Jewel Journal of Scientific Research (JJSR) 10(1): 10–19, 2025
©Federal University of Kashere-Nigeria (Print ISSN: 2384 – 6267, Online ISSN 2756-651X)
https://journals.fukashere.edu.ng/index.php/jjsr

Comparative Assessment of The Antibacterial Activity of Ethanolic and Aqueous Extract of *Annona muricata* against *Staphylococcus aureus*

***Mohammed, A. O.[1], Hassan, S. O.[1] and Yakubu, F. E.[1]**
[1]Department of Biological Sciences, Faculty of Natural and Applied Sciences, Al-Hikmah University, Ilorin, Nigeria.
***Corresponding Author**: muhdaeesha@gmail.com; +2347038948557

Abstract
In indigenous medicine, natural products particularly those derived from plants have long been utilized to support human health. *Annona muricata* is widely used in folkore medicine by indigenous people in South America and Africa because of its purported therapeutic benefits. This study was conducted to explore he phytochemical constituents and also ascertain the antibacterial activity of ethanol and aqueous extracts of *Annona muricata* leaf (soursop) leaves against *Staphylococcus aureus*. The extracts were screened qualitatively for the presence of phytochemicals and tannins, saponins, flavonoids and alkaloids were present. Antibacterial susceptibility profile of the isolate with standard antibiotics as well as the antibacterial activity of the plant extracts was determined using disc and agar diffusion method respectively. The inhibition zones of ethanolic and aqueous extracts of *Annona muricata* against *Staphylococcus aureus* were 37 ± 4.7 mm and 25 ± 3.75 mm respectively at 100 mg/ml concentration. The HPLC analysis of the phytochemicals revealed the presence of Limonime, Licorice, Geraniol, Strychnine, Chlorogenic acid, Quinine, Squalene and some unidentified compounds. This study revealed the antibacterial activity of crude *Annona muricata* leaf extracts against the clinical isolate in comparison to some standard antibiotics. Further research on the isolation and characterization of the bioactive agent in the plant is required to provide a variety of novel components for drug discovery.

Keywords: Phytochemicals, High Particulate Liquid Chromatography, Inhibition Zones.

Received: 15th March, 2025 *Accepted:* 28th April, 2025 *Published Online:* 30th April, 2025

Introduction

Annona muricata Lin., commonly referred to as soursop, is a member of the Annonaceae plant family and is extensively cultivated in tropical and subtropical regions, including Southeast Asia, South America, and the rainforests of Africa (Mutakin *et al*., 2022). The various plant parts of *A. muricata* Lin. including the leaves, bark, fruit and seeds have been traditionally used for ethnomedicinal purposes to address a diverse range of health issues (Mutakin *et al*., 2022; Nwonuma *et al*., 2023). The plant is known to contain compounds with pharmacological activity such as flavonoids, terpenoids, saponins, coumarins, lactones, anthraquinones, glycosides, tannins, and phytosterols (Gavamukulya *et al*., 2014). *Annona muricata* is an antioxidant-rich plant and this confers on it the ability to counteract free radicals and prevent oxidative stress at the cellular and tissue levels (Badmus *et al*., 2022). Secondary metabolites in *A. muricata* such as flavonoids, alkaloids, phenolics and tannins have been documented to possess therapeutic activity in biological systems (Olasehinde and Afolabi, 2023). Notably, all parts of *A. muricata* are known to possess therapeutic activity hence, the interest in the comparative assessment of the ethanolic and aqueous extracts of the plant leaf.

Materials and Methods

Collection and identification of plant sample

Fresh and mature leaves of *Annona muricata* were collected at University of Ilorin Senior Staff Quarters, Ilorin, Kwara State. The plant sample were rinsed under running tap water to remove debris or filth and undesirable materials (Farouk, 2009) and identified at the herbarium unit of Plant Biology Department, University of Ilorin with voucher number UILH/003/890/2021.

Preparation of Plant Material and Extraction

Fresh leaves of *A. muricata* were air-dried for seven (7) days and pulverized using an electrical blender (Marlex 500) and the powdered form was kept in an air tight container until required for extraction. The ethanol extract of the plant leaf was prepared via maceration of 50g of the powdered leaf in a 1000 ml conical flask containing 500 ml of absolute ethanol at room temperature for 24 h. The extract was filtered with muslin cloth and subsequently filtered using Whatman No 1 filter paper and concentrated using a rotary evaporator (Gallenkamp RE-52A) at 40^0C. This procedure was repeated for aqueous extraction.

