Volume 10, Issue 6, June – 2025 International Journal of Innovative Science and Research Technology
ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun1060

Antibacterial Activity and Bioactive Compounds of the *Adoyo* Beverage

Bolarinwa Oluwatoyin Olubisi[1]; Nwose Adaobi Maureen[2]; Onifade Deborah Ajoke[1]; Adesokan Isaac Ayanniran[1]; Obayiuwana, Amarachukwu Chigozie[2]

[1]Department of Biology, The Polytechnic Ibadan, Nigeria
[2]Department of Biological Sciences, Augustine University Ilara, Epe, Lagos- Nigeria

Publication Date: 2025/06/21

Abstract: *Adoyo* beverage is an herbal drink consumed in Nigeria that offers medicinal benefits. This study aimed to assess antibacterial activity and bioactive compounds of the *adoyo* beverage. The antibacterial potential, antioxidant, flavonoid, vitamin C content, and chemical composition of the *adoyo* beverage were evaluated. The *adoyo* beverage was inhibitory against the test pathogen with a zone of 1.4 to 9.5 mm. It had total antioxidant, total flavonoid, and vitamin C content of 84.18%, 56.77 mg/mL, and 42.01 mg/mL, respectively. The chemical compounds were elucidated by gas chromatography–mass spectrometry (GC-MS) and yielded 21 peaks. The highest occurring compound was quinolinecarboxylic acid, while the lowest was benzisothiazole. *Adoyo* beverage had appreciable antibacterial activity and several bioactive compounds, hence can serve as a medicinal beverage.

Keywords: *Adoyo Beverage, Antibacterial, Antioxidant, Herbal Drink, GC-MS.*

How to Cite: Bolarinwa Oluwatoyin Olubisi; Nwose Adaobi Maureen; Onifade Deborah Ajoke; Adesokan Isaac Ayanniran; Obayiuwana, Amarachukwu Chigozie (2025) Antibacterial Activity and Bioactive Compounds of the *Adoyo* Beverage. *International Journal of Innovative Science and Research Technology*, 10(6), 1375-1379. https://doi.org/10.38124/ijisrt/25jun1060

I. INTRODUCTION

Beverages are food consumed in a liquid state, either for their thirst-quenching properties or for their stimulating effects [1]. Herbal beverages are usually produced from the natural ingredients of different herbs and spices, such as leaves, stems, roots, fruits, buds, flowers, barks, and seeds. Herbal beverages emanate from traditions of healing, wellness, and cultural identity handed down via generations all over the world. These drinks are integral to different cuisine cultures of countries spread across different continents, especially Africa and Asia, where they serve as thirst-quenchers as well as home remedies for common sicknesses. Herbal drinks, whether derived from a single herb or a combination of herbs are most likely a rich source of phytochemicals such as flavonoids, phenolic compounds, carotenoids, plant sterols, alkaloids, polyacetylenes, coumarins, saponins and terpenoids, and other Sulphur-containing compounds [2]. These phytochemicals have been associated with antibacterial, anti-inflammatory, and antioxidant effects. Popular examples of such drinks in Africa include *Zobo* (*Hibiscus sabdariffa*), Kinkeliba tea (*Combretum micranthum*), *Umhlonyane* tea (*Artemisia afra*), Brukina', (millet and pasteurized milk), *Emuduro* (ginger drink), *Asaana* (caramelised corn drink), and *adoyo* beverage which is popular in Western Nigeria [3, 4].

Adoyo beverage is an herbal drink, made up of pineapple, lemon grass, lemon, and supernatant of fermented maize/sorghum slurry popularly known as omidun. It may be considered as fermented beverage and classified as ready-to-eat food [4]. It is rich in vitamins and minerals like B vitamins, magnesium, potassium, phosphorus, and zinc considering the blended herbs used. This beverage probably contains bromelain, dietary fiber, and antioxidants due to the use of pineapple [5].

