IN VITRO AND *EX-VIVO* ANTI-OXIDANT EFFECT OF *CORCHORUS OLITORIUS* (JUTE) SEEDS

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ABSTRACT

The need for more studies on antioxidant effects of Corchorus olitorius (Jute) commonly used in traditional western part of Nigeria, has been on the increase. Thus, the antioxidant effects of Jute seeds have been explained in this study. The ethanol and hexane extracts of Corchorus olitorius seeds were prepared and phytochemical constituents examined. Their ability to prevent oxidation was also examined in vitro by reacting with DPPH after observing the decolonization of the reaction. Ex vivo, the levels of MDA and the ability of the extracts to increase Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) activity was also examined. Qualitative phytochemical analysis showed that ethanol extracts had phenols, tannins, alkaloids, flavonoids, saponins and terpenoids, while hexane extracts showed alkaloids. flavonoids and terpenoids only. The results of in-vitro antioxidant assay result showed a dose dependent increase in DPPH free radical scavenging activity in both ethanol and hexane extracts. In the ex-vivo studies, Ethanol extract of MDA levels increased with increasing concentration while hexane extracts decreased with increasing concentration. Corchorus olitorius significantly increased the inhibitory percentage (%) of pyrogallol autoxidation SOD highest at ethanol extract 100mg and hexane extract 200mg, while the activity of GPx was increased significantly at hexane extracts 200mg.

Keywords: *Medicinal plants, antioxidants, Corchorus olitorius, jute*

1. INTRODUCTION

Plants have provided nourishment and other health benefits to humans and animals for as long as they have existed. According to reports, around 80% of the world's population rely on natural products and plant-based medicines as their primary source of medication [1]. About 25% of all medications are derived from 500 various herbal plant species [2]. Medicinal plants have a promising future because there are about a million plants species around the world, whose medicinal activities have not been investigated yet, could be decisive in the treatment of present or future diseases [3]. The use of medicinal plants in the treatment and management of diseases has gained prominence worldwide, especially in the developing countries where an estimated 80% of the African Member States population use traditional medicine as a primary source of health care [1][4].

Medicinal plants are increasingly gaining acceptance even among elites in urban settlements, probably due to the increasing ineffectiveness of many modern drugs used in the treatment of many infectious diseases, antimicrobial drug resistance and increasing cost of prescription drugs for the maintenance of personal health. Different plants parts and components (roots, leaves, stem barks, flowers or their combinations, essential oils) have been employed in the treatment of infectious pathogens in the respiratory, urinary tract, gastrointestinal and biliary systems as well as on the skin [5]. Like every other medicinal plant, *Corchorus olitorius* is a vast and common medicinal plant with much attention given to the leaves and stems. This has provided insight to the nutritional and medicinal potentials of the plant in recent years.

Oxidative stress in living organisms results from the imbalance between the production of reactive oxygen species (ROS) and the ability to neutralize them [6]. The long-term effect of ROS level includes damages to important cellular components especially protein, nucleic acids and polyunsaturated fatty acids in cell membranes and plasma

lipoproteins [7]). Research has also shown that superoxide (O_2^-) and hydroxyl radicals (OH^-), have been associated with heart disease and carcinogens.[8] Oxidative and nitrosative stress contribute to the development of many chronic diseases, including hypertension, diabetes melitus, atherosclerosis, disturbance of wound healing, neoplastic diseases, eye disorders, brain disorders, neurodegenerative diseases such as Alzheimer's or Parkinson's disease, autoimmune diseases, and aging [9]. There are many complications and side effects usually reported from many anti-inflammatory drugs such as the popular Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), aspirin which causes adverse gastric reactions, inhibit renal function, reduce the efficacy of the diuretics and retard the angiotensin converting enzyme inhibitors. This unpleasant side effects associated with prolonged use of current anti-inflammatory drugs have necessitated a need for alternate treatment regime with limited side effects. However, as vast and common as the jute plant has been in different regions in Nigeria and beyond, little attention has been given to the seeds. Hence, the need for the studies on antioxidant potentials of *Chorchorus olitorius* seeds.