Qualitative Phytochemical Screening

Phytochemical screening was carried out to ascertain the presence of bioactive phytochemical components in the ethanolic and aqueous extract of *Annona muricata* Linn. Components screened for were Alkaloids, Flavonoids, terpenoids, phenols, tannins, saponnins, anthraquinones and phlobatannins following the procedures as described by Geetha and Geetha (2014) and Anibijuwon *et al*. (2020) with slight modifications.

Test Organism

Staphylococcus aureus of clinical importance was collected from the Microbiology Laboratory of Al-Hikmah University, Ilorin, Nigeria, and sub-cultured for viability checking at 37 ℃ for 24 hours and sustained on nutrient agar slant at 4^{o}C till further use. Standardization of inoculum (Ochei and Kolhatkar, 2008) was achieved by picking colonies (3-4 colonies) of the strain from the sub-cultured agar plate using a sterile inoculating loop into the sterile normal saline in a sterilized test tube. This was standardized using a UV spectrophotometer (Lemfield Medical England) to get an absorbance value of 0.063 – 0.1 at a wavelength (600 nm) equivalent to 0.5 McFarland turbidity standard corresponding to 10^8 cells /ml.

Antibacterial susceptibility test

The disc diffusion method was used to determine the antibacterial activity of *A. muricata* method as described by Vineetha *et al.* (2014) with slight modifications. A sterile Pasteur pipette was used to transfer 0.5 ml of the standardized inoculum onto the surface of Mueller Hinton agar plates. Filter paper discs impregnated with concentration of the crude extracts were then placed at equidistant to each other using sterile forceps on the Mueller Hinton agar plates seeded with the test organism. Plates were incubated at 37^o C for 24 hours after which the mean diameter of the zones of inhibition of the extracts against the test organism were measured.

HPLC Assay

The compounds were identified using High Performance Liquid Chromatography (HPLC) analysis, a technique that separates, identifies, and quantifies compounds in a mixture. The method described by Sakakibara *et al*. (2003) was explored.

Data Analysis

Data were analyzed statistically and the results were expressed as mean±SEM (standard error of mean). With one-way analysis of variance (ANOVA) was used to evaluate of significant level at $p \leq 0.05$.

Results

Result of phytochemical screening of the aqueous and ethanol extracts of *Annona muricata* are shown in Table 1; the presence of Alkaloids and terpenoids in the aqueous extract and tannins, alkaloids, saponins, terpenoids, and flavonoids in the ethanol extracts were revealed. Tables 2 shows the MIC and MBC values respectively. Figure 1 shows the activity of the Ethanolic and Aqueous extract of *Annona muricata* against *Staphylococcus aureus* with varying zones of

inhibition. The activity of the test isolate in the presence of selected commercially available antibiotics showed resistance to Augmentin, Ceftazidine, Vancomycin, Meropenem and Ampicillin and susceptible to Erythromycin, Ciprofloxacin, Gentamycin, Co-trimoxazole were revealed in Figure 2. Figures 3, 4 and 5 show the HPLC chromatogram of Terpenoids, Alkaloids and Saponins of the leaf extract.

Table 1: Qualitative phytochemical composition of Ethanol and Aqueous Extracts of *Annona muricata* Linn. Leaf

Phytochemicals	Ethanol	Aqueous
Tannins	+	-
Flavonoids	+	-
Saponins	+	-
Steriods		-
Alkanoids	+	+
Anthraquinones	-	-
Terpenoids	+	+
Phlobatannins	-	-