Traditional medicine is used by about 60% of the world population, especially in developing countries [6]. *Adoyo* drink is traditionally used in the treatment of ailments such as typhoid and paratyphoid fever, dysentery, malaria, and diarrhea. Although interest in natural health products has grown globally, African herbal drinks lack scientific research and the quality control required to validate their efficacy and standardized use. The aim of this work is to assess the in vitro antibacterial activity of *adoyo* drink against pathogenic bacteria (*Staphylococcus* sp., *Salmonella* sp., *Pseudomonas* sp., and *E. coli).*

II. MATERIAL AND METHODS

- *Production of Adoyo Drink:*

Five liters of fermented maize slurry supernatant (*omidun*, 5 liters) was set to boil at medium heat. Properly

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun1060

rinsed 2 kg of large ripe pineapple with the bark, 3 medium sized sliced ripe lemons, and 1kg of lemongrass were added to the fermented maize water and allowed to boil for about 45 minutes. The mixture was scooped into a clean jug, and 400 g of sugar was added to sweeten beverage.

➢ *Test Organisms:*

The indicator organisms *Salmonella typhi, Pseudomonas aeruginosa,* and *Escherichia coli* were obtained from the Department of Biology, The Polytechnic Ibadan, Nigeria.

➢ *Standardisation of Inoculum for Antimicrobial Assay:*

The standardisation was done as outlined by the Clinical and Laboratory Standards Institute (CLSI) [7]. From an 18-hour culture plate, two colonies of each test isolate were mixed in 5 mL of normal saline. The mixture was adjusted to 0.5 McFarland standard by adding more inoculum or diluent as needed.

➢ *Screening for Antibacterial Activities:*

The antibacterial effects of the a*doyo* beverage were investigated using the agar well diffusion method [8]. The 0.5 McFarland standard test cultures were evenly swabbed on Muller Hinton agar. Wells were bored on the agar using a sterile cork borer of 8 mm diameter. About 100 µL of the *adoyo* beverage and positive control (10 mg/mL amoxicillin) were pipetted into the wells. The inoculated plates were incubated at 37ºC for 24 hours. The zone of inhibition diameter was taken in three different fixed directions, and the average values were recorded. The tests were done in duplicate.

➢ *The Total Antioxidant Activity:*

The antioxidant activity was carried out [9].The hydrogen atom donating ability of the plant extractives was determined by the decolourization of methanol solution of 2,2-diphenyl-1-picrylhydrazy l(DPPH). A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in methanol at different concentrations (12.5–150 µg/mL). The reaction mixture was vortexed and left in the dark at RT for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Butylated Hydroxyanisole (BHT) was used as a reference. Percentage DPPH radical scavenging activity was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \{(A0 - A1)/A0\} \times 100$$

Where

A0 is the absorbance of the control, and A1 is the absorbance of the extractives/standard.

➢ *Total Flavonoid Content:*

This was estimated by aluminum chloride colourimetric assay [9]. One mL of sample or standard solution of Quarcetin (20 – 100 µg/mL) was added to 10 mL volumetric flask containing 4 mL of distilled water. To the above mixture, 0.3 mL of 5% NaNO2 was added. After 5 minutes, 0.3 mL of 10% $AlCl_3$ was added. At the 6th minute, 2 mL of 1 M NaOH was added and the total volume was made up to 10 mL with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. The total flavonoid content was subsequently calculated using quercetin (10 mg/100 mL) as standard.

➢ *Vitamin C content:*

The sample (300 uL) was mixed with 100 uL of 4% trichloroacetic acid (TCA). This was stirred with a vortex mixer and allowed to stay for 15 minutes. The component was centrifuged at 2000 rpm for 5 minutes. Vitamin C colour reagent (Dichlorophenolindophenol, 500 uL) was added to 250 microliter of the supernatant. The orange colour that developed was measured at 700 nm. Blank was prepared the same way as the sample but TCA was used in place of the sample supernatant. The standard was prepared by using ascorbic acid at various concentrations. The vitamin C content in each sample was calculated from the standard curve prepared using the standard [10].

