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Brine shrimp lethality and antioxidant property of *Lagenaria breviflora* (Benth.) Roberty fruit crude extract and fractions

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ABSTRACT

Background: Oxidative injury plays a pivotal impact in the development of human diseases, and it is a major component of the pathophysiology of several inflammation-linked human medical disorders.

Objective: The focus of this study was to evaluate the cytotoxicity and antioxidant potential of the fruit of *Lagenaria breviflora* (Benth.) Roberty (LB).

Methods: The antioxidant capacity of *L. breviflora*'s crude and solvent fractions was assessed using standard techniques by measuring the total phenolic content, total flavonoid content, and DPPH radical scavenging activity. The data we0re analyzed using One-way ANOVA, and Dunnett's Multiple Comparison was used to establish the threshold.

Results: The total flavonoid contents of fruit methanol extract, LB hexane, chloroform, and ethyl acetate fractions were 32.00 ± 0.94, 224.32 ±0.05, 224.06 ±0.14, and 2615.67 ± 13.20 mg gallic acid equivalent/g of extract, respectively. In contrast, the total phenolic contents were 5218.83 ± 3.18, 2615.67 ± 13.20, and 4553.00 ± 24.27 mg gallic acid equivalent/g of extract, respectively. The crude extract of LB, hexane fraction, chloroform, and ethyl acetate fraction displayed IC_{50} values of 242.57 ± 8.90, 262.91 ± 6.49, 96.17 ± 2.74, and 221.45 ± 0.83 µg/mL, respectively, for their ability to scavenge DPPH radicals. Ascorbic acid and rutin had IC_{50} values of 2.76 ± 0.01 and 20.6 ± 9.26 µg/mL, respectively.

Conclusions: Fruit from *L. breviflora* (LB) exhibited strong antioxidant potential, which may have resulted from the fruit's phenolic and flavonoid content. This demonstrates the reason this herb is used in traditional medicine.

Keywords: Oxidative stress, brine shrimp lethality assay, total phenolic contents, total flavonoid contents, DPPH, *Lagenaria breviflora* fruit.

Table 1: The brine shrimp lethality effect of *L. breviflora* fruit

Extracts	LC_{50} (µg/mL)
Lagenaria breviflora	257.71 ± 0.12
Cyclophosphamide	224.74 ± 0.35

Table 2: The percentage yield of *L. breviflora* partitioned fractions

Extracts	Weight of methanol extract (g) partitioned	Solvent	Weight of fractions (g)	% yield (w/w)
L. breviflora	10.02	Hexane	1.67	16.67
		DCM	0.14	1.40
		Ethyl acetate	8.76	87.43

Table 3: The DPPH (IC_{50}), TPC, and TFC values of the extract and fractions

Extracts	Solvents	DPPH (IC_{50}) (µg/mL)	TPC (µg GAE/g)	TFC (mg QE/g)
L. breviflora fruit	Crude	242.57 ± 8.90***/***	ND	87.15 ± 9.01***
	Hexane	262.91 ± 6.49***/***	2615.67 ± 13.20***	224.32 ±0.05^{H}
	DCM	96.17 ± 2.74***/***	5218.83 ± 3.18^{H}	224.06 ± 0.14
	Ethyl acetate	221.45 ± 0.83***/***	4553.00 ± 24.27***	32.00 ± 0.94***
Ascorbic acid		2.76 ± 0.01		
Rutin		20.6 ± 9.26		

Graphical Abstract: Brine Shrimp Lethality and Antioxidant property of *Lagenaria breviflora* (Benth.) Roberty fruit crude extract and fractions

INTRODUCTION

Oxidative stress damages the chemical composition and biological properties of biomolecules in the body. It occurs due to excessive free radical formation or insufficient antioxidant defenses [1,2,3]. This damage contributes to the pathophysiology of various inflammation-related human diseases [4]. Uncontrolled lipoxidation is caused by Reactive Oxygen Species (ROS). After hydroxyalkenals from lipid hydroperoxides are broken down, malonyldialdehyde (MDA) is the byproduct. MDA functions as a lipid peroxidation indicator since it is a persistent consequence of the process [5]. Superoxide dismutase (SOD) is an antioxidant enzyme that defends the body, while MDA, produced during lipid peroxidation, serves as a marker of oxidative stress [6]. Elevated levels of MDA can be attributed to intracellular and cell wall damage caused by elevated ROS [7]. An increase in ROS production may be the cause of the rise in MDA levels in these patients, as ROS production led to excessive oxidative damage. Increased ROS harms all body cells, including mononuclear cells, which in turn induces the damaged cells to produce more inflammatory markers like TNF-α and NF-Kappa B [8,9]. Phytochemicals are necessary as antioxidants to prevent and treat ROS-related illnesses caused by redox imbalances in the body [10]. Diverse phytochemical compounds, such as flavonoids and phenolic compounds, have been identified in medicinal plants. These bioactive compounds act as antioxidants, protecting against ROS-induced oxidative damage [11]. Herbs from African countries have been found to possess these valuable compounds, which are utilized in phytomedicine [12].