2. MATERIAL AND METHODS

2.1 Plant Sample Collection and Extraction

The whole dried seeds of *Corchorus olitorius* was purchased from Orange Market, Mararaba, Karu LGA, Nasarawa State (9.0267° N, 7.6074° E), and taken to the Department of Plant Science and Biotechnology, Nasarawa State University Keffi for identification. The dried seeds were grinded and sieved using a filter of 0.5mm mesh size to provide a greater surface area [10] and weighed. An average of 14g of the dried powder was filled in the porous cellulose thimble and subjected to soxhlet extraction using 98% ethanol and hexane [11] for 12 hours at 70^{o}C. The extracts obtained were concentrated to dryness at 45^{o}C using a rotary evaporator under reduced pressure and the extracts were weighed and then stored at 4^{o}C for further use [12]. The percentage yield was calculated as follows:

$$\text{Percentage Yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}} \times 100$$

2.2 DPPH Radical Scavenging Activity Assay

The DPPH free radical scavenging activity assay was performed according to a method described by [13]. 200 µL of 0.1 mM DPPH prepared in methanol will be added to 100 µL of the plant extract. The resulting mixture was incubated at room temperature in the dark for 15 minutes. Absorbance was observed at 517 nm. The experiment was carried out in triplicates and percentage inhibition of the DPPH radical scavenging activity was calculated.

2.3 Preparation of Rat Liver Homogenate (LH) for Ex-Vivo Antioxidant Assay

Five Rats weighed, anaesthetized using ether and dissected. Livers were isolated and washed in normal saline. Ten grams (10g) of livers were cut into pieces and homogenized in 100ml PBS at 700g in ice for 10min, centrifuged at 5000rpm for 15min. the supernatant was stored at -20^{0}C for further use.

2.4 Determination of Malondialdehyde (MDA)

Lipid Peroxidation (LPO) could be initiated invitro by Ferrous Sulphate through hydroxyl radical in Fenton's reaction [15]. The extent of LPO could be estimated by the amount of MDA generated [16]. One molecule of MDA reacts with two molecules of TBA with the production of pink pigment which absorbs at 552 nM

2.5 Determination of Superoxide Dismutase Activity (SOD)

Twenty microliters (20ml) of LH, buffer and 20µl of each extract separately and completed to 1ml buffer solution tris buffer, the incubated at 37^{0}C for 45min. after which 10µl of pyrogallol were added (reaction took place in quartz cuvette with 1cm light path) and the absorbance was read at 420 nm against air. Afterward, the change in absorbance per minute was determine

2.6 Determination of Glutathione Peroxidase Activity (GPx)

Fifty microliters of LH were added to equal volumes of each extract and incubated for 45 min at 37 ºC. Subsequently, 100 µl GSH, 100 µl stock solution of cummen H_2O_2 and 750 µl Tris-HCl (pH 7.6), were added and incubated at 37 ºC for 10 min. One milliliter TCA was added then centrifuged at 300 rpm for 20 min and the supernatant was collected. One milliliter of the supernatant was added to 2 ml Tris-HCl (pH 8.9) and 100 µl DTNB, then incubated for 5 min at 25 °C. The absorbance was measured at 412 nm against distilled water**.**

2.7 Ethical Clearance

Ethical approval was obtained from the NSUK Animal care and use research ethics committee, Nasarawa State University, Keffi, Nasarawa State (Approval No.: NSUK-ACUREC/BCH/23/10-07/03/2023).

3. Results and Discussion

3.1 Total Percentage Yield

The percentage yield recovered of the ethanol and hexane extract from *Corchorus olitorius* is presented in Figure 1.0. The ethanol and hexane extracts of *Corchorus olitorius* were each weighed immediately after evaporation in water bath and compared to the original weight of the dried powdered sample. Weight of Dried powdered sample before extraction= 200g. Weight of ethanol extract after extraction= 34g. Weight of hexane extract after extraction=19g