Key: (+) Present (-) Absent

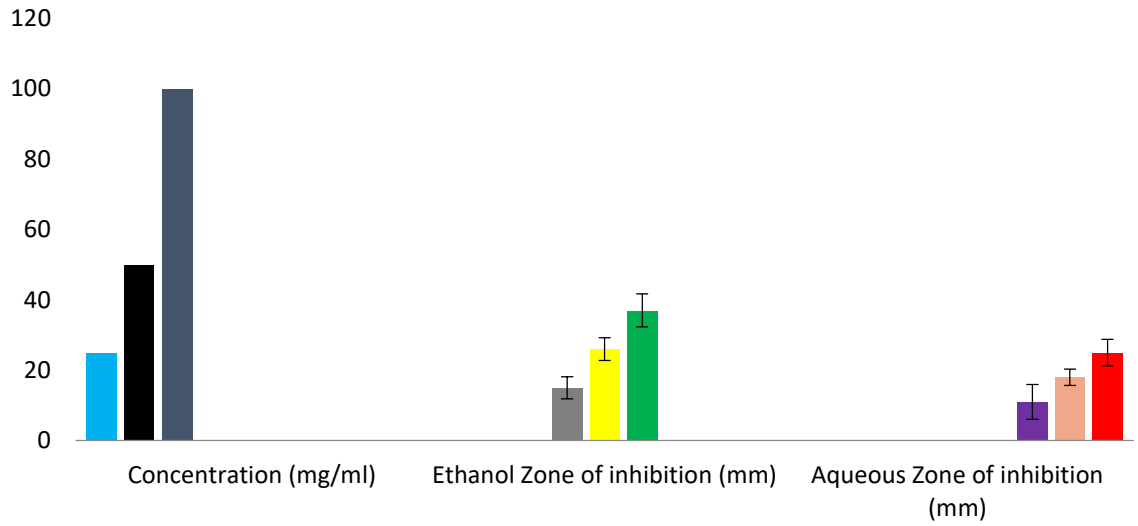


Figure 1: Antibacterial activities of ethanolic and aqueous leaf extract of *Annona muricata* against *Staphylococcus aureus*

Table 2: MIC and MBC of the *A. muricata* leaf extracts

Extracts	MIC	MBC
Ethanolic	50 mg/ml	100 mg/ml
Aqueous	50 mg/ml	100 mg/ml

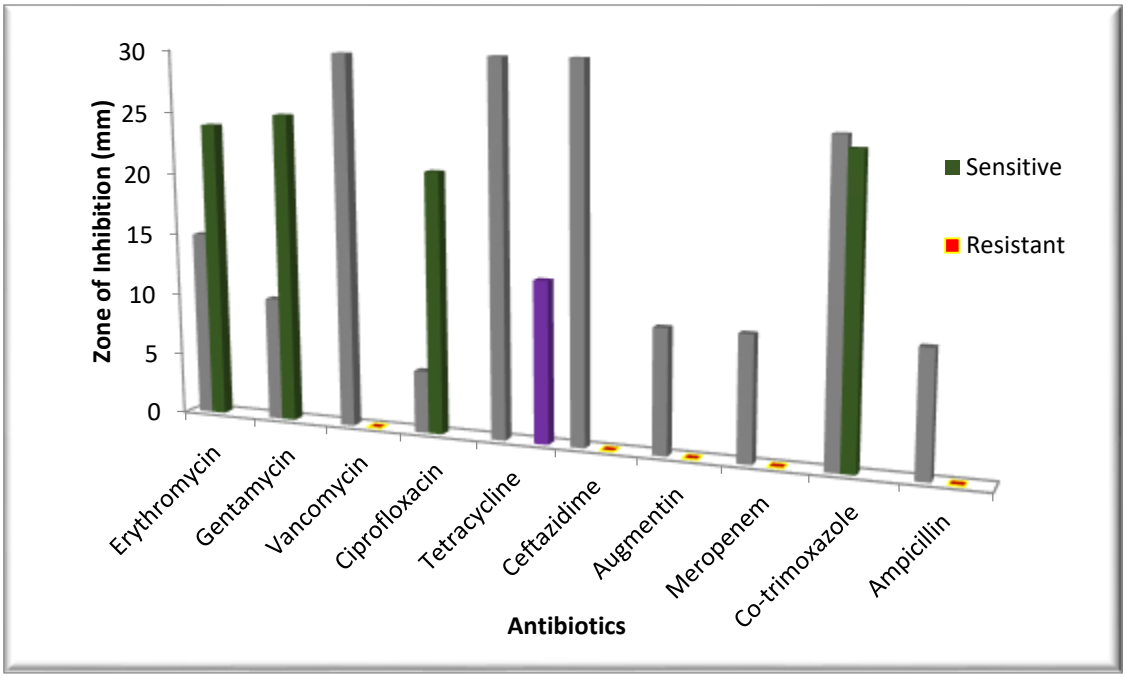
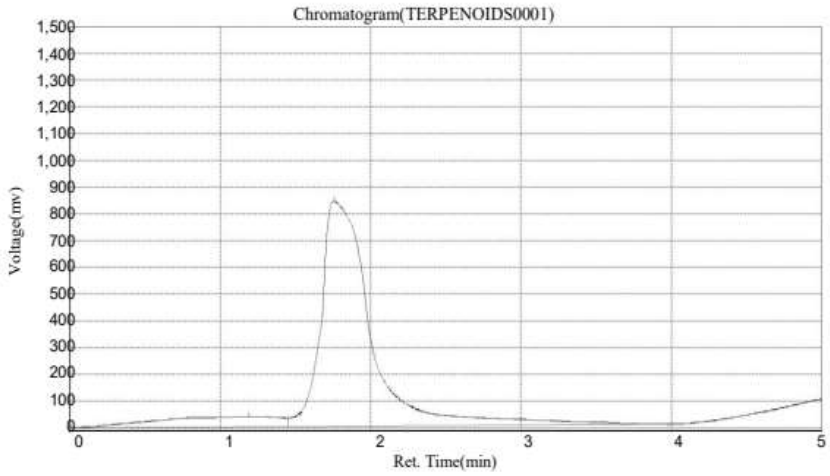


