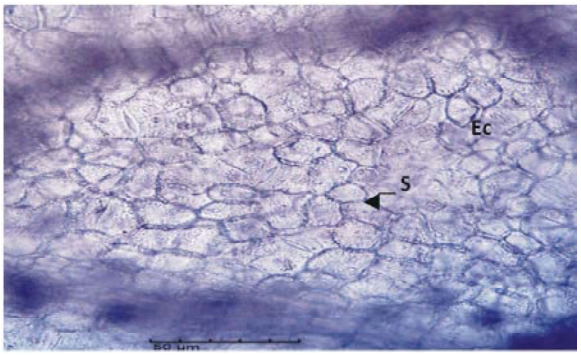
(i.)



X400

Plate 1: Adaxial surface of the leaf of *Margaritaria discoidea* showing polygonal shaped epidermal cells (Ec) and absence of stomata

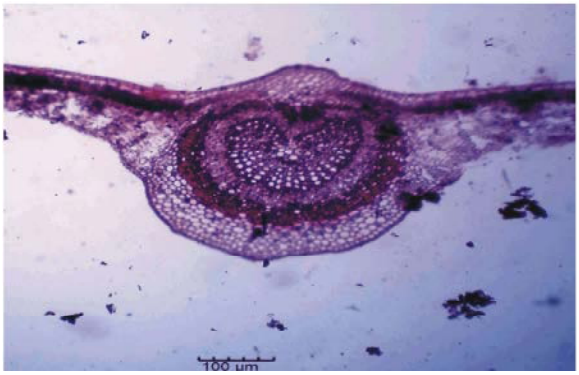
(ii.)



X400

Plate 2: Abaxial surface of the leaf of *Margaritaria discoidea* showing epidermal cells (Ec) and anomocytic type of stomata (St)

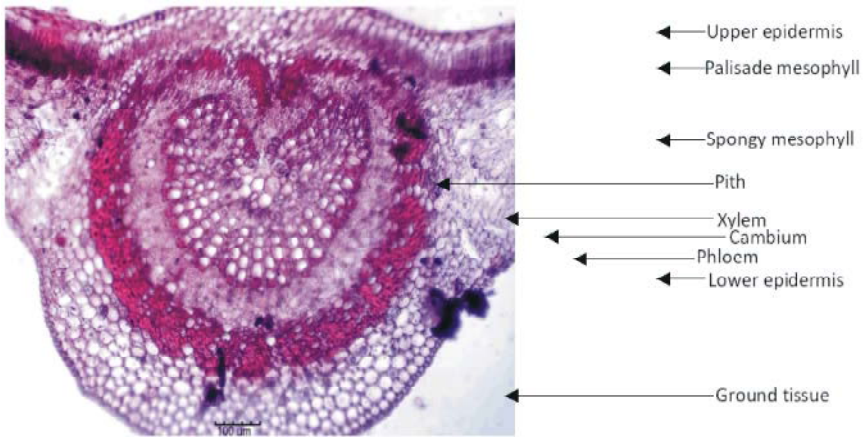
(iii.)



X40

Plate 3: Transverse section of the leaf of Margaritaria discoidea

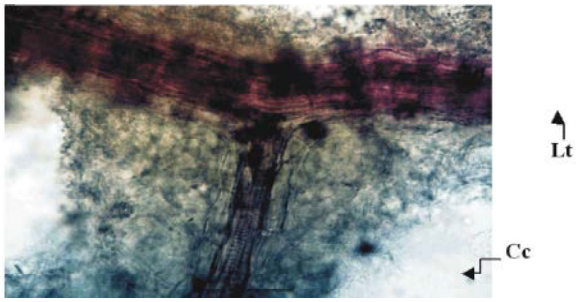
(iv.)