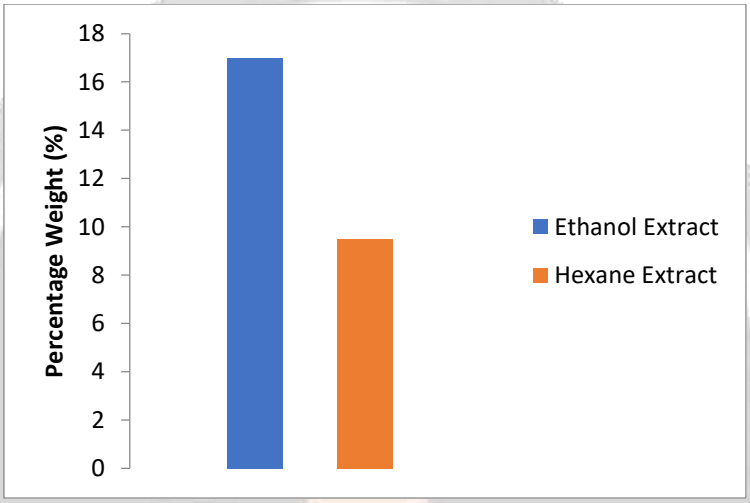

Chart-1: Total Percentage (%) Yield of Ethanol and Hexane Extracts after Extraction

Table 1: Phytochemical screening of ethanol and hexane extract of *Corchorus olitorius* seeds.

Phytochemicals Presence	EtOH Extract(mg/100g)	Hexane Extract(mg/100g)
Phenols	+	-
Tannins	+	-
Alkaloids	+	+
Flavonoids	+	+
Cardiac glycosides	+	-
Saponins	+	-
Glycosides	-	-
Terpenoids	+	+
Steroids	-	-

"-", and"+"indicate negative andpositive reaction respectively

Phytochemical analysis revealed the presence of phenols, tannins, alkaloids, flavonoids, cardiac glycosides, sapponins and terpenoids through the ethanol extract while only alkaloids, flavonoids and terpenoids were present in the hexane extract.

3.2 Radical Scavenging Activity of *Corchorus olitorius* Against DPPH

Figure 2 shows the results of the free radical (DPPH) scavenging activity inhibition. The result revealed that the ethanol extract of *Corchorus olitorius* seeds exhibited the highest DPPH radical scavenging activity with 98.15% at 500 µg/ml concentration which appears to be slightly above the value of vitamin C i.e., 90.20% at 500 µg/ml while hexane extract exhibited highest DPPH radical scavenging activity with 35.37% at 500 µg/ml concentration which appears to be very far below the value of vitamin C i.e., 90.20% at 500 µg/ml. The same is followed by 89.64%, 80.12%, 79.59% and 79.23% for ethanol and 29.8%, 29.17%, 27.6% and 25.83% for hexane at the concentrations of 250 µg/ml, 125 µg/ml, 62.5µg/ml and 31.25 µg/ml l respectively. The percentage inhibition of the ethanol extracts as shown in table above appears to vary widely across concentrations more than the way it did with the standard vitamin C. At 250 µg/ml, the percentage inhibition of *Corchorus olitorius* and Vitamin C are much closer than decreasing from 125 µg/ml concentration down to 31.25 µg/ml

The plot of percentage inhibition against concentration of the DPPH comparison between *Corchorus olitorius* and the standard vitamin C has shown that *Corchorus olitorius* proves more effectiveness with higher concentration. The effect also decreased with decreasing concentration