➢ *Gas Chromatography*

Mass Spectrometry analysis: GC-MS analysis was carried out using the GCMS-QP2010 PLUS SHIMADZU. The column used was Perkin Elmer Elite - 5 capillary column measuring 30 m × 0.25 mm with a film thickness of 0.25 mm composed of 95% Dimethyl polysiloxane. The carrier gas used was Helium at a flow rate of 0.5 mL/min. Exactly 1 µL sample injection volume was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed initially at 80°C for 4 min, then an increase to 200°C, and then programmed to increase to 280°C at a rate of 20°C ending at 5 min. Total run time was 28 min. The MS transfer line was maintained at a temperature of 200°C. The source temperature was maintained at 180°C. GC-MS was analyzed using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library [11]

III. RESULTS

The antibacterial activity of the *adoyo* beverage against selected pathogenic microorganisms is shown in Figure 1. The antibacterial activity of the extract ranged from 1.4 to 9.5 mm. The most susceptible was *E. coli,* while *S. aureus* was the least susceptible. The isolates were more susceptible to the standard antibiotic compared to the *adoyo* beverage.

The total antioxidants, flavonoids, and vitamin C content of the *adoyo* beverage are shown in Table 1. The total antioxidant content of the samples was 84.1%. The flavonoid content is 56.7 mg/mL, while the vitamin C content is 42.0 mg.

The results of Gas Chromatography mass analysis of the a*doyo* beverage (Figure 2, Table 2, showed different chemical components. A total of 21 peaks representing different chemical compounds (appendix) were obtained. quinolinecarboxylic acid had the highest intensity at peak 21, while the lowest was benzisothiazole at peak 18. The

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun1060

compounds were further grouped into 7 classes of chemical compounds, namely, organosilicons, esters, phenols/ aromatics, heterocyclics, alkane /alkene, nitrogenous bases /alkaloid, and ketones/aldehydes.

IV. DISCUSSUION

The increase of drug-resistant pathogens, especially bacteria, which is among the major contributors to morbidity and mortality in sub-Saharan Africa, is a cause of concern. A potential solution is the utilization of medicinal plants processed into beverages these have been used to treat many ailment in the time past. Herbal teas or beverages have gained attention as natural products due to the increasing antibiotic resistance [12]. This study examined the antibacterial potential of herbal beverages and the bioactive compounds present in a*doyo* beverage. The *adoyo* beverage showed varied antibacterial activities against the test organisms. The *adoyo* drink contain organics acid probably produced during fermentation of the maize slurry and phytochemical from the additives. These could have reduced the pH. Besides, on interaction with other metabolites, a*doyo* beverage could have inhibited the metabolic activity of the microbes. This result is in accordance with the study of [13] and [14]. Both authors examined the antibacterial potential of herbal tea and beverages.

The Total anti-oxidants, flavonoids and vitamin C content of the *adoyo* beverage values indicate the potential of the *adoyo* beverage in combating oxidative stress and as well as enhance immunity. Other herbal blends have shown high antioxidant, flavonoid and vitamin C content [9, 11].