Figure 2: Antibiotic susceptibility test against *Staphylococcus aureus*

Instrument:LC
Column Temp.(¡æ)£°35

Gradient:

Detector:UV
Wavelength(nm)£°210



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc µg/100g
1	Limonime	1.190	37148.703	2160451.500	9.9416
2	Geraniol	1.757	840199.563	19571066.000	80.0584

System Evaluation

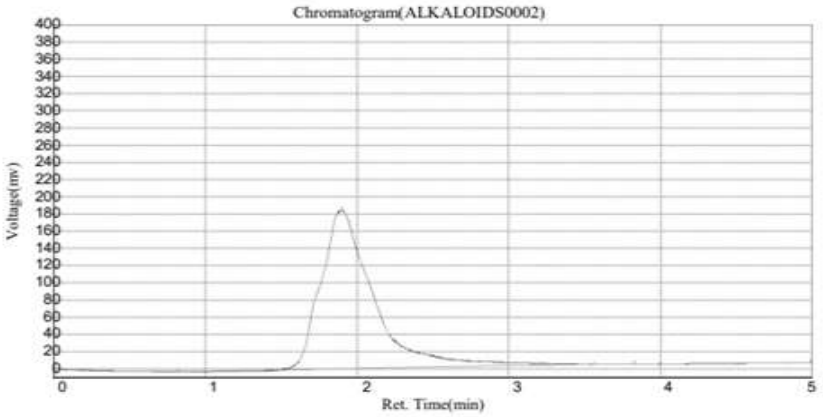
Peak No.	Peak ID	Ret Time	Half-Peak Width	Theoretical Level	Resolution	Tail Factor	Asymmetry

Figure 3: HPLC Chromatogram of Terpenoids components in *A. muricata* leaf

Instrument:LC
Column Temp.(¡æ)£°35

Gradient:

Detector:UV
Wavelength(nm)£°245



Results

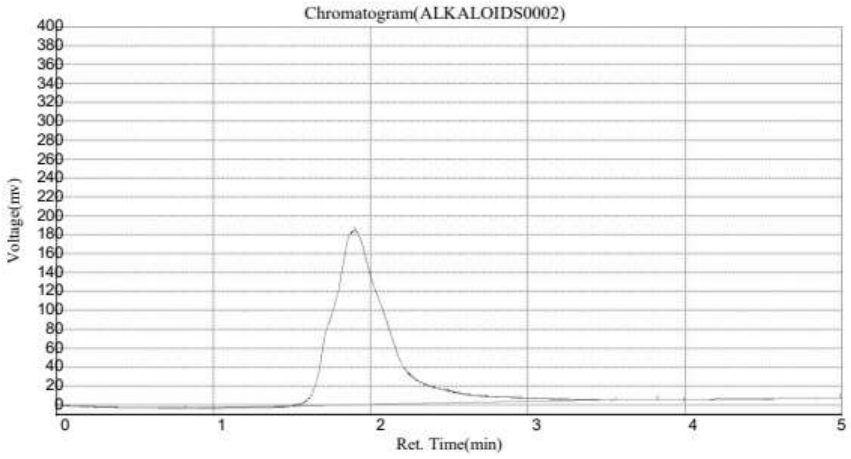
Peak No.	Peak ID	Ret Time	Height	Area	Conc µg/100g
1	Strychnine	1.898	184007.766	4397584.500	59.8255
2	Quinine	3.823	55.846	701.300	0.0159
3	Chlorogenic acid	4.990	288.915	1672.987	0.0380
4	Unidentified	5.348	327.593	5312.816	0.1206