X100

Plate 4: Transverse section of the leaf of *Margaritaria discoidea*

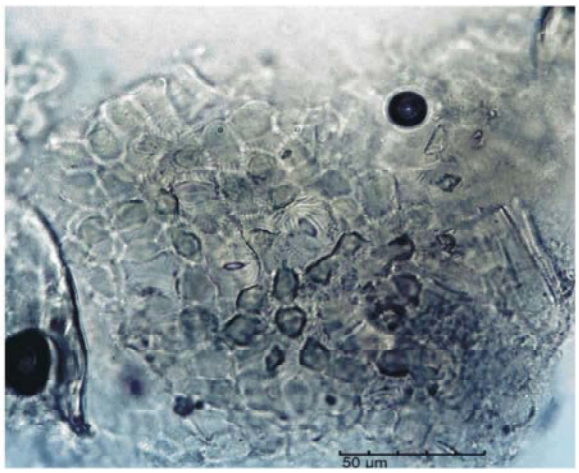
(v.)



X400

Plate 5: Chemo-microscopy of the powdered leaf of *Margaritaria discoidea* showing lignified tissues (Lt - vessels and fibres) and cork cells (Cc)

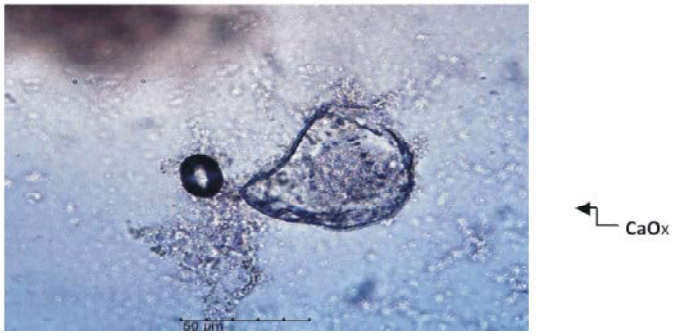
(vi.)



X400

Plate 6: Fragment of the leaf of *Margaritaria discoidea* in chemo-microscopy showing anomocytic type of stomata (St), epidermal cell (Ec) and crystal of calcium oxalate (CaOx)

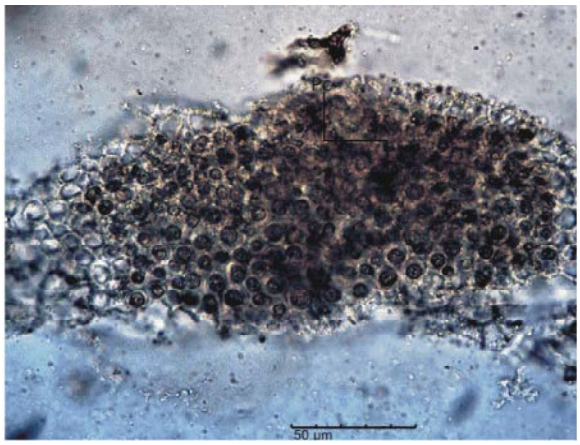
(vii.)



X400

Plate 7: Chemo-micrograph of the powdered leaf of *Margaritaria discoidea* showing a large crystal of calcium oxalate (CaOx)

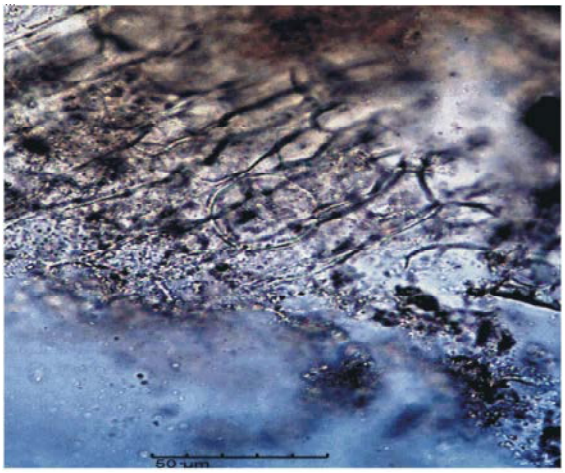
(viii.)



X400

Plate 8: Chemo-micrograph of the powdered leaf of *Margaritaria discoidea* showing a mass of palisade cells (Pc)

(x.)