Tropical African woods are home to *L. breviflora*, a perennial climber in the Cucurbitaceae family. The fruits of this plant have an ovoid shape of about 9 cm long and feature creamy dots on a dark green background. The leaves have a unique scabrid and sandpapery texture. The fruit has been used for medicinal purposes for a long time to treat human measles, gastrointestinal problems, and microbiological ailments in West Africa [13]. Common in West Tropical Africa, particularly Nigeria, it is known for its distinct antibacterial and antiviral properties [13] Among other Cucurbitaceae species, the *L. breviflora* plant is distinctive among Nigerian indigenous communities due to its considerable therapeutic potential [14]. Additionally, it is known that the fruit of *L. breviflora* contains compounds that can serve as environmentally friendly antibacterial agents that protect poultry species from bacterial disease [15]. According to Ezim et al. [16] *L. breviflora's* phytochemical analysis indicates a range of chemical components, such as saponins and phenolic acids. In West Africa, *L. breviflora* is widely recognized for its application in the treatment of microbiological illnesses and gastrointestinal issues. However, there has not been any information released regarding the fruit's overall toxicity to brine shrimp or the antioxidant qualities of the extract and its fractions. Thus, the cytotoxicity and antioxidant qualities of the fruit were investigated in this present research.

MATERIALS AND METHODS

Plant collection, extraction, and partitioning: Fresh fruits of *L. breviflora* were collected in Oluponna (7° 35′ 34.7″ N 4° 11′ 27.5″ E), Osun state of Nigeria on 19th of February 2024. The plant was identified and validated in Bowen University Herbarium, Iwo where a voucher specimen (BUH: 091) was deposited. Using a grinding machine, *L. breviflora* was gathered, dried, and ground into a coarse powder. Following a 72-hour maceration period at room temperature (27±2 ºC) with periodic shaking and stirring, the powdered samples were filtered using Whatman (Number 1) filter paper and a new wool plug. Using a rotating evaporator (Buchi Rotavapour R-210, Switzerland), the filtrates were concentrated in vacuo. The yield (%) was computed and the weights of the crude extracts were established.

Solvent-solvent partitioning: The modified guidelines [17] were followed for solvent-solvent partitioning. Crude extracts of the fruit *L. breviflora* were redissolved in a 3:1 methanol-to-water ratio and then transferred into separating funnels. The n-hexane, dichloromethane (DCM), and ethyl acetate extracts were each combined with 50 mL aliquots of the respective solvents. The combined layers were then evaporated separately under a vacuum to obtain dried fractions, which were stored in an airtight container. The yield percentage was then calculated.

Brine shrimp lethality assay: 30 mg of cyclophosphamide (standard) and the dried methanol extracts were redissolved in 3 mL of methanol to yield a 10 mg/mL (10,000 µg/mL) extract solution for the toxicity test. Subsequently, concentrations of 1, 2, 5, 10, 100, and 500 µg/mL were prepared [17,18]. Forty-eight hours post-hatching, ten nauplii were transferred into each sample vial using a 23 cm disposable Pasteur pipette and exposed to light for 24 hours. The experiment was set up in triplicates, with seawater serving as the sole control. When nauplii remained at the bottom of test tubes and were motionless, they were deemed dead. Finney's Probit analysis was used to estimate the lethal concentration of plant extract (LC_{50}) at 95 percent confidence intervals that cause 50% death of brine prawns based on 24-hour counts of surviving nauplii.

Diphenyl picryl hydrazyl (DPPH) antioxidant assay: With minor adjustments, the methanol extract's and the fractions' capacity to scavenge free radicals were assessed using the techniques outlined by Ogunlakin et al. [19]. For the DPPH test, 3 mL (0.004%) of 1, 1-diphenyl-2-picryl-hydrazyl-hydrate (DPPH) was mixed with 2 mL of methanol solution of *L. breviflora* fruit raw extract, solvent divisions, and reference standards (rutin and vitamin C) at different concentrations (200, 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL) added separately. A test sample of 2 mL of methanol was added to the control. After giving the reaction mixtures a good shake, they were left in the darkroom for half an hour at 27 °C. With the aid of a UV–VIS spectrophotometer (Spectrumlab 752S), the value of the absorption rate of the sample was measured at 517 nm. The absorbance was then translated into a percentage inhibition using the formula [1-absorbance of solution with sample and DPPH/absorbance of solution with DPPH] × 100. Plotting the sample scavenging activity versus the test sample concentration (using linear regression analysis) allowed for the calculation of the percentage inhibition concentration (IC_{50}) value.

