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Original Research Article

Free Radical Scavenging Potential and Bioactive Phytochemical Profile of *Lagenaria Breviflora*

*[1]Banso Aderotimi, [2]Dachi Stephen, [3]Usman, J. Idris, [1]Ajewole Elijah, [1]Nma Etsu-Musa

[1]*Department of Biological Sciences, Federal Polytechnic, Bida Niger State Nigeria*
[2]*Department of Agriculture, University of Jos, Jos Plateau State Nigeria*
[3]*Department of Biological Sciences, Federal Polytechnic, Nasarawa, Nasarawa State Nigeria*

ABSRTACT

Free radicals which are normal byproducts of normal metabolism, can cause oxidative stress resulting in DNA damage and gene mutation. When there is an excess of oxygen or insufficient reduction, reactive oxygen species (ROS) like superoxide anions, hydroxyl radicals and hydrogen peroxide are produced. ROS are essential for various important biological processes in organisms including phagocytosis, cell growth regulation, intracellular signaling and synthesis of biologically active substances. Leaf extract of *Lagenaria breviflora* was examined for its secondary metabolites, antioxidant properties and antibacterial effects on *Escherichia coli* (ATCC28923), *Staphylococcus aureus* (ATCC28923), *Enterococcus faecalis* (ATCC29212) and *Bacillus subtilis* (ATCC6051). Chemical analysis of the plant leaves showed that they contained sesquiterpens, steroids, terpenoids, alkaloids and tannins. The quantitative assessment of the secondary metabolites showed specific concentrations of various compounds in the leaf extract of *Lagenaria breviflora*. This study showed that the antioxidant activity demonstrated using DPPH (2, 2-diphenyl-1-1-picrylhydrazyl) scavenging assay increased with higher concentrations of the leaf extract. The susceptibility of the test organisms to the plant extract was evidenced by the zones of inhibition they produced. Zones of inhibition of 16.0±1.2mm, 17.5±0.3, 19.2±0.4mm, and 16.5±2.3mm were recorded against *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus subtilis* respectively when methanol extract of *Lagenaria breviflora* were assayed against the bacteria. The results indicated that the bacteria were vulnerable to the plant extract. This study suggests that the compounds present in leaf extract leaf extract *Lagenaria breviflora* have the potential for use in chemotherapy applications.

Keywords: *Lagenaria breviflora***,** free radicals, oxidative stress, vulnerable, phagocytosis.

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Introduction

Lagenaria breviflora, a perennial climber belonging to the *Cucurbitaceae* family, is characterized by its scabid and sandpapery leaves. The plant is commonly used for its herbal and medicinal properties in both humans and animals particularly in West Africa. Medicinal plants are made up of organic compounds known as phytochemicals , which cause specific physiological effects in human body.[1] The use of medicinal plants which are locally sourced, cheap and easily accessible can be a safe and alternative means of combating the problem of antibiotic resistance.[2] While it is widely utilized for food and medicinal purposes in Africa.[3]

*Corresponding author. E mail: drbanso@yahoo.com
Tel: 08060775952

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There has been limited scientific research conducted to verify its efficacy against various diseases. During the raining season, *Lagenaria breviflora* blooms and it bears fruits during the dry season. Among the many species of *Cucurbitaceae*, Lagenaria *breviflora* stands out for its antibacterial and antiviral harbal remedies with its extract being popular in local communities in Nigeria.[4] Cucurbitacine, a compound found in many *Cucurbitaceae* plants has attracted attention for its diverse biological activities in humans and animals,[5] including potential anticancer activities.[6] Patents exist for cucurbitacin in preparing medicine used for treating acne[7] and cancer.[8] The plant has about 960 species and 130 genera distributed mostly in Tropical and subtropical regions of the world.[9, 10]

Free radicals which are normal byproducts of normal metabolism, can cause oxidative stress resulting in DNA damage, gene mutation and cross-links between DNA strands and protein. When there is an excess of oxygen or insufficient reduction, reactive oxygen species (ROS) like superoxide anions, hydroxyl radicals and hydrogen peroxide are produced. ROS are essential for various important biological processes in organisms including phogocytosis, cell growth regulation, intracellular signaling and synthesis of biologically active substances.[11] Reactive oxygen species (ROS) have been associated with the development of various diseases such as cancer, malaria, hearth diseases, atherosclerosis, diabetes and other age- related health issues.[12, 13] The involvement of ROS in the cause of and progression of these