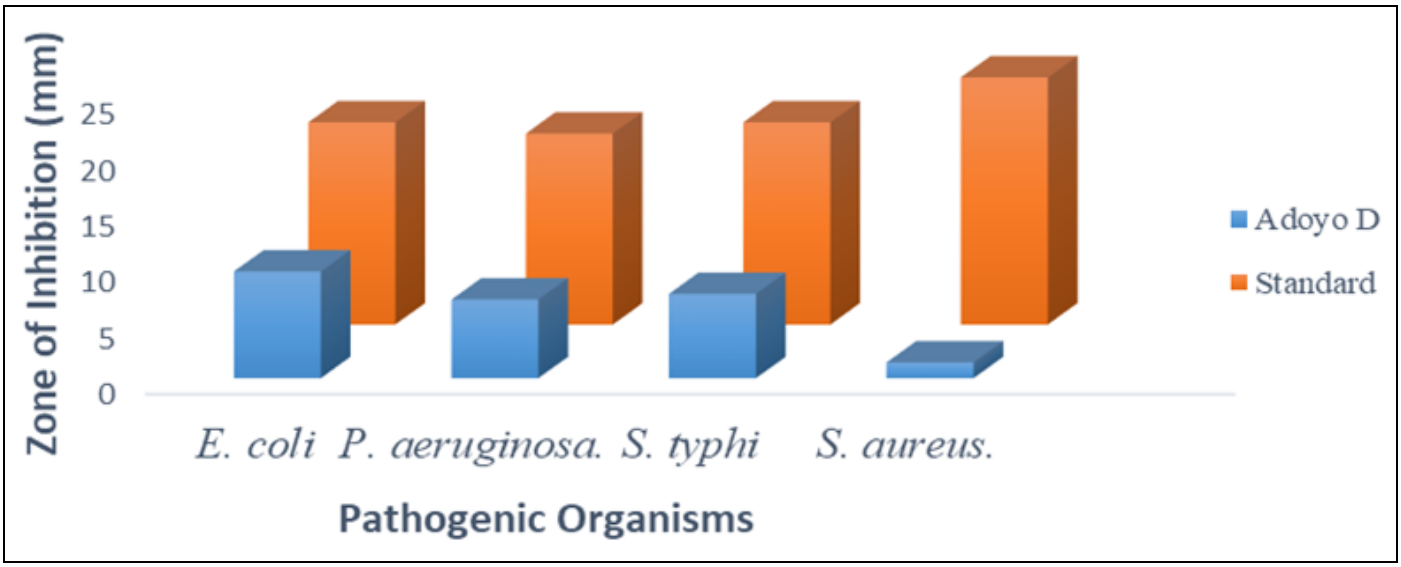

Fig 1 Inhibitory Activity of Adoyo against Selected Pathogen

Table 1 Total Antioxidant, Total Flavonoid, Total Sugar and Vitamin C

Beverage	Total Antioxidant 200mg/mL	Total Flavonoid mg/100g	Vitamin C mg/100g
Adoyo	84.1825	56.7667	42.010

Fig 2 GC-MS Chromatogram of Adoyo Beverage

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun1060

Table 2 Bioactive Compounds of the *Adoyo* beverage

Peaks	Retention Time (RT)	Area %	Compound Name	Chemical Group	Biological Importance
1	4.066	1.48	Methylene chloride	Chlorinated hydrocarbon	Non bioactive
2	4.586	9.49	Tetrachloroethylene	Chlorinated alkene	Antibacterial, antifungal Potential toxicant at high dose
3	4.901	14.93	Cyclotrisiloxane, hexamethyl-	Siloxane	Fragrance carrier, cosmetics
4	5.662	1.96	Methylene chloride	Chlorinated hydrocarbon	Non bioactive
5	6.229	4.31	p-Xylene	Aromatic hydrocarbon	Antimicrobial / Flavour
6	7.539	4.02	6-Methyl-2-Heptanol, acetate	Ester	Flavoring agent /Antimicrobial
7	7.648	1.94	Cyclohexane	Cycloalkane	Aromatic carrier
8	8.111	4.60	Benzene, 1,2,3-trimethyl-/1,2,4-trimethyl-	Aromatic hydrocarbons	Aroma, precursor to antioxidants
9	8.220	7.05	Cyclotetrasiloxane, octamethyl-	Siloxane	Emollient, Fragrance carrier
10	9.221	5.06	2-Pyrrolidinone, 1-methyl- Piperazine,	Lactam (heterocycle)	Nootropic, Antibacterial neuroprotective
11	10.743	3.29	2-Pyrrolidinone, 1-methyl- Imidosulfurous difluoride,	Lactam (heterocycle)	Non bioactive
12	12.843	1.48	Syringic acid (TMS derivative)	Phenolic acid	Antioxidant, Antimicrobial
13	13.210	1.98	Cyclohexasiloxane, dodecamethyl-	Siloxane	Emollient, cosmetic use
14	14.079	1.72	Triazine derivative	Polyheterocyclic (N-rich)	cytotoxic/antitumor
15	15.367	2.67	Adamantanecarboxamide derivative	Tricyclic alkaloid-like	Antiinflammatory, Antiviral, neuroactive,
16	15.407	3.26	Phenytoin (TMS derivative)	Hydantoin derivative	Anticonvulsant, neuroprotective
17	16.551	1.91	Fumaric acid esters	Purine base (alkaloid-like)	Antioxidant, anti-inflammatory, immunomodulatory, antiviral
18	17.398	1.38	Benzisothiazole + derivative	Fused heterocycle	Antibacterial, antioxidant, Antipsychotic
19	19.040	2.64	1H-Pyrazole-3-carboxylic acid ester	Heterocyclic ester	Anti-inflammatory, enzyme inhibitor potential
20	20.122	7.04	Hexadecanoic acid, methyl ester	Fatty acid ester	Antimicrobial, anti-inflammatory
21	25.832	17.80	Quinolinecarboxylic acid Octasiloxane, hexadecamethyl	Heteroaromatic Siloxane	Antimalarial/antibacterial potential Emollient