Measurement of total phenolic content (TPC): In the analysis, Folin-Ciocalteu spectrophotometric technique was used to determine the overall phenolic compound of the methanol extracts and partitions [20]. Each test sample consisted of one milliliter (aliquot) mixed with five milliliters of diluted Folin-Ciocalteu's phenol reagent (100 µg/mL). After a 5-minute incubation, 4 mL of Na_2CO_3 solution in purified water (7.5 g/100 mL) was added to each vial, and the mixture was then incubated in the darkroom for half an hour at a temperature of 27 °C. A blank sample was prepared using 1 mL of methanol. Each sample was tested three times, and the absorbance of the mixture was measured at 765 nm using a UV-VIS spectrophotometer (Spectrumlab 752S) after a 30-minute incubation period. The straight-line dose-effect relationship regression curve (Figure 1) produced from the absorbance of gallic acid was used to quantify the total phenolic content. Gallic acid equivalent to mg/g of dry mass of extracts was the TPC result. The following formula was used to determine the TPC in the plant extract:

$$TPC = \frac{CV}{M}$$

Where V = extract volume (mL), M = weight of plant extract (0.03 g), and C is the corresponding Gallic acid concentration determined using calibration curve µg/mL.

Measurement of total flavonoid content (TFC): The colorimetry technique using aluminum chloride in this investigation was adapted from a method described by Sonibare et al. [21]. To create the calibration curve (Figure 2), quercetin was dissolved in ethanol at concentrations ranging from 100 to 6.25 µg/mL and used as the standard. Test samples, consisting of thirty milligrams of the substance, were dissolved in 30 mL of methanol. Each test solution was then mixed individually with 2.8 mL of methanol, 0.1 mL of 1% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 1 mL of either the reduced quercetin solution or the test sample. Instead of adding 1% aluminum chloride, an equivalent amount of distilled water was added to the mixture. Each of the samples underwent three examinations. After the reaction was incubated for half an hour at a temperature of 27°C, the absorbance of the reaction at 415 nm was measured using the UV-VIS spectrophotometer (Spectrumlab 752S). The overall flavonoid concentration for every examined sample was quantified through the use of the comparable amount of quercetin (mg Quercetin equivalent per gram of extract), using the calculation developed from the quercetin curve of calibration.

Statistical analysis: Each test result is presented as the mean ± standard deviation.The trials were performed three times for maximum accuracy. The data obtained were analyzed using GraphPad (Version 9.0). Statistical differences between the categories were examined using a one-way ANOVA and Dunnett's Multiple Comparison Test with a significance threshold of $p < 0.05$.

RESULTS

According to Table 1, the methanol extract and cyclophosphamide (standard) had LC_{50} values of 257.71 ± 0.12 µg/mL and 224.74±0.35 µg/mL, respectively, in the brine shrimp lethality assay (BSLA). Table 2 shows the yields of the pure extract, hexane, DCM, and ethyl acetate fractions, which were 1.90%, 1.67%, 0.14%, and 87.6%, respectively. The extract of *L. breviflora* fruit, hexane, DCM, and ethyl acetate fractions had DPPH IC_{50} levels of 242.57 ± 8.90, 262.91 ± 6.49, 96.17 ± 2.74, and 221.45 ± 0.83 µg/mL, respectively (Table 3). The total phenolic contents of LB hexane, chloroform, and ethyl acetate fractions were 2615.67 ± 13.20, 5218.83 ± 3.18, and 4553.00 ± 24.27 mg gallic acid equivalent/g of extract, respectively. The fruit methanol extract, hexane, chloroform, and ethyl acetate fractions had total flavonoid contents of 87.15 ± 9.01, 224.32 ±0.05, 224.06 ± 0.14, and 32.00 ± 0.94 mg gallic acid equivalent/g of extract, respectively (Table 4).

Table 1: The brine shrimp lethality effect *of L. breviflora* fruit

Extracts	LC_{50} (µg/mL)
L. breviflora	257.71 ± 0.12
Cyclophosphamide	224.74 ± 0.35

The means ± SD for the data sets are presented. P<0.05 was used for Dunnett's Multiple Comparison Test after a one-way ANOVA. There is no significant difference between the values.