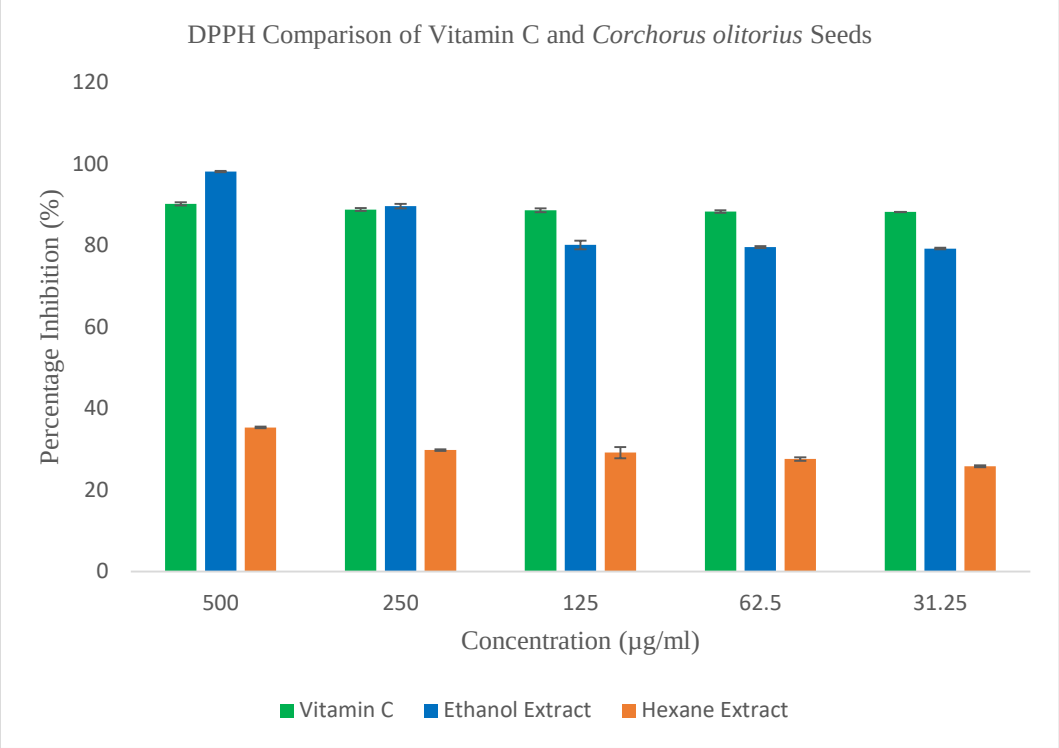

Chart-2: Comparison of %Radical Scavenging Activity against Concentrations of Ethanol and Hexane Extract

3.3 *Ex Vivo* antioxidant activity of Ethanol and Hexane Extract of Corchorus Olitorius