Regardless of the concentration (high or low) of compounds present in the a*doyo* beverage, majority of its components have been reported to possess some pharmacological or other biological activity of which include antitumour, antimicrobial, CNS activity which is responsible for its medicinal value and its use as local drug. The compounds identified using GCMS are bio-actively relevant. Siloxanes are the highest occurring compounds, and are highly of use in the cosmetics and pharmaceutical industries, especially in drug delivery [15]. Esters and fatty acid esters facilitate interactions with the bacterial cell components, resulting in the loss of cell viability, prevents bacterial growth and biofilm formation and also acts as surfactants [16]. Phenols and alkaloids have been reported to inhibit bacterial growth. Phenols cause leakage of ions and hinder the absorption of essential nutrients inhibiting cell wall synthesis and causing cell death [17]. The alkaloid and alkaloid like derivatives quicken membrane damage and protein unfolding, thereby interfering with cell metabolic activity and eventual death [18]. Heterocyclic and poly-heterocyclic compounds have been noted to exhibit analgesic, anthelmintic, antitumor, antiviral, antifungal, and anticancer effects [19]. Their antimicrobial activity is attributed to the interaction with electrophiles or nucleophiles of cells, preventing cell wall synthesis, breach of the cell membrane integrity and inhibition of metabolic processes [18].

V. CONCLUSION

Adoyo beverage is a blend of nature, tradition, and healing. This study showed that *adoyo* beverage can inhibit selected microbes and contain high antioxidant and free radical scavenging abilities. *Adoyo* beverage is made up of several beneficial compounds to enhance total well-being. To precisely establish the antagonistic effects and package the a*doyo* beverage, there is a need to further this study by determining the best means of extracting the bioactive compounds and studying the molecular variation of the pathogens based on their susceptibility.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun1060

REFERENCES

[1]. Olufunke AP, Oluremi KO (2015) Assessment of nutritional and sensory quality of *Kunu-zaki* - A home-made traditional nigerian beverage. *J Nutr Food Sci* 5: 358.

[2]. Shaik, M. I., Hamdi, I. H., and Sarbon, N. M. (2023). A comprehensive review on traditional herbal drinks: physicochemical, phytochemicals and pharmacology properties. *Food Chemistry Advances*, *3*, 100460.

[3]. Obatolu V. A., Adeniyi P. O., Ashaye O. A. (2016). Nutritional, sensory and storage quality of sekete from zea mays. *International Journal of Food Science and Nutrition Engineering*, 6(4): 73-80.

[4]. Nwose, A. M., Bolarinwa, O. O., Obayiuwana, A. C., and Nwadike, B. I. (2025). Bacterial contamination of fermented saps of raphia hookari obtained from selected markets in epe, lagos nigeria. *dutse journal of pure and applied sciences*, *11*(1c), 1-7.