Table 2: The percentage yield of *L. breviflora* partitioned fractions

Methanol Extract (% yield)	Weight of methanol extract (g) partitioned	Solvent	Weight of fractions (g)	% yield (w/w)
L. breviflora (1.90)	10.02	Hexane	1.67	16.67
		DCM	0.14	1.40
		Ethyl acetate	8.76	87.43

Table 3: The DPPH (IC_{50}), TPC, and TFC values of the extract and fractions

Extracts	Solvents	DPPH (IC_{50}) (µg/mL)	TPC (µg GAE/g)	TFC (mg QE/g)
L. breviflora fruit	Crude	242.57 ± 8.90***/***	ND	87.15 ± 9.01***
	Hexane	262.91 ± 6.49***/***	2615.67 ± 13.20***	224.32 ±0.05^{H}
	DCM	96.17 ± 2.74***/***	5218.83 ± 3.18^{H}	224.06 ± 0.14
	Ethyl acetate	221.45 ± 0.83***/***	4553.00 ± 24.27***	32.00 ± 0.94***
Ascorbic acid		2.76 ± 0.01		
Rutin		20.6 ± 9.26		

The data is presented as mean ± SEM for n = 3. An ANOVA is conducted first, followed by Dunnett's Multiple Comparison Test at P<0.05. The extract or fraction with the highest TPC and TFC from each solvent, marked with the letter "H," was compared with other extracts. Symbols *, **, and *** denote different levels of significance. If there is no discernible change between "H" or the same solvent extracts and fractions, it is indicated by NS. The IC_{50} DPPH of each extract was compared to standards (rutin and ascorbic acid), and the results showed that the levels of significance differed from the standards in the following order: ND (not detected), NS (no significant difference from the standards), **, ***, or an asterisk separated by (/).

DISCUSSION

Many human diseases, including cancer, diabetes, atherosclerosis, and inflammatory arthritis, are made worse by oxidative damage [22,23]. Due to the strong antioxidant activity, poor solubility, and potential health risks of synthetic antioxidants, their usage has been restricted. This has led to the investigation of several natural compounds and antioxidants as first-line therapies for polycystic ovarian syndrome (PCOS) [27]. To discover new antioxidants, a variety of plant species have recently been studied [28, 29]. When food lacks adequate phyto-antioxidants, which are crucial for medicinal purposes, the body becomes more vulnerable to ROS-induced damage.

The evaluation of *L. breviflora's* cytotoxicity indicated the presence of potential toxicity in addition to other biological activities, such as anticancer activity [31–35]. Together with other principles important to medicine,

the bulk of detrimental bioactive principles are infrequently biosynthesized in therapeutic herbs [35]. Recognizing that the toxicity assessment is not the only factor in determining the plant's overall safety profile is crucial, and it should be done with caution [36]. Meyer's cytotoxicity index [37] indicated that the methanol extract of *L. breviflora* is non-toxic, with an LC_{50} > 1000 µg/mL. The cytotoxicity level of this plant extract was categorized further using Clarkson's criterion [38]. Research has shown that potentially toxic bioactive compounds are sometimes produced along with other important medicinal principles in plants [39]. The recent emphasis on assessing the toxicity of medicinal herbs and phytomedicines suggests that phytotoxicity should be seen as a valuable aid in drug research and development, rather than a drawback of herbal medicine. Many medical conditions including diabetes, atherosclerosis, inflammatory arthritis, and cancer, are aggravated by oxidative damage. Artificial antioxidants have been used cautiously due to their high activity, low solubility, and potential health risks [40, 41]. Consequently, there is a need for safer, more soluble organic antioxidants. Recent investigations have focused on exploring a wide variety of plant species to discover novel antioxidants [42–44]. Phyto-antioxidants, which are important therapeutic benefits in food, have been found to effectively reduce the susceptibility to ROS [45]. They are easily obtainable and pose minimal risk.

The remarkable antioxidant qualities of *L. breviflora* fruit may be attributed to its high flavonoid and phenol content. Because of their high total phenolic content (TPC), these two fractions—DCM and ethyl acetate—are potent sources of antioxidants. These results, which are consistent with previous studies relating antioxidant capabilities to phenolic and flavonoid components, support the potential health benefits of *L. breviflora* fruit in avoiding disorders associated with oxidative stress.

The ability of *L. breviflora* fruit and its solvent fractions to scavenge DPPH radicals further supports their antioxidant activity. The plant's relatively low IC_{50} values demonstrate strong antioxidant activity comparable to benchmarks like rutin and ascorbic acid, indicating its effectiveness as a natural antioxidant. Phenols, which are important components of medicinal plants because of their -OH functional group, have numerous therapeutic benefits, one of which is free radical scavenging [46]. Several studies confirm a strong correlation between phenolic content and antioxidant capacity. The remarkable scavenging properties of the extract and solvent fractions investigated during this work may be attributed to the hydroxyl functional groups present in the structural framework of the phenolic compound molecules. [48]. The plant's rich phytochemical profile is confirmed by the observed phytochemical composition, which includes phenolic acids, following previous studies on *L. breviflora* [49]. The plant has long been used to cure a wide range of ailments, including gastrointestinal problems and microbial infections, and these bioactive compounds support this use. They also enhance the medicinal properties of the plant [50,51].