X400

Plate 9: Chemo-micrograph of the powdered leaf of *Margaritaria discoidea* showing cork cells and epidermal cells

Table 2: Quantitative leaf microscopic features of *Margaritaria discoidea*

Features	Characteristics
Stomata Frequency	133.33 ± 7.08
Stomata index (%)	11.88 ± 0.55
Stomata length	20.80 ± 2.60
Stomata width	11.82 ± 0.63
Stomata size	249.10 ± 45.36
Vein-islet number	24.25 ± 3.46
Veinlet termination number	25.14 ± 3.72
Palisade ratio	10.23 ± 1.05

Table 3: Physicochemical constants from the leaves of *Margaritaria discoidea*

Parameter	Mean values (%)
Total Ash	4.36 ± 0.05
Acid-insoluble Ash	0.81 ± 0.02
Water-soluble Ash	2.02 ± 0.11
Water-soluble extractives	4.52 ± 1.02
Alcohol-soluble extractives	6.15 ± 1.51
Moisture content	4.71 ± 0.92

n=3

Phytochemical Analysis Result

Table 4: Qualitative Phytochemical Analysis

Test	Result
Alkaloids	+
Flavonoids	+++
Tannins	++
Phenols	++
Saponins	+
Terpenoids	+
Steroids	+
Reducing Sugars	+
Glycosides	+

+ = mildly present

++ = moderately present

+++ = highly present

DISCUSSION AND CONCLUSION

The yield of 7.6% of the extract was appreciable with the use of methanol as the solvent of extraction. This makes methanol economically viable and efficient.

In DPPH Radical Scavenging Assay, the results obtained showed that all the concentrations of the sample had above 87% inhibition of DPPH, showing a substantially very high DPPH scavenging activity. DPPH is a stable nitrogen- centered free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [22]. The results also revealed that the inhibiting ability of the sample peaked at the 62.5µg/ml concentration and achieved equilibrium at 125µg/ml and 250µg/ml, then decreases at 500 and 1000. This shows that highest beneficial activity on DPPH is achieved at 62.5, beyond which it turns pro-oxidant.

The decrease in absorbance of DPPH caused by antioxidants is due to the reaction between the antioxidant and radical, which results in the scavenging of the radical by hydrogen donation [23].

From the DPPH bar chart, the antioxidant radical power of sample on DPPH was found to be far more that of Ascorbic acid standard, which lends credence to its good potential use in the near future.

In the Ferric reducing Antioxidant Power Assay model, the ability of *Margaritaria discoidea* extract to reduce Fe^{3+} to Fe^{2+} was determined and compared with that of a standard, gallic acid. Fe^{2+} transformation in the presence of the extract was investigated and all the concentrations of the extract used for experimentation were found to have significant reducing ability. Nevertheless, it was shown from the results obtained that Ferric reduction occurs at lower concentration of gallic acid than the extract, proving gallic acid to be more effective in FRAP analysis and potentially, a more effective antioxidant. The reducing power of compound is associated with its electron donating capacity [24, 25]. It may serve as a significant indicator of its potential antioxidant activity [26].

From the Scatter plot of the FRAP Assay on the extract, the EC50 was shown to be 531.34, while the Scatter plot of the FRAP Assay on the standard drug, gallic acid, showed its EC50 to be 156.07g. This shows gallic acid to be more effective than the sample at lower concentrations to reduce Fe3+ to Fe2+.

The Total Antioxidant Capacity (TAC) of *Margaritaria discoidea* extract which was expressed as ascorbic acid equivalents showed that *Margaritaria discoidea* has significant antioxidant activity as concentration increases. The TAC Assay is a measure of the ability of the sample to reduce Mo (VI) to Mo (V) and the subsequent formation of a green phosphate Mo (V) at acidic pH. The antioxidant activity increased steadily with increase in concentration until at 500µg/ml where activity peaked and began to decrease at 1000µg/ml. This shows that the extract turns pro-oxidant at high concentration. In comparison with the standard ascorbic acid, the capacity of ascorbic acid decreased with increasing concentration while that of the extract increased with increasing concentration, until at high concentration of 1000µg/ml, where the capacity of the extract began to decrease. With good reduction effect of Mo (VI) to Mo (V) at very low concentration by ascorbic acid relative to the extract, Ascorbic acid proved to be more effective.

Considering the phytochemical analysis, Jones [13] reported that flavonoids, triterpenes and tannins were antioxidant agents and that they interfere with free radical formation. From the result of the Qualitative Phytochemical analysis, it showed that flavonoids were highly present, while tannins and phenols were moderately present, with saponins and terpenoids mildly present. This shows that flavonoids, tannins and phenols were majorly responsible for the antioxidant activity of the extract.