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diseases has lead to the suggestion that antioxidants could serve as beneficent preventive agents. While aerobic organisms, including humans posses natural antioxidant defenses that safeguards against oxidative damage and aid in the repair of compromised molecules, these innate mechanisms may sometimes proof insufficient. Therefore there is considerable interest in the intake of antioxidants through dietary sources as a means to support overall health and wellbeing.[10, 11] Phytochemical compounds with antioxidant properties can minimize or inhibit the damage caused by reactive oxygen species.[12] The objective of this study is to determine the free radical scavenging potential and phytochemical constituents of *Lagenaria breviflora.*

Materials and Methods

Collection of plant material

Fresh leaves of *Lagenaria breviflora* were collected from the Botanical Garden Forestry Reseach Institute (Latitude 9° 55' 42.56′ N Longitude 8° 53′ 31.62′ E) Jos North local government Plateau State, Nigeria on the 5th of December, 2023. The leaves were authenticated according to the guidelines set by the International Committee for Botanical Nomenclature (ICBN). The fresh leaves were carefully collected, placed in polythene bags, labeled and stored for future use.

Extraction of plant material

The plant leaves were air- dried in a shaded area at room temperature, and the plant material was extracted using a method similar to the one outlined by[13] with minor modifications. Initially 10g of plant powder was combined with 200ml ethanol, methanol or distilled water in a conical flask. The flask was then covered with aluminum foil, sealed using sterile cotton wool and, positioned on a rotary shaker at 37°C for 24h to ensure thorough mixing. Following the extraction procedure, the resulting extract was filtered through Whatman no 1 filter paper and kept in the refrigerator until needed.

Qualitative phytochemical screening

The leaf extracts were assayed for the presence of glycosides saponins, steroids terpenoids, tannins and sesquiterpenes as outlined by [14, 15, 16, 17]

Quantitative analysis

Determination of total phenolic content

The total phenolic content of the ethanolic leaf extract was quantified using the Folin-Ciocalteu method with a UV-Vis spectrophotometer (6705 UV/Vis, JEN-WAY, France). Initially 1ml of methanol leaf extract was mixed with 9mL of distilled water in a 25mL volumetric flask and thoroughly combined. After a 5 minutes interval, 10ml of 7% sodium carbonate was added and the mixture was vigorous shaken. To reach a final volume of 25ml, 4ml of distilled water was added, and the flask was then placed in the darkness for incubation. Gallic acid standard solutions of different concentrations (20, 40, 60, 80, and 100µg/mL) were prepared and also incubated for 90 minutes alongside with the test sample. A blank solution was prepared using 1ml of distilled water instead of the extract. After the incubation period, the absorbance was measured using UV-Vis spectrophotometer (6705 UV/Vis, JEN-WAY, France).[18, 19, 22]

Determination of total flavonoid content

The total flavonoid content in the leaf extract was determined adding 1ml of a 1mg/ml extract to a 10ml volumetric flask. Following this, 4ml of distilled water, 0.3mL of 5% sodium nitrate and 0.3ml of 5% aluminum chloride were added. Subsequently, 2mL of 1M sodium hydroxide was introduced to the mixture and shaken thoroughly, and the final volume was adjusted to 10ml by adding 2.4ml of distilled water. A blank solution was created by substituting 1ml of distilled for the extract. Moreover, standard solutions of quercetin at concentrations of 20, 40, 60, 80, and 100µg/ml were prepared by dissolving quercetin in methanol. After a 30-minute incubation period, the absorbance of both the standard and test solutions was measured at 510nm using a UV-Vis spectrophotometer (6705 UV/Vis, JEN-WAY, France). [20]

Antioxidant activity

DPPH (2, 2-diphenyl-1-1-picrylhydrazyl) Radical scavenging assay

In order to evaluate the antioxidant activity using a DPPH (2, 2-diphenyl-1-1-picrylhydrazyl) radical scavenging assay, a DPPH stock solution was created by dissolving 2.4 mg DPPH in 100mL of methanol. Different concentrations (50, 100, 150, 200, and 250µg/mL in ethanol) of the plant extract were then prepared. Each concentration (100µl) of the leaf extract was mixed with 3ml of DPPH in a 25ml volumetric flask. Similarly standard solution of ascorbic acid (at concentrations of 50, 100, 150, 200, and 250µg/mL in distilled water) were prepared using the same pmethod. A control sample containing only DPPH was prepared, with methanol used as a blank. After a 30-minute incubation period, the absorbance of both the standard and test solutions was measured at 515nm using a UV-Vis spectrophotometer (6705 UV/Vis, JEN-WAY, France).. The radical scavenging activity of the leaf extract was calculated using the following formula adapted from [20]

$$\text{DPPH \%} = \frac{\text{Ab(control)} - \text{Ab(sample)}}{\text{Ab(control)}} \text{ X } 100$$