CONCLUSION

As a naturally occurring source of antioxidants, the fruit of *L. breviflora* was found to have medicinal potential. However, more research is required to clarify its modes of action, evaluate its *in-vivo* safety profile, and investigate its possible use in nutraceutical and pharmaceutical formulations.

List of abbreviations: MDA, Malonyldialdehyde; ROS, Reactive Oxygen Species; SOD, Superoxide dismutase; cm, Centimeters; mg/mL, Milligram per milliliter; µg/mL, Microgram per milliliter; nm, nanometer; $IC_{50,}$ Inhibition concentration; mL, milliliter; g, Gram; equivalent/g, Equivalent per gram.

Competing Interest: The authors have declared that the study was carried out in the absence of any relationship that can be interpreted as competing interests.

Authors Contribution: ADO analyzed data and edited and revised the manuscript. ADO, FIO, SAA, and OGA designed the study. ADO designed the study, performed the experiments, analyzed data, wrote the manuscript, and MAS provided overall supervision. All authors have read and approved the final manuscript.

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REFERENCES

1. Olanrewaju AA, Ibeji CU, Oyeneyin OE. Biological evaluation and molecular docking of some newly synthesized 3d-series metal (II) mixed-ligand complexes of fluoro-naphthyl diketone and dithiocarbamate. SN Applied Sciences. 2020 2:1-1. DOI: https://doi.org/10.1007/s42452-020-2482-0
2. Fathima SA, Maurya R, Naqvi S. Oxidative Stress and Metals in Alzheimer's Disease. In Natural Product-based Synthetic Drug Molecules in Alzheimer's Disease: Therapeutic & Theranostic Agents. 2023 15: 17-4. Singapore: Springer Nature Singapore.
DOI: https://doi.org/10.1007/978-981-99-6038-5_2
3. Beyatli A, Gül N, Coşkun Cevher Ş., Arı N. Antihyperglycemic, Antihyperlipidemic, and Antioxidant Effects of Morin on Streptozotocin-Induced Diabetic Rats. Eurasian Chemico-Technological Journal 2024 27;26(2):85-92.
4. Chaudhary P., Janmeda P., Docea A.O., Yeskaliyeva B., Ahmad Abdull Razis F., Modu B., Calina D., and Sharifi-Rad J. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases Frontier Chemistry, 2023 Secondary Medicinal and Pharmaceutical Chemistry 11-2023
DOI: https://doi.org/10.3389/fchem.2023.1158198
5. Ogunlakin AD, Onifade TR, Ojo OA, Adesanya EO, Berena GA, Ayeni PO, Omolekan TO, Ogunlakin MA, Iyinkristi DA, Sonibare MA, Fategbe MA. Antidiabetic potential of Carica papaya L. and its constituents: From folkloric uses to products development. Bioactive Compounds in Health and Disease. 2023; 6(6):126-44.
6. Jomova K, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. Archives of Toxicology. 2024 14:1-45. DOI: https://doi.org/10.1007/s00204-024-03696-4
7. Chen Y, Wan Y, Cai W, Liu N, Zeng J, Liu C, Peng H, Fu G. Effects on cell membrane integrity of *Pichia anomala* by the accumulating excessive reactive oxygen species under ethanol stress. Foods. 2022 21;11(22):374.;
DOI: https://doi.org/10.3390/foods11223744
8. Albano GD, Gagliardo RP, Montalbano AM, Profita M. Overview of the mechanisms of oxidative stress: impact in inflammation of airway diseases. Antioxidants. 2022 13;11(11):2237.
DOI: https://doi.org/10.3390/antiox11112237
9. Bezerra FS, Lanzetti M, Nesi RT, Nagato AC, Silva CP, Kennedy-Feitosa E, Melo AC, Cattani-Cavalieri I, Porto LC, Valenca SS. Oxidative stress and inflammation in acute and chronic lung injuries. Antioxidants. 2023 21;12(3):548. DOI: https://doi.org/10.3390/antiox12030548.
10. Ogunlakin AD, Akinwumi IA, Ambali OA. Ethnomedicinal application, phytochemistry and therapeutic effects of genus clerodendrum. Functional Food Science. 2023 3(10):228-47.
11. Li Y., Zhang J., Zhang J.J., Fan J.Y., Zhao Q., Chu Q., Zhong S., Gu R. Comprehensive comparison on antioxidant properties and UPLC-Q-TOF/MS-based metabolomic discrimination between *Gentiana veitchiorum* and *G. szechenyii*. 2024 17(4): 105695
DOI: https://doi.org/10.1016/j.arabjc.2024.105695
12. Babalola O. Y., Lawal I. O., Akinwumi I. A. Comparative evaluation of phytochemical, in vitro antioxidant activities and elemental composition of the fruit, leaves, and stem bark of *Tetrapleura tetraptera*. Functional Food Science 2023; 3(12): 317-328.
DOI: https://www.doi.org/10.31989/ffs.v3i12.1263
13. Mutmainah M., Mayangsari Y., Santoso U., Chansuwan W., Sirinupong N. Phytochemical Profile and Antioxidant Activity of Torch Ginger (*Etlingera elatior*) Inflorescence Extract after In vitro Simulated Digestion. Functional Foods in Health and Disease 2024; 14(7): 528-545.
DOI: https://doi.org/10.31989/ffhd.v14i7.1382
14. Adeyemi MA, Ekunseitan DA, Abiola SS, Dipeolu MA, Egbeyale LT, Sogunle OM. Phytochemical analysis and GC-