Figure 4: HPLC chromatogram of Alkaloids components in *A. muricata* Leaf

Instrument:LC
Column Temp.(¡æ)£°35

Gradient:

Detector:UV
Wavelength(nm)£°245



Results

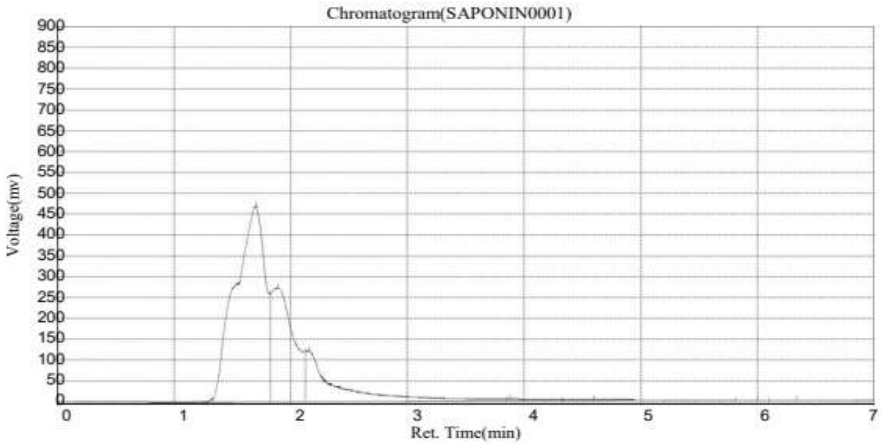
Peak No.	Peak ID	Ret Time	Height	Area	Conc µg/100g
1	Strychnine	1.898	184007.766	4397584.500	59.8255
2	Quinine	3.823	55.846	701.300	0.0159
3	Chlorogenic acid	4.990	288.915	1672.987	0.0380
4	Unidentified	5.348	327.593	5312.816	0.1206

Figure 4: HPLC chromatogram of Alkaloids components in *A. muricata* Leaf

Instrument:LC
Column Temp.(¡æ)£°35

Gradient:

Detector:UV
Wavelength(nm)£°205



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc µg/10g
1	Unidentified	1.698	469942.313	8426534.000	58.6246
2	Unidentified	1.890	273071.219	3616740.000	25.1622
3	Flavouring	2.157	122107.344	2186316.750	15.2105
4	Unidentified	3.882	3370.261	76242.789	0.5304
5	licorice	4.598	1772.942	59030.758	0.4107
6	Unidentified	5.998	150.295	1899.166	0.0132
7	Squalene	6.332	313.377	6949.531	0.0483

Figure 5: HPLC chromatogram of Saponins components in *A. muricata* Leaf

Discussion

Phytochemicals play vital role in the bioactivity of medicinal plants (Ekwenye and Elegalam, 2005; Cao *et al*., 2022). The presence of phytochemicals in medicinal plants has been linked to antibacterial efficacy due to their biologically active pharmacological properties (Adebayo and Ishola, 2009). The qualitative screening for bioactive phytochemicals of the ethanolic extract of *A. muricata* leaf revealed the presence of alkaloids, tannins, flavonoids, saponins, and terpenoids which is in agreement with similar research done by Menut *et al.* (2016) while the aqueous extract revealed the presence of Alkaloids and Terpenoids only. The disparity in phytochemicals from the two solvents (ethanol and aqueous) could be due to the polarity of the solvents and a solvent's polarity influences the solubility of target phytochemicals. Ethanol is a polar solvent hence, its ability to extract bioactive substances such as flavonoids and alkaloids may be linked to its nature and potential (Jeyachandran *et al*., 2010; Dieu-Hien, 2019).

Alkaloids were detected and they are known to possess antimicrobial and cytotoxic properties (Mohammed *et al.*, 2025). Tannins act as antibacterial agent through the deactivation of microbial adhesives, enzymes, cell envelopes and transport (Hashem and El-Kiey, 1982; Ishtiaq *et al.*, 2013). Flavonoids are known for their scavenging skills which is a mechanism that prevents tumor invasion in cells (Alan and Miller, 1996; Williams *et al.*, 2004). Saponins are known to possess anti-inflammatory and antimicrobial properties (Soeten, 2006). Terpenoids have shown antimicrobial properties in studies as reported by Mohammed *et al.* (2025).