Further quantitative analysis was done to verify the quantity of total phenolics, tannins and flavonoids present in the sample. The result showed that a 100g of the sample yields 11013.44mg, 23.76mg and 299.69mg of total phenolics, tannins and flavonoids. This shows the sample has huge potential for antioxidant effect.

The pharmacognostic analysis of *M. discoidea* done will assist in stamping its botanical identity. The standardization of the herbal medicines is important to assure the quality of the drug and will also help in checking and preventing substitution and adulteration of foreign material, that is by mixing or substituting the original drug material with other spurious, substandard, defective, spoiled, useless other parts of the same or different plants [23]. It is well known that plant drugs are used locally for treatment of various disease conditions without recourse to standardization in other to establish the correct identity of the drug [20].

The Microscopic evaluation of the leaf of *M. discoidea* showed that the leaf has anomocytic stomata occurring at both adaxial and abaxial epidermis thus indicating efficient gaseous exchange for photosynthesis and loss of water.

Chemo- microscopic evaluation of the leaf showed starch, tannins including calcium oxalate crystals and lignins which are indications of the presence of alkaloids, flavonoids and glycosides in the leaves.

Quantitative microscopic evaluation of the leaf of *M. discoidea* has provided values for palisade ratio, stomatal number, epidermal layer, stomatal indices, vein islet number and veinlet termination number. These information set genuity and standard for the herb as well as distinguishing it from co-generic species that may be closely related and cannot easily be characterized by general microscopy.

The physico-chemical evaluation of the leaves of *M. discoidea* could serve as a significant role in standardization and quality control by means of purity, stability and phytochemical composition of the herb. The moisture content for *M. discoidea* calculated by loss was 4.71 ± 0.92% which is less than 14% standard requirement for crude drugs. This is an indication that the powder of this herb can be stored for a longer period of time without spoilage [11]. The total cash value for *M. perotitii* lies within fair limits and thus signified its quality and purity and gives idea about the total inorganic content in it [9]. The acid- insoluble ash value of 0.81±0.02% obtained for the leaves is an indication that the herb was in good physiological ash produced due to adherence of inorganic dirt and dust to the crude drug.

CONCLUSION

In conclusion, the results of the present study indicated that under the present experimental conditions, methanol leaf extract of *Margaritaria discoidea* showed significant antioxidant activities against oxidation induced by DPPH radical, Ferric ion and molybdenum (VI), with that against DPPH, proving strongly that it should vehemently be considered for future development in this field.

Also, the pharmacognostic parameters of the leaf of *Margaritaria discoidea* which have been evaluated and the results obtained can be considered as distinctive characters of this plant part and serves as standards to authenticate and assure the quality of the drug in herbal industry to prevent adulteration. The findings from the phytochemical analysis showed that there was a significant presence of Flavonoids and Total phenolics, both in the qualitative and quantitative analysis, supporting its antioxidant potential. These findings will contribute to solving the problem of lack of information on herbal medicinal materials and form part of a monograph for leaf of *Margaritaria discoidea* in a future African Herbal Pharmacopeia.

REFERENCES

1. Abdulrahman, A.A. and F.A. Oladele, 2005. Stomata, trichomes and epidermal cells as diagnostic features in six species of the genus *Ocimum* L. (*Lamiaceae*). Niger. J. Bot., 18: 214-223.
2. Alam, N., N.Y. Ki, J.S. Lee, H.J. Cho and T.S. Lee, 2012. Consequences of the antioxidant activities and tyrosinase inhibitory effects of various extracts from the fruiting bodies of *Pleurotus ferulae*, Saudi. J. Biol. Sci., 19: 111-118.