MS determination of *Lagenaria breviflora* R. fruit. International Journal of Pharmacognosy and Phytochemical Research. 2017;9(7):1045-50.
DOI: https://doi.org/10.25258/phyto.v9i07.11178.

15. Aderotimi B, Stephen D, Usman JI, Elijah A, Etsu-Musa N. Free Radical Scavenging Potential and Bioactive Phytochemical Profile of *Lagenaria breviflora*. Tropical Journal of Phytochemical and Pharmaceutical Sciences. 2024. 1 ;3(5):309-13.
DOI: https://doi.org/10.26538/tjpps/v2i4.3
16. Ezim OE, Okoye FN, Krukru VS, Ogbonnaya EA. Evaluation of the nutritional potentials of *Lageneria breviflora* seeds. World Journal of Advanced Research and Reviews. 2024;21(2):1071-7.
DOI: https://doi.org/10.30574/wjarr.2024.21.2.0330
17. Olakojo, T., Oridupa, O., Saba, A. In-vitro and in-vivo antibacterial and therapeutic activity of methanol extract of whole fruit of Lagenaria breviflora against Salmonella species in broilers. *SVU-International Journal of Veterinary Sciences*, 2024; 7(3): 51-63.
DOI: https://doi.org/10.21608/svu.2024.302808.1329
18. Ogunlakin AD, Sonibare MA. Antioxidant and Ameliorative Effects of Basella alba L. On Letrozole-Induced Polycystic Ovarian Syndrome in Rats. Tropical Journal of Natural Product Research. 2023 1;7(4).
DOI: https://doi.org/10.1080/07391102.2023.2293271
19. Ogunlakin AD, Odugbemi AI, Omolekan T, Adaramoye OA, Abiola OO, Akinola A, Akinsete A, Alabi T, Alade FF, Ahossinme HE, Ajiboye A. Elemental and In vitro Antioxidant Studies of Some Bracharia species and Milk from Bowen University Dairy Farm. InIOP Conference Series: Earth and Environmental Science 2023a 1219: 1, 012003. DOI 10.1088/1755-1315/1219/1/012003
20. Fadogba OA, Ogunlakin AD, Ajayi AM, Sonibare MA. Antioxidant and anti-arthritic activity of Bombax buonopozense P. Beauv. Leaves. InAnnales Pharmaceutiques Françaises 2024 16. Elsevier Masson.
DOI: https://doi.org/10.1016/j.pharma.2024.02.008
21. Sonibare MA, Onifade TR, Ogunlakin AD, Akinmurele OJ, Adebodun SA. Microscopic Evaluation and Antioxidant Activity of *Glyphaea brevis* (Spreng.) Monach.(Family Tiliaceae). Free Radicals and Antioxidants. 2022 2;12(1):27-32. DOI: https://doi.org/10.5530/fra.2022.1.5
22. Olanrewaju Adesoji A., Festus S. Fabiyi, Rajeev Gupta, Emmanuel G. Kolawole. "New Transition Metal (II) Mixed-Ligand Complexes of Phenylbutanedione and Dithiocarbamate: Synthesis, Characterization, Thermal and Antioxidant Studies". Chemistry Research Journal. 2018. 3(5): 103-120
DOI: https://doi.org/10.1016/j.molstruc.2020.12805
23. Ojo OA, Ogunlakin AD, Akintayo CO, Olukiran OS, Adetunji JB, Ajayi-Odoko OA, Ogwa TO, Molehin OR, Ojo OO, Mothana RA, Alanzi AR. *Spilanthes filicaulis* (Schumach. & Thonn.) CD Adams leaves protect against streptozotocin-induced diabetic nephropathy. Plos one. 2024 19;19(4): e0301992.
DOI: https://doi.org/10.1371/journal.pone.0301992
24. Leyane TS, Jere SW, Houreld NN. Oxidative stress in aging and chronic degenerative pathologies: molecular mechanisms involved in counteracting oxidative stress and chronic inflammation. International journal of molecular sciences. 2022 30;23(13):7273.
DOI: https://doi.org/10.3390/ijms23137273
25. Stoia M, Oancea S. Low-molecular-weight synthetic antioxidants: classification, pharmacological profile, effectiveness and trends. Antioxidants. 2022 26;11(4):638.
DOI: https://doi.org/10.3390/antiox11040638
26. Pedro AC, Paniz OG, Fernandes ID, Bortolini DG, Rubio FT, Haminiuk CW, Maciel GM, Magalhães WL. The importance of antioxidant biomaterials in human health and technological innovation: a review. Antioxidants. 2022 24;11(9):1644.
DOI: https://doi.org/10.3390/antiox11091644
27. Tkaczewska J, Kulawik P, Jamróz E, Čagalj M, Matas RF, Šimat V. Valorisation of prawn/shrimp shell waste through the production of biologically active components for functional food purposes. Journal of the Science of Food and Agriculture. 2024 30;104(2):707-15.
DOI: https://doi.org/10.1002/jsfa.12969
28. Motta AB. Polycystic Ovary Syndrome and Oxidative Stress. Natural Treatments. Current Medicinal Chemistry. 2024. 16.
DOI: 10.2174/0109298673270372231130071320.
29. Kumar V, Prasher IB. Phytochemical Analysis and Antioxidant Activity of Endophytic Fungi Isolated from *Dillenia indica* Linn. Applied Biochemistry and Biotechnology. 2024 ;196(1):332-49.
DOI: https://doi.org/10.1007/s12010-023-04498-7
30. Fayyazi M, Esmaeili H, Moridi Farimani M, Mirjalili MH. Variations in phytochemical traits, total carbohydrate, and antioxidant activity of Iranian wild populations of greater burdock (*Arctium lappa* L.). Genetic Resources and Crop Evolution. 2024 ;71(2):915-27.
DOI: https://doi.org/10.1007/s10722-023-01672-y