3.31 Determination of Malondialdehyde (MDA) levels

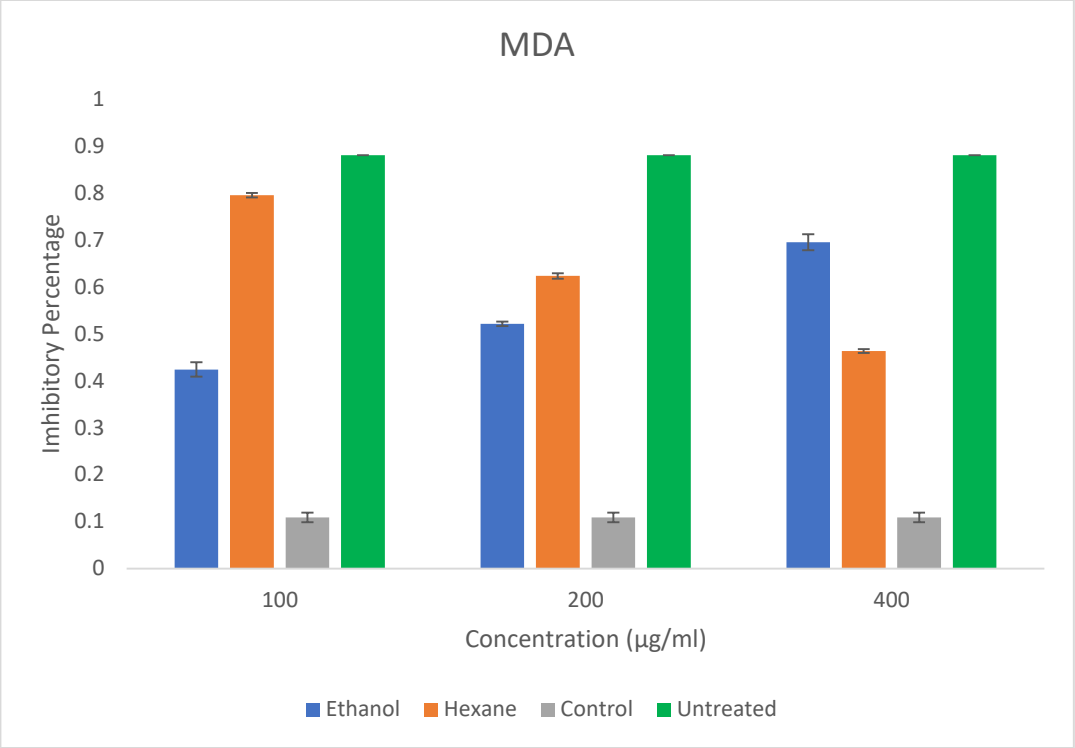

Chart-3: Malondialdehyde (MDA) levels

3.32 Glutathione Peroxidase Activity

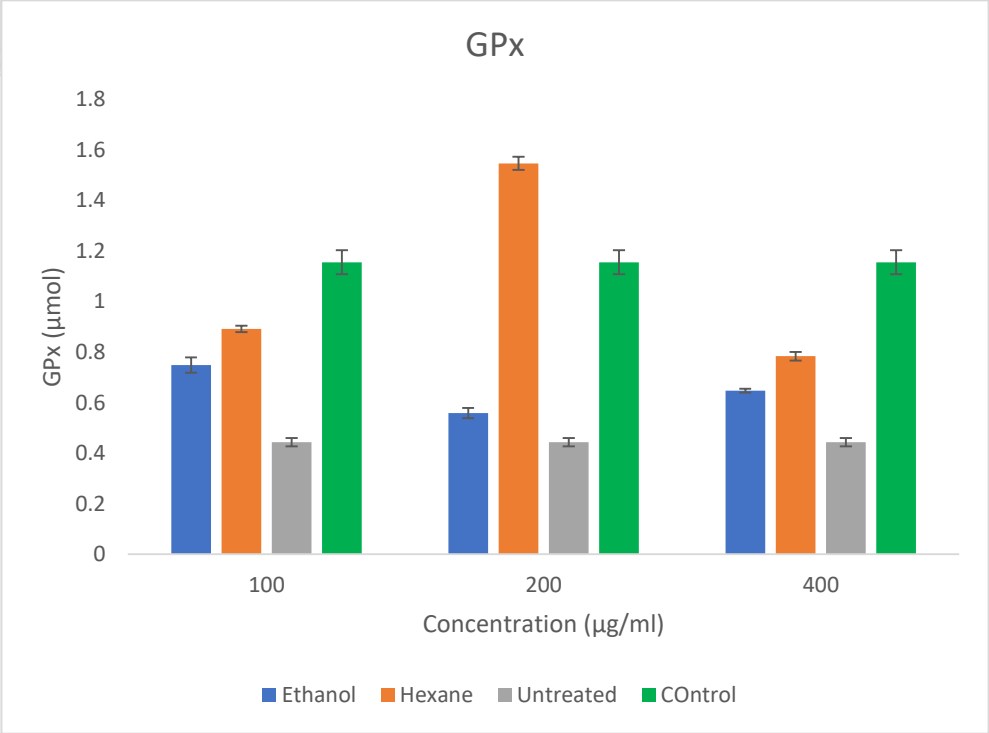

Chart-4: Glutathione Peroxidase Activity

3.33 Superoxide Dismutase Activity

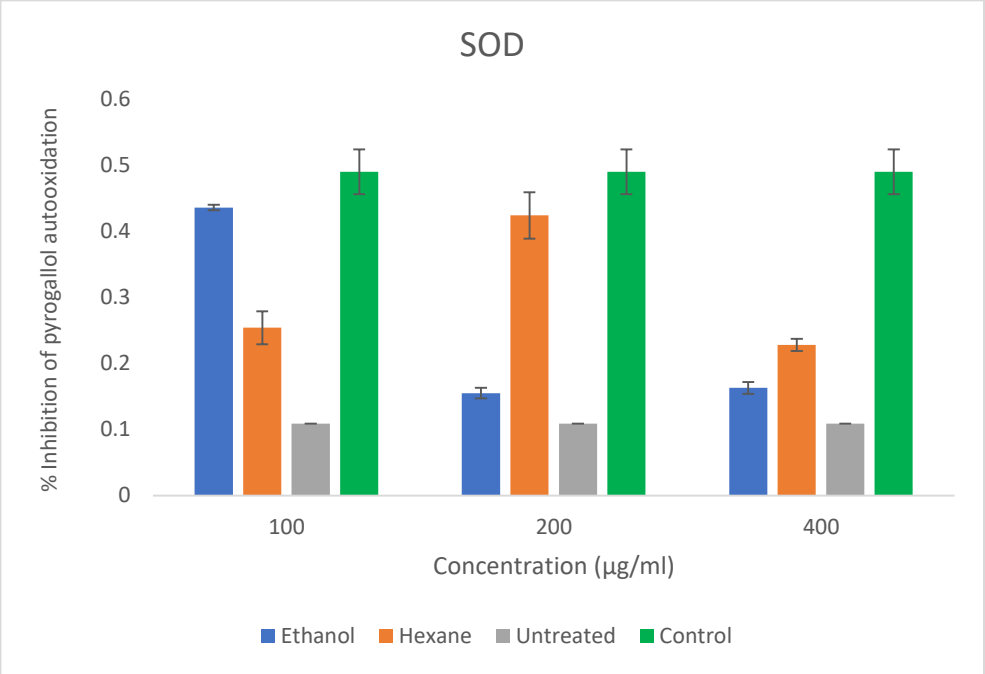

Chart-5: Percentage inhibition of pyrogallol autooxidation against concentration

4. Discussion

According to previous studies, the amount of the antioxidant components extracted from a plant material is mainly affected by the vigour of the extraction procedure [17], which may probably vary from sample to sample and amongst other contributing factors. The efficiency of the extracting solvent to dissolve endogenous compounds might also be very important [17]. Many phytochemicals in plants are soluble in polar solvents. In this study, ethanol and hexane were used as preferred solvents for extraction and a yield of 17% and 9.5%, respectively were obtained after the extraction process (Figure 1). The presence of secondary metabolites including phenols, alkaloids, and flavonoids, saponins and terpenoids were identified in the phytochemical screening of *Corchorus olitorius*. Ethanol extraction of *Corchorus olitorius* exuded more yield than the Hexane extraction, which may explain why some phytoconstituents were not shown in the phytochemistry of *Corchorus olitorius* using hexane extracts. The phytochemical compounds in the plants included alkaloids, carboxylic acid derivatives, carbohydrates, glycosides, flavanoids, saponins, and so on, are all linked to plants having medicinal efficacies [13]. In research carried out by Egua *et al* (2018), all phytochemicals except Saponins, Anthraquinones and Cardiac Glycosides were found present in the ethanolic seeds extracts. Previous reports have also shown that alkaloids possess antidiabetic activity and show free