The aqueous and ethanolic extracts of *A. muricata* showed significant antibacterial efficacy against *Staphylococcus aureus* as revealed by the observed zones of inhibition in Figure 1. This finding may imply that *A.*

muricata extracts contain antimicrobial components that could be employed as antimicrobial agents in novel therapeutic formulations to treat staphylococcal infections in people.

The aqueous and ethanolic extracts of *A. muricata* both showed antibacterial activity against the test organism and the organism showed different response for each extract as revealed by the observed zones of inhibitions. The antibacterial effect was shown to be concentration-dependent and the ethanol extract revealed wider zones of inhibition across the varied concentrations, this may be due to the fact that ethanol is a better extractant than water because of its short carbon chains. The zones of inhibition of ethanol and aqueous extracts at 100 mg/ml were 37 ± 4.7 mm and 25 ± 3.75 mm respectively albeit there were lesser zones of inhibition for ethanol and aqueous extracts at lower concentrations such as 50 mg/ml showing 26 ± 3.2 mm and 18 ± 2.31 mm respectively. This study corresponds with the work of Gleye *et al.* (1999) who found that the ethanolic and aqueous extract of *Annona muricata* have antibacterial effects against *Staphylococcus aureus*.

The result of the antibacterial testing of the plant was carried out using agar well diffusion method while MIC was carried out using broth dilution method which shows the sensitivity of *Staphylococcus aureus* to *Annona muricata*. MIC and MBC of both extracts were at 50mg/ml and 100mg/ml respectively as shown in Tables 2 and 3 respectively.

The antibiotic susceptibility pattern revealed the sensitivity of the test organism to Co-trimaxazole, Erythromycin, Gentamicin and Ciprofloxacin and resistance to Vancomycin and Ceftazidime. Among the antibiotics tested against the organism, Gentamicin shows the widest zone of inhibition compared to the other antibiotics used. This confirms the effectiveness of the drug in treating diseases associated with the test organism. The components separated by HPLC in ethanolic and aqueous extract *Annona muricata* leaf were; Limonene, Geraniol, Strychnine, Chlorogenic acid, Licorice, Quinine, Squalene and some unidentified compounds.

Limonene belongs to class terpenes and it possess anti-inflammatory, antioxidant, anti-stress, and disease-preventing properties (John *et al.*, 2018). Geraniol belongs to the terpenes family. It possesses antibacterial activity, antioxidant, antifungal and anti-inflammatory properties (John *et al.*, 2018). Quinine interferes with the growth and reproduction of *Plasmodium falciparum* and serves as an antimalarial (Othman *et al.*, 2019). Squalene belongs to class of triterpenes. It possesses its anti-cancer, antioxidant and skin- protecting properties (De Vita *et al.*, 2019).

Strychnine contains high quantity of alkaloids and it possesses antimicrobial properties (De Vita *et al.*, 2019). Licorice possesses antiviral and antimicrobial properties. Licorice is effective against *S. aureus* which has been implicated in skin infections such as folliculitis, impetigo etc (Preena *et al.*, 2020).

Chlorogenic acid possesses hypoglycemic, hypolipidemic, anti-inflammatory and antioxidant properties. It also increases plasma membrane permeability leading to the breakdown of membrane function of the bacteria (Balcerczyk *et al.*, 2019). These aforementioned compounds revealed the efficacy of *A. muricata* in therapeutic formulations for treating Staphylococcal infections.

Conclusion

Annona muricata has been used for several decades in medicine. This study further shows that based on the antimicrobial potential and importance of the plant's phytoconstituents, it is regarded as valuable plant that has a promising use as an antibacterial agent to compliment treatment of various diseases caused by *Staphylococcus aureus.* Research into isolation and identification of the active compounds in the plant is encouraged for potential ingredient in drug discovery.