(Ab(control)) = Absorbance of the control sample (DPPH only)
(Ab(sample)) = Absorbance of the test sample (DPPH + leaf extract)

Antimicrobial activity

Test organism

The study utilized strains of *Escherichia coli* (ATCC28923), *Staphylococcus aureus* (ATCC28923), *Enterococcus faecalis* (ATCC29212) and *Bacillus subtilis* (ATCC6051) obtained from National Institute of Pharmaceutical Research, Abuja, Nigeria.

Standardization of bacteria

The bacteria suspension was standardized using a McFarland standard to reach a turbidity level equivalent to$1X10^6$ bacteria cell/ml (0.5 McFarland standards) and this level was maintained consistently during the entire study.

Antibacterial bioassay

The agar diffusion method described by [14] was used to assay for the effect of the leaf extract on *Escherichia coli* (ATCC28923), *Staphylococcus aureus* (ATCC28923), *Enterococcus faecalis* (ATCC29212) and *Bacillus subtilis* (ATCC6051). The bacterial cultures were inoculated onto separate nutrient agar plates and evenly spread using a sterile glass spreader. Using a sterile pipette, 200µl of 50mg/ml solution of the plant extract in DMSO was introduced into wells created on the agar surface. The plates were allowed to sit at room temperature for an hour to facilitate diffusion before incubating the plates at 37°C for 24hours to allow bacterial growth. The zones of inhibition were then measured, with positive control wells containing antibiotics for comparison. The antibacterial activity was determined by measuring the diameter of the zones of inhibition and compared them to the zone produced by Amoxillin in the positive control.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the approved broth microdilution method outlined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The

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tested extract, dissolved in 10% DMSO, was serially diluted by half directly in a microtiter plate containing Mueller-Hinton broth (M-H Broth), resulting in a concentration of $5x10^6$cfu/mL in each well for evaluation. A positive control containing amoxillin at a final concentration of 10µg/ml was also included. The plate was then sealed and incubated for 24h at 37°C. MIC was defined as the lowest concentration of the extract that completely inhibited bacterial growth, with a lower MIC indicating higher extract activity.[17]

Data analysis

The results were subjected to analysis of variance, and mean comparisons were conducted using Turkey's multiple range tests in SPSS version 20.0 (IBM Corporation, Armonk, NY, USA). Statistical significance was determined at a p-value of less than 0.05.

Results and Discussion

Phytochemical study of the plant leaf used in this study showed that *Lagenaria breviflora* contained sesquiterpens, steroids, terpenoids, alkaloids, tannins. Saponins and general glycoside were however absent in the plant leaf (Table 1). These findings align with those of, [21] who suggested that certain secondary metabolites could be present only in specific plant parts, with environmental stress potentially influencing the abundance of different secondary metabolites in plants. The presence of bioactive compounds with free radical scavenging abilities is suggested by antioxidant capabilities.[23]

The total terpenoids, steroids, sesquiterpens, tannins and alkaloids detected in the leaf extract of *Lagenaria breviflora* were 12.0±0.2, 13.5±0.1, 11.5±2.0, 15.5±3.0 and 19.0±2.0 mg/100g respectively (Table 2)

Table 1: Secondary metabolite constituent of leaf extract of *Lagenaria breviflora*

Constituent	leaf extract
Sesquiterpens	+
Steroids	+
Saponins	-
Glycoside	-
Terpenoids	+
Alkaloids	+
Tannins	+

(+) = Detected (-) = Not detected

Table 2: Total secondary metabolites detected in ethanol leaf extract of *Lagenaria breviflora*

Active principle	Quantity (mg/100g) ± SD
Terpenoids	12.0±0.2
Saponins	ND
Steroids	13.5±0.1
Sesquiterpens	11.5±2.0
Tannins	15.5±3.0
Alkaloids	19.0±2.0
Glycoside	ND