radical scavenging action [18]. Glycosides were said to be involved in enhancing pancreatic β cells function in some research [19]. Flavonoids on the other hand, one known for numerous medicinal properties including hypoglycemic and free radical scavenging action. These phytochemicals have been quoted as having therapeutic effects on conditions such as cancer, liver cirrhosis, chronic renal disease, chronic obstructive lung disease, diabetes and Alzheimer's disease which have been linked to antioxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, antihypertensive, cardioprotective, anti-arthritic and antimicrobial activities (Ande *et al*, 2022). The presence of these phytochemicals in this plant points to a possibility of it being used in traditional medicine.

The radical scavenging activity of the extracts of *Corchorus olitorius* against DPPH increases proportionally with increased concentrations for both ethanol and hexane extracts (Figure 2). The high exhibition of antioxidant activity of ethanol extract is probably due to the fact that it has more phytochemicals especially flavonoids than there are in hexane extracts. One of the important underlying mechanisms of action of dietary flavonoids and related polyphenols is associated with their inhibition of oxidative stress and related downstream responses including inflammatory diseases [20]. A similar work recorded high DPPH radical scavenging capacity in the

leaf extract of flowering stages, seedling stages of stems, and post-flowering stages of roots [21]. Flavonoids scavenge free radicals and their subsequent damage by forming relatively stable phenoxy radicals and also by metal chelation [20]. *Corchorus olitorius* extracts quenched DPPH free radical in dose-dependent manner as their activities were lower than that of Vitamin C, pointing towards a hint about the ability of these extracts to scavenge the free radical.