31. Hou Y, Wang H, Wu J, Guo H, Chen X. Dissecting the pleiotropic roles of reactive oxygen species (ROS) in lung cancer: From carcinogenesis toward therapy. Medicinal Research Reviews. 2024 29.
DOI: https://doi.org/10.1002/med.22018
32. Shrestha D, Magar AB, Pakka S, Sharma KR. Phytochemical analysis, antioxidant, antimicrobial, and toxicity studies of *Schima wallichii* growing in Nepal. International Journal of Food Properties. 2024 31;27(1):273-85.
DOI: https://doi.org/10.1080/10942912.2024.2304267
33. Iwansyah AC, Latifatunnajib S, Kumalasari ID, Ariani D, Wardhani R, Herawati ER, Juliagani B. Nutrition and toxicity properties of fried grasshopper (*Oxya chinensis*): The effect of air frying temperature and time of cooking. InAIP Conference Proceedings 2024 6:2957. AIP Publishing.
DOI: https://doi.org/10.1063/5.0184025
34. Cavò E, Taviano MF, Davì F, Cacciola F, Oulad El Majdoub Y, Mondello L, Ragusa M, Condurso C, Merlino M, Verzera A, Miceli N. Phenolic and volatile composition and antioxidant properties of the leaf extract of *Brassica fruticulosa* subsp. *fruticulosa* (Brassicaceae) growing wild in Sicily (Italy). Molecules. 2022 26;27(9):2768.
DOI: https://doi.org/10.1063/5.0184025
35. Ungureanu AR, Popovici V, Oprean C, Danciu C, Schröder V, Olaru OT, Mihai DP, Popescu L, Luță EA, Chițescu CL, Gîrd CE. Cytotoxicity Analysis and In Silico Studies of Three Plant Extracts with Potential Application in Treatment of Endothelial Dysfunction. Pharmaceutics. 2023 11;15(8):2125.
DOI: https://doi.org/10.3390/pharmaceutics15082125
36. Lediga ME, Malatjie TS, Olivier DK, Ndinteh DT, Van Vuuren SF. Biosynthesis and characterization of antimicrobial silver nanoparticles from a selection of fever-reducing medicinal plants of South Africa. South African journal of botany. 2018 1; 119:172-80.
DOI: https://doi.org/10.1016/j.sajb.2018.08.022
37. Sharma A, Lee BS. Toxicity test profile for deep eutectic solvents: A detailed review and prospects. Chemosphere. 2024 1:141097.
DOI: https://doi.org/10.1016/j.chemosphere.2023.141097
38. Thomas A, Kweka EJ, Ogwang PE. Laboratory and simulated semi-field larvicidal efficacy of *Aframomum angustifolium* (Sonn.) K. Schum and *Tagetes patula* essential oils against Anopheles gambiae. Journal of Natural Pesticide Research. 2024 1;7: 100067.
DOI: https://doi.org/10.1016/j.napere.2024.100067
39. Hamid HA, Navaranjan N, Sulaiman Z. Application of Three of Selected Medicinal Plant Extracts in Brunei Darussalam to Improve Shelf Life of Poultry Product. ASEAN Journal on Science and Technology for Development. 2024;40(2):10.
DOI: https://doi.org/10.61931/2224-9028.1533
40. Alamgir AN, Alamgir AN. Medicinal, non-medicinal, biopesticides, color-and dye-yielding plants; secondary metabolites and drug principles; significance of medicinal plants; use of medicinal plants in the systems of traditional and complementary and alternative medicines (CAMs). Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1: Pharmacognosy. 2017:61-104.
DOI: https://doi.org/10.1007/978-3-319-63862-1_3
41. Kurek M, Benaida-Debbache N, Elez Garofulić I, Galić K, Avallone S, Voilley A, Waché Y. Antioxidants and bioactive compounds in food: Critical review of issues and prospects. Antioxidants. 2022 8;11(4):742;
DOI: https://doi.org/10.3390/antiox11040742
42. Lourenço SC, Moldão-Martins M, Alves VD. Antioxidants of natural plant origins: From sources to food industry applications. Molecules. 2019 15;24(22):4132.
DOI: https://doi.org/10.3390/molecules24224132
43. Deshmukh RK, Gaikwad KK. Natural antimicrobial and antioxidant compounds for active food packaging applications. Biomass Conversion and Biorefinery. 2024 ;14(4):4419-40.
DOI: https://doi.org/10.1007/s13399-022-02623-w
44. Ahmed MH, Karkush SI, Ali SA, Mohammed AA. Phytochemicals: a new arsenal in drug discovery. International Journal of Medical Science and Dental Health. 2024 1;10(01):29-44.
DOI: https://doi.org/10.55640/ijmsdh-10-01-03
45. Ferreira-Sousa D, Genisheva Z, Rodríguez-Yoldi MJ, Gullón B, Costa CE, Teixeira JA, Botelho CM, Ferreira-Santos P. Exploration of Polyphenols Extracted from Cytisus Plants and Their Potential Applications: A Review. Antioxidants. 2024 2;13(2):192.
DOI: https://doi.org/10.3390/antiox13020192
46. Gauttam VK, Munjal K, Chopra H, Ahmad A, Rana MK, Kamal MA. A Mechanistic Review on Therapeutic Potential of Medicinal Plants and their Pharmacologically Active Molecules for Targeting Metabolic Syndrome. Current Pharmaceutical Design. 2024 1;30(1): 10-30.DOI: https://doi.org/10.2174/0113816128274446231220113957
47. Chanthasri W, Puangkeaw N, Kunworarath N, Jaisamut P, Limsuwan S, Maneenoon K, Choochana P, Chusri S. Antioxidant capacities and total phenolic contents of 20