3. Alam, M.N., N.J. Bristi and M. Rafiquzzaman, 2013. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharm. J., 21: 143-152.
4. Antolovich, M., P.D. Prenzler, E. Patsalides, S. McDonald and K. Robards, 2002. Methods for testing antioxidant activity. Analyst, 127: 183-198.
5. Arulmozhi, S., P.M. Mazumder, L.S. Narayana and P.A. Thakurdesai, 2010. *In vitro* antioxidant free radical scavenging activity of fractions from *Alstonia scholars* Linn. R.Br. Int. J. Pharma Tech. Res., 2: 18-25.
6. Babu, B.H., B.S. Shylesh and J. Padikkala, 2001. Antioxidant and hepatoprotective effects of *Acanthus ilicifolius*. Fitatherapia, 72: 272-277.
7. Benzie, I.F.F. and J.J. Strain, 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol., 299: 15-27.
8. Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28: 25-30.
9. Burkill, H.M., 1994. The useful plants of West Tropical Africa. 2nd Edition. Volume 2, Families E-I. Royal Botanic Gardens, Kew, Richmond, United Kingdom, pp: 636.
10. Chemwiki, 2011. Section 16:1: Oxidant and reduction of organic compounds - an overview. Retrieved from http://chemwiki.uc.davis.edu/organic chemistry/organic chemistry with a biological basis/chapter 16 oxidation and reduction reactions/section 16.1: oxidation and reduction of organic compounds an overview.
11. Halliwell, B. and J.M. Gutteridge, 1995. The definition and measurement of antioxidants in biological systems. Free Radic. Biol. Med., 18: 125-126.
12. Jan, S., M.R. Khan, U. Rashid and J. Bokhari, 2013. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. Osong Public Health Res. Perspect., 4: 246-254, doi:10.1016/j.phrp.2013.09.003.
13. Jones, D.P., 2008. Radical free biology of oxidative stress: Am. J. Physiol. Cell. Physiol., 295(4): C849-C868
14. Lobo, V., A. Phatak and N. Chandra, 2010. Free radicals and functional foods: Impact on human health. Pharmacogn. Rev., 4: 118-126.
15. Mubo, A.S., A.O. Tolulope and O.S. Mike, 2014. A Pharmaco-botanical Study of two Medicinal species of *Fabaceae*. Asian Pac. J. Trop. Biomed., 4(2): 131-136.
16. Mukherjee, P.W., 2002. Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. New Delhi, India: Business Horizons Publishers.
17. Nain, P., K. Ashok, S. Sunil and N. Jaspreet, 2011. *In vitro* evaluation of antimicrobial and antioxidant activities of methanolic extract of *Jasminum humile* leaves. Asian pacific J. Trop. Med., pp: 804-807.
18. Palaniswany, R. and P.R. Padma, 2011. Free radical scavenging activity of *Majorana hortensis* leaves. Anc. Sci. Life, 30(4): 96-99.
19. Periyanayagam, K. and V. Karthikeyan, 2013. Pharmacognostical, SEM and XRF profile of the leaves of *Artocarpus heterophyllus* L. (*Moraceae*) - A contribution to combat the NTD. Innov. J. Life Sci., 1(1): 23-28.
20. Prieto, P., M. Pineda and M. Aguilar, 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem., 269: 337-341.
21. Prior, R.L., X. Wu and K. Schaich, 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem., 53: 4290-4302.
22. Rokad, N., J. Pande and S. Chanda, 2018. Pharmacognostic and phytochemical studies of Ipomoea pes-caprae, An halophyte from Gujarat. J. Pharmacog. Phytochem, 7(1): 11-18.
23. Sahreen, G.K., R. Jagonmohen and K.K. Sakariah, 2004. Antioxidant activities of flavidin in different in vitro model systems. Bioorg. Med. Chem., 12: 5141-5146.
24. Siddhuraju, P. and K. Becker, 2002. Effect of phenolic nonprotein amino acid L-dopa (L-3, 4-dihydroxyphenylalnine) on growth performance, metabolic rates and feed nutrient utilization of common carp (*Cyprinus carpio* L.). Aquacult. Nutr., 8(1): 69-77.
25. Ugur, C. and K. Selima, 2013. Nitrate, Moisture and Ash Contents of Edible wild plants. J. Cell. Plant Sci., 2(1): 1-5.
26. Wagg, S.S., G.P. Vadnere, A.V. Patil and S.K. Jain, 2012. *In vitro* free radical scavenging and antioxidant activity of *Cicer arietinum* L. (*Fabaceae*). Int. J. Pharm. Tech. Res. CODEN (USA), 4(1): 343-350.