[5]. Kansakar, U., Trimarco, V., Manzi, M. V., Cervi, E., Mone, P., and Santulli, G. (2024). Exploring the therapeutic potential of bromelain: applications, benefits, and mechanisms. *Nutrients*, *16*(13), 2060.

[6]. World Health Organization. (2023). Integrating Traditional Medicine in Health Care. https://www.who.int/southeastasia/news/feature-stories/detail/integrating-traditional-medicine

[7]. Altuner, E. M. (2025). Inoculum standardisation: ensuring reliable antimicrobial study outcomes. *Altuner. me*, *5*(29), 1-5.

[8]. Salaam, A. M., Adebayo-Tayo, B. C., and Ajibade, A. A. (2019). Phytochemical analysis, antioxidant, antibacterial potentials and chemical composition of methanol extract of Oscillatoria sp. *Nigerian Journal of Pharmaceutical Research*, *15*(2), 219-227.

[9]. Adewole S. A., Lawal T. F., Adeoye B. K., Adewole O. A., Olusanya P. A., Oyesiji O. O., Adebiyi-Olabode A. O., Ukangwa N. A. (2024), Quality evaluation of ginger-spiced hibiscus sabdariffa drink sweetened with dates and sucrose. *African Journal of Agriculture and Food Science* 7(3), 70-80.

[10]. Oboh, H. A., Osagie, A. U., and Esewe, R. E. (2012). Evaluation of antioxidant and free radical scavenging abilities of some Nigerian packaged fruit juices. *Nigerian Journal of Nutritional Sciences*, *33*(1), 31-36.

[11]. Ikyenge, B. A., Samoh, F. T., Agbidye, I. G., and Asogwa, N. (2024). Antifungal activity of ethanolic extract of potato peels on fungi causing rot in mango fruits. *Journal of Chemical Society of Nigeria*, *49*(6).

[12]. Seukep, A.J., Mbuntcha, H.G., Zeuko'o, E.M., Woquan, L.S., Nembu, N.E., Bomba, F.T., Watching, D. and Kuete, V. (2023). Established antibacterial drugs from plants. In *Advances in Botanical Research* (Vol. 106, pp. 81-149). Academic Press.

[13]. Hacioglu M., Dosler S., Birteksoz Tan A. S., Otuk G. (2017). Antimicrobial activities of widely consumed herbal teas, alone or in combination with antibiotics: *Anin Vitrostudy*. *PeerJ*. 5:p. e3467. doi: 10.7717/peerj.3467

[14]. Seukep, A. J., Ojong, O. C. G., Mbuntcha, H. G., Matieta, V. Y., Zeuko'o, E. M., Kouam, A. F., Kuete, V., and Ndip, L. A. (2025). In vitro antibacterial potential of herbal beverage extracts from cinnamon, clove, and thyme and their interactive antimicrobial profile with selected antibiotics against drug-resistant clinical pathogens. *Journal of tropical medicine*, 9916282. https://doi.org/10.1155/jotm/9916282

[15]. Chen, J., Fine, J. D., and Mullin, C. A. (2018). Are organosilicon surfactants safe for bees or humans? *Science of the total environment*, *612*, 415-421.

[16]. Lei, Z.; Heyan, Z.; Tianyang, H.; Siran, L. (2015) In vitro antibacterial activities and mechanism of sugar fatty acid esters against five food-related bacteria. Food Chem., 187, 370–377.

[17]. Ecevit, K., Barros, A. A., Silva, J. M., and Reis, R. L. (2022). Preventing microbial infections with natural phenolic compounds. *Future Pharmacology*, *2*(4), 460-498.

[18]. Al-Mohammadi A-R, Ibrahim RA, Moustafa AH, Ismaiel AA, Abou Zeid A, Enan G. (2021) chemical constitution and antimicrobial activity of kefir fermented beverage. *Molecules*, 26(9):2635.

[19]. Kabir, E., and Uzzaman, M. (2022). A review on biological and medicinal impact of heterocyclic compounds. *Results in Chemistry*, *4*, 100606.