References

Adebayo, E.A. and Ishola, O. R. (2009). Phytochemical and antimicrobial screening of the crude extracts from the

root, stem bark and leaves of *Bridelia ferruginea*. *African. Journal of Biotechnology*. 8(4): 650-653

Adedapo, A. A., Oni, O. A., Falayi, O. O., Ogunmiluyi , I. O., Ogunpolu, B.S., Omobowale, T.O., Oyagbemi, A. A., Oguntibeju, O.O. and Yakubu, M. A. (2022). *Annona muricata* mitigates glycerol-induced nephrotoxicities in male albino rats through signaling pathways of angiotensin conversion enzyme, kidney injury molecule-1, and antioxidant properties. *Scientific African* 16: e01225, ISSN 2468-2276, https://doi.org/10.1016/j.sciaf.2022.e01225

Anibijuwon, I.I., Adetutu A., Suleiman, M.M., Olafimihan, C.A. and Olanrewaju, S.O. (2020). Multi-Drug Resistant Salmonella typhi among Out-Patients in Hospitals within Ilorin, Nigeria and their Susceptibility to *Cymbopogon citratus* (Lemon grass). *Nigerian Journal of Pure and Applied Sciences (NJPAS)* 33(1): 3628-3638. http://dx.doi.org/10.48198/NJPAS/20.A16

Badmus, J. A., Rafiu, M. A. and Fatoki, J. O. (2022). The protective effect of ethanol leaf extract of *Annona muricata* against doxorubicin toxicity via modulations of hematological, serum biochemical, antioxidant enzymes, and lipid peroxidation *Phytomedicine Plus*. 2(3): 100328 https://doi.org/10.1016/j.phyplu.2022.100328

Balcercyk, A., Biesiekierska, M., Vialichka, V. and Pirola, L. (2019). Histone acylation in the epigenomic regulation of Insulin Action and Metabolic disease. *Nutritional epigenomics* 14: 101-117 https://doi.org/10.1016/B978-0-12-816843-1.00007-2

Cao, Z., Ma, J. and Shen, X. (2022). Alpinetin suppresses cell proliferation and metastasis in osteosarcoma by inhibiting PI3K/AKT and ERK pathways. *Qual. Assur. Saf. Crops Foods*, 14(2): 112-118.

De Vita, D.; Messore, A.; Toniolo, C.; Frezza, C.; Scipione, L.; Bertea, C.M.; Micera, M.; Di Sarno, V.; Madia, V.N. and Pindinello, I. (2019). Towards a new application of amaranth seed oil as an agent against *Candida albicans*. *Natural Product Research*, volume 35, issue 22:4621-4626 https://doi.org/10.1080/14786419.2019.1696335

Dieu-Hien, T., Dinh, H.N., Nhat, T.A., Ta, A., Tuong H.D. and Hoang, C.N. (2019). Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of *Severinia buxifolia*. *Journal of Food Quality*. 2019(1): 8178294 https://doi.org/10.1155/2019/8178294

Ekwenye, U. N. and Elegalam, N. N. (2005). Antibacterial activity of ginger (*Zingiber officinale* Roscoe) and garlic (*Allium sativum* L.) extracts on *Escherichia coli* and *Salmonella typhi*. *International Journal of Molecular Medicine and Advance Sciences*, 1(4): 411-416.

Farouk, L. A., Laroubi, R. A., Benharref, A. and Chait, A. (2009). Antinociceptive activity of various extracts of *Peganum harmala* L. and possible mechanism of action. *Iranian J. Pharmacol. Therap* 8: 29-35.

Gavamukulya, Y., Abou-Elella, F., Wamunyokoli, F. and AEl-Shemy, H. (2014). Phytochemical screening, anti-oxidant activity and in vitro anticancer potential of ethanolic and water leaves extracts of *Annona muricata* (Graviola). *Asian Pacific J. of Tro. Med.* 2014: S355-363 *https://*doi:org/10.1016/S1995-7645(14)60258-3

Geetha, T.S. and Geetha, N. (2014). Phytochemical Screening, Quantitative Analysis of Primary and Secondary Metabolites of *Cymbopogan citratus* (DC) Stapf. leaves from Kodaikanal hills, Tamilnadu. *International Journal of Pharm Tech Research* 6: 521-529.

Gleye, C., Laurensa, A., Laprevoteb, O., Serani, L. and Hocquemiller, R. (1999). Isolation and structure elucidation of Sabadelin, an acetogenin from roots of *Annona muricata*, *Phytochemistry* 5:

1403-1408 https://doi.org/10.1016/S0031-9422(99)00423-9

Hashem, F. M. and El-Kiey, M. A. (1982). Nigella sativa seeds of Egypt. *Journal of Pharmaceutical Sciences of the United Arab Republic*, 3: 121-133.