Human body has an effective defense system to protect and neutralize free radical-induced damage. This is accomplished by endogenous antioxidant enzymes such as CAT, SOD, and GPx. These enzymes constitute a mutually supportive team of defense against ROS [22]. The investigated activities of the following antioxidants enzymes: SOD and GPx and. SOD and GPx enzymes catalyze the complete chain reaction in which superoxide anion, the first produced ROS, is sequentially converted into water [23]. In the *ex vivo* studies, the enzymes SOD and GPx act by reducing the rate of production of new radicals; therefore, they are referred to as preventive antioxidants [24].*Corchorus olitorius* significantly increased the activity of SOD highest with ethanol extract 100mg and hexane extract 200mg (see Table 8), while the activity of GPx was increased significantly at hexane extracts 200mg.Ethanol extract MDA levels increased with increasing concentration while hexane extracts decreased with increasing concentration. MDA levels of ethanol extract increased with increasing concentration of sample (0.424 ± 0.015, at 100mg, 0.521 ± 0.005 at 200mg and 0.695 ± 0.017 at 400mg) which implies that ethanol extract is efficacious at lower dosages. On the other hand, MDA levels acted in an opposing manner in hexane extract, decreasing with increasing concentrations (0.795 mg/dm^3 at 100mg, 0.623 mg/dm^3 at 200mg and 0.463 mg/dm^3 at 400mg) which can be said that hexane extract inhibits MDA production best at higher concentrations.

5. References

1. Beyene, B., Beyene, B. and Deribe, H. (2016) Review on Application and Management of Medicinal Plants for the Livelihood of the Local Community. Journal of Resources Development and Management
2. Shariati, M., & Meric-Bernstam, F. (2019). Targeting AKT for cancer therapy. *Expert opinion on investigational drugs*, *28*(11), 977–988. https://doi.org/10.1080/13543784.2019.1676726BassamA,Rasool H. Medicinal Plants (importance and uses) (2012). Pharmaceutica Analytica Acta.3; e139
3. World Health Organization (WHO (2018)): Medicinal Plants for Holistic Health and Well-Being
4. Mohamed Eddouks, Debprasad Chattopadhyay, Vincenzo De Feo, William C. Cho, (2012) "Medicinal Plants in the Prevention and Treatment of Chronic Diseases", *Evidence-Based Complementary and Alternative Medicine*, Article ID 458274, 2 pages, 2012. https://doi.org/10.1155/2012/458274
5. Vona, Rosa, Lucia Pallotta, Martina Cappelletti, Carola Severi, and Paola Matarrese. (2021). "The Impact of Oxidative Stress in Human Pathology: Focus on Gastrointestinal Disorders" *Antioxidants* 10, no. 2: 201. https://doi.org/10.3390/antiox10020201
6. E.S. Omoregie and A.U. Osagie (2012) Antioxidant Properties of Methanolic Extracts of some Nigerian Plants on Nutritionally-Stressed Rats. Nigerian Journal of Basic and Applied Science, 20(1): 7-20
7. Tsatsakis, A. M., Docea, A. O., Calina, D., Buga, A. M., Zlatian, O., Gutnikov, S., et al. (2019). Hormetic Neurobehavioral effects of low dose toxic chemical mixtures in real-life risk simulation (RLRS) in rats. *Food Chem. Toxicol.* 125, 141–149. doi: 10.1016/j.fct.2018.12.043
8. Toma, C., De Cillà, S., Palumbo, A., Garhwal, D. P., & Grossini, E. (2021). Oxidative and Nitrosative Stress in Age-Related Macular Degeneration: A Review of Their Role in Different Stages of Disease. *Antioxidants (Basel, Switzerland)*, *10*(5), 653. https://doi.org/10.3390/antiox10050653
9. Deli, M., Ndjantou, E. B., Ngatchic Metsagang, J. T., Petit, J., Njintang Yanou, N., & Scher, J. (2019). Successive grinding and sieving as a new tool to fractionate polyphenols and antioxidants of plants powders: Application to *Boscia senegalensis* seeds, *Dichrostachys glomerata* fruits, and *Hibiscus sabdariffa* calyx powders. *Food science & nutrition*, *7*(5), 1795–1806. https://doi.org/10.1002/fsn3.1022
10. K.Gopalasatheeskumar (2018) Significant Role of Soxhlet Extraction Process in Phytochemical: Research https://www.researchgate.net/publication/332407655_
11. Omoregie, E.S., & Osagie, A.U. (2012). Antioxidant Properties of Methanolic Extracts of some Nigerian Plants on Nutritionally-Stressed Rats. *Nigerian Journal of Basic and Applied Sciences, 20*, 7-20.
12. Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African journal of traditional, complementary, and alternative medicines: AJTCAM*, *10*(5), 210–229. https://doi.org/10.4314/ajtcam.v10i5.2