ND = Not detected

The qualitative and quantitative production of secondary plant metabolites can be affected by various agronomic factors such as developmental stage, plant organs, fertilization and soil pH. Climatic conditions like light intensity and water availability, as well as genetics, can also impact both the quantity and quality of the phytochemicals. [20]

DPPH (2, 2-diphenyl-1-1-picrylhydraxyl) scavenging activity

The DPPH scavenging activity of ascorbic acid and leaf extract of *Lagenaria breviflora* are shown in Table 3. The results showed that the DPPH scavenging activity is concentration dependent. Values recorded for ascorbic acid at concentrations of 50, 100, 150, 200 and 250µg/mL were 36.00±1.30, 65.00±2.32, 68.56±3.09, and 84.50±2.20 and 95.45±4.21 respectively. However values of 9.32±1.35, 16.70±0.40, 30.10±3.70, and 40.32±4.01 and 43.93±2.10% respectively were recorded for *Lagenaria breviflora*. This study demonstrated that the DPPH (2, 2-diphenyl-1-1-picrylhydrazyl) scavenging activity of leaf extract of *Lagenaria breviflora* extract increased with higher concentrations, albeit with lower antioxidant potential compared to ascorbic acid. Antioxidants inhibit oxidation in molecules, preventing oxidative chain reactions. Oxidative prowess of *Lagenaria breviflora* leaf likely stems from flavonoids phenolics, tannins and glycosides present within the leaf. This compound particularly phenolics, exhibit redox properties, crucial for neutralizing free radicals, oxygen singlets, triplets and decomposing peroxides. Phenolic compounds acting as natural antioxidants, transform DPPH from a purple stable radical to a colourless form, indicating their ability to donate hydrogen and reduce DPPH to DPPH-H. This is observed through the bleaching of purple DPPH solution. [24]

Antimicrobial properties of leaf extract of Lagenaria breviflora.

Diameter of zones of inhibition recorded against the test organisms ranged between 12.0±0.2and 19.2±0.4. Zones of inhibition of 12.0±0.2, 16.0±1.2and 14.5±0.2mm were recorded against aqueous, methanol and ethanol extracts respectively when assayed against *E. coli* (Table 4) Values of 15.2±0.1, 19.2±0.4and 17.5±3.1mm were recorded against *E. faecalis* when the extracts were assayed against the organism. Aqueous, methanol and ethanol extracts produced 14.5±2.0, 16.5±2.31 and 16.1±2.7mm respectively when the extracts were assayed against *B. subtilis* (Table 4).

This study found that leaf extracts of *Lagenaria breviflora.*exhibited antibacterial properties against *E. coli* (ATCC28923), *S. aureus* (ATCC28923) *E. faecalis* (ATCC29212)) and *B. subtilis* (ATCC6051). The size of the inhibition zones varied among the tested organisms, suggesting different levels of susceptibility to the plant extracts.[16] noted that the effectiveness of an agent can differ depending on the target species. Additionally, [17] emphasized that factors such as initial population density, growth rate and diffusion rate of the antimicrobial agent can influence the position of the zone of inhibition. . These variations underscore the potential of the plant extract and support its use in medicinal applications, likely due to the presence of observed phytochemicals, which are significant sources of pharmaceutical compounds. [20]

The result of the minimum inhibitory concentration indicates that the minimum inhibitory concentration of methanol leaf extract of *Lagenaria breviflora* against *E. coli* was 25mg/mL; however the minimum inhibitory concentration recorded against *S. aureus, E. faecalis, B. subtilis* were 40, 30 and 35mg/ml respectively (Fig. 1). In this study, the ethanol leaf extract of *Lagenaria breviflora.*displayed the lowest minimum inhibitory concentration against *Escherichia coli* (ATCC28923) and highest against *Staphylococcus aureus.* [16] proposed that antimicrobial agents with lower activity tend to have highest minimum inhibitory concentration values, while highly effective agents exhibit lower values. This research suggests that the compounds found in *Lagenaria breviflora* leaf extract hold promise for potential application in chemotherapy.