Ishtiaq, S., Ashraf, M., Hayat, M.Q. and Asrar, M. (2013). Phytochemical analysis of *Nigella sativa* and its antibacterial activity against clinical isolates identified by ribotyping. *International Journal of Agriculture and Biology*, 15(6): 1151-1156 http://www.fspublishers.org

Jeyachandran, R., Baskaran, X. and Cindrella, L. (2010). Screening of phytochemical and Antibacterial potential of four Indian medicinal plants. *Libyan Agriculture Research Center Journal Internation. Tripoli* 1(5): 301-306.

John, I.O., Awodele, O., Olusayero, A.M. and Ochieng, C.O. (2018). Mechanisms of analgesic and anti-inflammatory properties of *Annona muricata* Linn. (Annonaceae) fruit extract in rodents. *Journal of Medicinal Food*, 17: 1375–1382.

Menut, L., Siour, G., Mailler, S., Couvidat, F. and Bessagnet, B. (2016). Observations and regional modeling of aerosol optical properties, speciation and size distribution over Northern Africa and western Europe. *Atmospheric Chemistry and Physics.* 16(20): 12961–12982. https://doi.org/10.5194/acp-16-12961-2016

Mohammed A. O., Mansur M. M., Abdulsalam, H., Amina I. J., Haris N. G. Abubakar A. W., and Adeniyi, K. A. (2025). Comparative Efficacy of Pods, Leaves, Stem Bark of *Acacia nilotica* against *Aspergillus niger*. *Dutse Journal of Pure and Applied Sciences (DUJOPAS) 11(1d):* https://dx.doi.org/10.4314/dujopas.v11i1d.22

Mutakin, M., Fauziati, R., Fadhilah,F. N., Zuhrotun, A., Amalia, R. and Hadisaputri, Y. E. (2022). Review on Pharmacological Activities of Soursop (*Annona muricata* Lin.) *Molecules* 27: 1201. https://doi.org/10.3390/molecules27041201

Nwonuma, C. O., Balogun, E. A. and Gyebi, G.A. (2023). Evaluation of Antimalarial Activity of Ethanolic Extract of Annona muricata L.: An in vivo and an in silico Approach. *Journal of Evidence-Based Integrative Medicine* 28: 1-30. DOI: 10.1177/2515690X231165104

Ochei, J. O. and Kolhatkar, A. A. (2000). Medical laboratory science: theory and practice. McGraw Hill Education.

Olasehinde, O. R. and Afolabi, O.B. (2023). Identification of bioactive constituents of chloroform fraction from *Annona muricata* leaf, its antioxidant activity and inhibitory potential against carbohydrate-hydrolyzing α-amylase and α-glucosidase activities linked to type II diabetes mellitus: In vitro study. *Journal of Herbmed Pharmacol.* 12(1):100-108. doi: 10.34172/jhp.2023.09.

Othman, L., Sleiman, A. and Abdel-Massih, R.M. (2019). Antimicrobial activity of polyphenols and alkaloids in Middle Eastern Plants. *Frontiers* 10: 911 https://doi.org/10.3389/fmicb.2019.00911

Preena, P. G., Swaminathan, T. R., Rejish Kumar, V. J. and Bright Singh, I. S. (2020). Antimicrobial resistance in aquaculture: a crisis for concern. *Biologia* 75: 1497–1517 https://doi.org/10.2478/s11756-020-00456-4

Sakakibara, H., Honda, Y., Nakaqawa, S., Ashida, H. and Kanazawa, K. (2003). Simultaneous Determination of All Polyphenols in Vegetables, Fruits, and Teas. *J. Agric. Food Chem.* 51(3): 571–581

Soetan, K.O. and Aiyelaagbe, O.O. (2009). The need for bioactivity-safety evaluation and conservation of medicinal plants - A review *J. of Med.l Plants Res*. 3(5): 324-328

Vineetha, M.S., Bhavya, J., Mirjakar, K.M. and More, S.S. (2014). In vitro evaluation of active phytochemicals from *Tabernaemontana alternifolia* (Roxb) root against the *Naja naja* and *Echis carinatus* Indian snake venom. *J. Biol. Active Prod. Nature* 4(4): 286–294.

Williams, J.W., Shuman, B. N., Webb, T., Bartlein, P.J. and Leduc, P. L. (2004). Late-quaternary Vegetation Dynamics in North America: scaling from Taxa to Biomes. *Ecological Monographs* https://doi.org/10.1890/02-4045