polyherbal remedies used as tonics by folk healers in Phatthalung and Songkhla provinces, Thailand. BMC complementary and alternative medicine. 2018;18:1-1. DOI: https://doi.org/10.1186/s12906-018-2131-y

48. Bey D, Mahfoudi R, Benalia M, Djeridane A, Ami Y, Yousfi M. Inter-and intraspecific variability of phenolic content, antioxidant activities and α-amylase inhibitory potential of different bran and husk extracts from Algerian durum wheat (*Triticum turgidum* Desf.), barley (Hordeum vulgare L.) and their derived products (frik and mermez). Journal of Food Measurement and Characterization. 2024 ;18(2):1158-74. DOI: https://doi.org/10.1007/s11694-023-02240-9
49. Oridupa O, Omobowale TO, Oyagbemi AA, Danjuma NO, Obisesan AD, Olakojo TA, Saba AB. Antioxidant activity enhancement and oxidative damage inhibition by *Lagenaria breviflora* fruit and Xanthosoma sagittifolium corm in hypertensive Wistar rats. Nigerian Journal of Physiological Sciences. 2023 30;38(1):101-6. DOI: https://doi.org/10.54548/njps.v38i1.14
50. Fajinmi OO, Olarewaju OO, Van Staden J. Propagation of Medicinal Plants for Sustainable Livelihoods, Economic Development, and Biodiversity Conservation in South Africa. Plants. 2023 3;12(5):1174. DOI: https://doi.org/10.3390/plants12051174
51. Fayemi OE, Elegbeleye JA, Akanni GB, Olaleye ED, Ogunremi OR, Kaindi DW, Okunbi FO, Anyasi JA. Bioactive Phytochemicals in the Development of Alternative Medicine. In Plant Food Phytochemicals and Bioactive Compounds in Nutrition and Health 2024 27:446-497. CRC Press. DOI: https://doi.org/10.1201/9781003340201