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Table 3: DPPH Scavenging activity of ascorbic acid and leaf extract of *Lagenaria breviflora*

Concentration (µg/ml)	Percentage of DPPH scavenged by ascorbic acid ±SD	Percentage of DPPH scavenged by leaf extract ±SD
50	36.00±1.30	9.32±1.35
100	65.00±2.32	16.70±0.40
150	68.56±3.09	30.10±3.70
200	84.50±2.20	40.32±4.01
250	95.45±4.21	43.93±2.10

SD = Standard deviation

Table 4: Susceptibility pattern of leaf extracts of *Lagenaria breviflora.*

	Leaf extract (50mg/mL)			Standard antibiotic
	Diameter of zone of inhibition (mm) ± SD			
Bacteria strain	Aqueous Ex	Methanol Ex	Ethanol Ex	Amoxillin
E. coli	12.0±0.2	16.0±1.2	14.5±0.2	29.7±2.3
S. aureus	14.4±0.2	17.5±0.3	15.3±1.3	34.8±0.2
E. faecalis	15.2±0.1	19.2±0.4	17.5±3.1	38.3±1.6
B. subtilis	14.5±2.0	16.5±2.3	16.1±2.7	35.0±0.4

SD = Standard deviation, Ex = extract

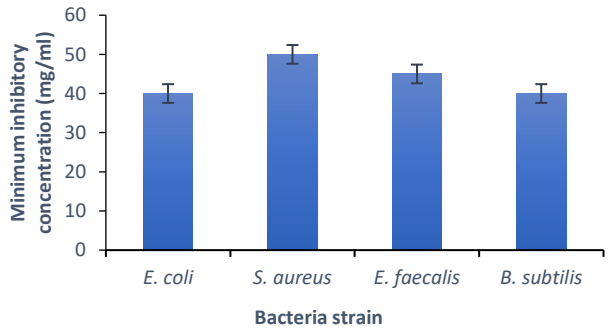

Fig 1: Minimum inhibitory concentration (mg/ml) of ethanol extract of Lagenaria breviflora againstthe test bacteria strains

Conclusion

This research profiles Leafy Vegetable Farmers and their Profit Efficiency during COVID-19 Lockdown in Oyo State. In accordance to study's findings, leafy vegetable farming is not only feasible but also profitable in the study area. The research revealed the there was good prospects for leafy vegetable production in Egbeda L.G., the entire State and Nigeria, even during the COVID-19 lockdown and the factors that contributed to profit included educational level, size of farm, farm income and farming experience. The study also showed that leafy vegetable farmers are educated in some way, thus any effort to educate them in modern and improve methods of production will translate into a proportional rise in the amount of farmers' participation in leafy vegetable production in the study area. It can therefore be concluded that since vegetables, particularly leafy vegetables are perishable and the deterioration had an impact on farm families' revenue, leafy vegetable farmers should constantly be assisted most especially because of the significant benefit eating more fruits and vegetables as part of a balanced diet lowers the risk of developing various chronic diseases for example COVID-19, which is one of the health benefits of doing so. This study recommends that an encouragement to farmers in growing more vegetable by agricultural stakeholders (Governmental and Non-Governmental Organisation) is sufficient in bridging the gap between demand and supply of food and also a means of cheap source of access to the nutrients the body needs to fight against diseases (COVID-19 and others).

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Oyawaluja B O, Oyawaluja A A, Akinyimika D E, Odukoya O A, Coker H A B. Antioxidant profiling , phytochemical investigation and pharmacognostic evaluation of *Nephralepis biserrata* (SW) Schott (Nephrolepidaceae) Trop J Phytochem Pharm Sci 2024, 3(2):208-215
2. . Oiseoghaede J O, Oyawaluja A A, Sowemimo A A, Odukoya, O A, Motaudra guineensis (Thonn) A.D.C (Apocynaceae) against human melanoma and ovarian cancer cells. Trop J Nat Prod Res 2024; 8(3): 6700-6704
3. Obajuluwa A F, Dani C L, Adeshina G O, Param S K, Bello, M. Phytochemical and antimicrobial studies of stored dried leaves of *Alchornea cordifolia* (Schum & Thonn) Mull. Arg (Euphobiaceae) Trop J Phytochem Pharm Sci 2024; 3(2):196-200
4. Olanrewaju O O, Fajinmi O O, Arthur G D, Coopoosamy R.M Naidoo K K. Food and medicinal relevance of *Cucurbitaceae* species in Eastern and SouthernAfrica. Bulletin of the national Res Cent 2021; 45(1)208
5. Popoola O K, Faleye F J, Adebowale, A A, Adebayo, A A, Dauda O S, Fadahunsi O N. Invitro assessment of skin enzyme inhibitory activities, total antioxidant potentials and ultraviolet spectral characteristics of *Chromolaena odorata* methanol extract at its column chromatographic fraction. Tropical J Phytochem Pharm Sci 2024; 3(2):189-195