13. Yamaguchi, T., Takamura, H., Matoba, T. and Teroa, J. (1998). HPLC method for evaluation of the free radical scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. Biosci, Biotechnol, Biochem, 62, pp: 1201-1204
14. Gutteridge J. M. (1984). Lipid peroxidation initiated by superoxide-dependent hydroxyl radicals using complexed iron and hydrogen peroxide. *FEBS letters*, *172*(2), 245–249. https://doi.org/10.1016/0014-5793(84)81134-5
15. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, *95*(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3
16. Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules (Basel, Switzerland)*, *14*(6), 2167–2180. https://doi.org/10.3390/molecules14062167
17. Bikash Adhikari, (2021) "Roles of Alkaloids from Medicinal Plants in the Management of Diabetes Mellitus", *Journal of Chemistry*, vol., Article ID 2691525, 10 pages, 2021. https://doi.org/10.1155/2021/2691525
18. Philippaert, K., Pironet, A., Mesuere, M., Sones, W., Vermeiren, L., Kerselaers, S., Pinto, S., Segal, A., Antoine, N., Gysemans, C., Laureys, J., Lemaire, K., Gilon, P., Cuypers, E., Tytgat, J., Mathieu, C., Schuit, F., Rorsman, P., Talavera, K., Voets, T., … Vennekens, R. (2017). Steviol glycosides enhance pancreatic beta-cell function and taste sensation by potentiation of TRPM5 channel activity. *Nature communications*, *8*, 14733. https://doi.org/10.1038/ncomms14733
19. Safe, S., Jayaraman, A., Chapkin, R. S., Howard, M., Mohankumar, K., & Shrestha, R. (2021). Flavonoids: structure-function and mechanisms of action and opportunities for drug development. *Toxicological research*, *37*(2), 147–162. https://doi.org/10.1007/s43188-020-00080-z
20. Ashok Biswas, Susmita Dey, Aiping Xiao, Siqi Huang, Ziggiju Mesenbet Birhanie, Yong Deng, Liangliang Liu, Defang Li (2023): Phytochemical content and antioxidant activity of different anatomical parts of Corchorus olitorius and C. capsularis during different phenological stages. Heliyon, Volume 9, Issue 6, e16494, ISSN 2405-8440, https://doi.org/10.1016/j.heliyon.2023.e16494.
21. Al-Gubory, K. H., Garrel, C., Faure, P., & Sugino, N. (2012). Roles of antioxidant enzymes in corpus luteum rescue from reactive oxygen species-induced oxidative stress. *Reproductive biomedicine online*, *25*(6), 551–560. https://doi.org/10.1016/j.rbmo.2012.08.004
22. Kurutas E. B. (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition journal*, *15*(1), 71. https://doi.org/10.1186/s12937-016-0186-5
23. Liege Cristina Garcia Silva, Daniel Souza Ramos Angrimani, Fernanda Machado Regazzi, Cristina Fátima Lúcio, Gisele Almeida Lima Veiga, Claudia Barbosa Fernandes & Camila Infantosi Vannucchi (2020) Pulmonary changes and redox status after fractionalized dose of prophylactic surfactant treatment in preterm neonatal lambs, Journal of Applied Animal Research, 48:1, 220-227, DOI: 10.1080/09712119.2020.1771344