6. Dhiman K, Gupa A, Sharma D K, Gill NS Goyal A A. review on the medicinally important plants of the family *Cucurbitaceae.* Asian J Clin Nut 2012; 4(1)16-26.
7. Lee D H , Iwanski G B and Thoennissen N H. *Cucurbitacin:*Ancient compound shedding new light on cancer treatment . The Scient World J 2010; 10 413-418
8. Deng Y , She Z. Application of cucurbitacins in preparing medicine used for treating acnes, Shenyang Pharma Uni, Shenyang. 2011
9. Deng Y , Meixia Z. New medical use of Cucurbitacin in treating cancer. Europe PMC , Shenyang Pharma Uni , Shenyang.2017
10. Schaffer A A, Paris H S. Melons, sqashes and gourds in B. caballero, P.finglas and F. Toldra (eds). Encyclopedia of food Sci and Nutri 2003; 3rd edn,.pp 3817 – 3827, Academic press, Cambidge , MA.
11. Honda K, Casadesus G, Paterson R B, Perry G , Smith M A. Oxidative stress and redox iron in Alzheimer's disease. Ann. New York Aca Sci. 2005;1012: 179-182
12. Espin J C, Soler-rivas C , Wichers H J. Characterisation of the total free radical scanvenging capacity of vegetable oils and oil fractions using 2,2 diphenyl-1-picrylhydrazyl radical. J Food Chem 2000;48:648-656
13. Greenwald P, Clifford C K, Miner J A. Diet and cancer prevention. Euro J Cancer 2001;37:948-965
14. Banso A, Dachi S, Ajewole A E, Etsu-Musa N , Ajayi M A. Impact of spices and aromatic plants on the growth and mycotoxin production in fungi. J Curr Opin Crop Sci 2004a;5(1) 1-5
15. Banso A, Ajeigbe S O, Koleola A A , Banso B F. Evaluation of phytochemical constituents and antimicrobial activity of *Vernonia amygdalina* leaf extracts. West Afri J Pharmacol and Drug Res 2020; 34:16-20
16. Banso A, Dachi S, Ajewole A E, Etsu-Musa N , Ajayi M A. Evaluation of antioxidant and bioactive phytochemical properties of *Achyranthes aspera* L. J Curr Opin in Crop Sci 2024c; 5(1):13-20
17. Banso A, Banso F, Ajeigbe S O, Koleola A.A. Optimisation of physical parameters for enhanced antimicrobial activity of *Acalypha hispida* leaf extract. Nigerian J Pure and Appl Sci 2021; 34(1)3844-3850
18. Chakraborty A, Brantnar A, Mukuinaka T, Nobukuni Y , Kuchido M. Activity of *Achyranthes aspera* leaves on esteinbarr virus activity and two stage mouse skin carcinigenasis. Cancer Lett 2002; (177)1-5
19. Geetha P, Narayanan K. R., Murugesan A G. Screening the anticancerous efficiency of *Achyranthes aspera* Linn using animal model Swiss albino mice. J. Biomed Sci Res 2010; (2)231-235
20. Malarvili T, Ganathi N. Effect of *Achyranthes aspera* (Linn)) seed on redox and oxidative status in plasma and selected tissues of rats fed with high dosages of fructose. Biosci Biotechnol Res. Asia 2009; (6)659-664
21. Banso A, Dachi S, Ajewole A E, Etsu-Musa N , Ajayi M A. Screening of antiaflatoxin, anti-inflammatory performance and biosafety of *Spondias mombi* Linn. (Hog Plum) leaf extract. Pharma Inn J. 2024b; 13(3):189-192
22. Yang L, Wen K.S, Ruan X, Zhao Y.X, Wei F , Wang A. Response of plant secondary metabolites to environmental factors. Molecles 2018; 27(4)762
23. Shaikh J R, Patril M. Qualitative test for preliminary phytochemical screening : An overview . Int J Chem Stud 2020;8(2) :603-608
24. Vazquez-Leon L.A, Pramo-Calderon D.E, Robles-Olvera V.J, Valdes-Rodrigez O.A, Perez-Vazquez A, Garcia-Alvarado M.A, Rodriquez-Jimenes G C. Variation in bioactive compounds and antiradical activity of *Moringa oliferaleaves*: Influence of climatic factors, tree age and soil parameters. Euro Food Res Tech 2017; (243) 1595-1608