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Phytochemicals Screening and Anti-bacterial Effect of Aqueous Leaves Extract of *Cola acuminata*

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ABSTRACT

Bacterial infections remain endemic in various societies causing more number of deaths worldwide. Cola acuminata has been used traditionally in treatment of many diseases including coughs, gastric ulcer, diarrhea, and dysentery. This study aimed at evaluating the phytochemicals constituents and anti-bacterial effect of aqueous leaves extract of Cola acuminata. Qualitative and quantitative determination of phytochemicals was carried out using standard methods. The susceptibility test of the bacterial isolates was conducted using agar diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was estimated using broth microdilution and sub-culturing method, respectively.

The results revealed that the extract contains 49.09%, 33.11% 20.41%, 12.08%, 34.13%, 7.74%, and 1.81% of flavonoids, alkaloids, tannins, steroids, glycosides, cardiac glycosides, and saponins, respectively. The extract showed a significant ($p < 0.05$) inhibition of the growth of Staphylococcus aureus, Streptococcus agalactiae, and Lactobacillus rhamnosus at 25 mg/mL (9.77 mm, 7.23 mm, and 3.86 mm), 50 mg/mL (17.93 mm, 13.63 mm, and 11.73 mm), 75 mg/mL (25.75 mm, 18.75 mm, and 17.04 mm), and 100 mg/mL (32.02 mm, 24.50 mm, and 21.61 mm), respectively. The extract exhibited MIC value of 8.41, 11.04, and 25.23 mg/mL on the growth of S. aureus, S. agalactiae, and L. rhamnosus, respectively. The observed MBC value of the extract on the growth of S. aureus, S. agalactiae, and L. rhamnosus was 15.01, 20.84, and 34.63 mg/mL, respectively. The aqueous leaves extract of Cola acuminata contains significant amounts of phytochemicals. The extract exhibited inhibitory effect on the growth of S. aureus, S. agalactiae, and L. rhamnosus with low minimum inhibitory concentration and minimum bactericidal concentration values.

Keywords: *Antibacterial activity, Bacteria, Cola acuminate, Infectious diseases, Phytochemicals*

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INTRODUCTION

Microbial infections remain endemic in several societies especially in Tropical countries where more number of deaths due to infections were recorded. Microbial infections are the second leading causes of death in the worldwide (WHO, 2017). Globally, more number of deaths due to microbial infections was recorded and predicted to reach ten million deaths by 2050 annually (Luo *et al.,* 2024; Halawa *et al.,* 2024). The prevalence rate of bacterial infections has increased due to multi-drug-resistant bacteria leading to a number of deaths (WHO, 2017; 2014). Antibiotic resistance remains a major public health problem. Most of the bacterial species remain resistance to a number of antibiotics currently available in the market (Pacheco *et al.,* 2022; Tiseo *et al.,* 2022). Report showed that almost all bacterial species might be resistant to most of the available antibiotics in the next twenty five years (Decker *et al.,* 2024; Luo *et al.,* 2024). Also, synthetic drugs used in treatment of bacterial infections have adverse effects and cannot be afforded by majority of people in the world. Thus, research should be done to find new effective and safe conventional medication against resistant microorganims.

About 80 % of people in African countries relied on plants and herbs for treatment of diseases (Ngbolua *et al.,* 2018; Ngbolua *et al.,* 2019). Medicinal plants have many medicinal properties and exhibited pharmacological activities (Goronyo *et al.,* 2022; Shubham *et al.,* 2021; Abubakar *et al.,* 2020a). Studies showed that different pants extracts exhibited antibacterial properties and antibiotics resistance (Dagne *et al.,* 2023; Gonfa *et al.,* 2022; Degu *et al.,* 2021). Plants and herbs have been used locally for treatment of diseases in Africa continent especially in West African countries many years ago. Traditional therapy is effective, safe, and affordable by almost all local communities worldwide. Hence, pharmaceutical industries have concentrated more on the use of bioactive compounds isolated from plants extracts in drug production and development (Berhanu and Kinfe, 2018). Phytochemicals are naturally found in different parts of plants (Pallavi *et al.,* 2017) and could be responsible for their medicinal and pharmacological properties (Abubakar *et al.,* 2024; Abubakar *et al.,* 2022). Phytochemicals isolated from different plants extracts demonstrated pharmacological activities and have significant applications in drugs production (Kumar *et al.,* 2021; Abubakar *et al.,* 2021).

The genus Cola consists of many species but the most significant cultivated species are *Cola acuminata* and Cola nitida (Adelusi *et al.,* 2020; Gbedie *et al.,* 2019; Ouattara *et al.,* 2018). Almost all species in the genus Cola have medicinal properties (Sery *et al.,* 2019; Erukainure *et al.,* 2019). *Cola acuminata* is an evergreen tree formerly belonging to the family Sterculiaceae (Dah-Nouvlessounon *et al.,* 2015; Salahdeen *et al.,* 2015) and now to the family Malvaceae (Ekalu and Habila, 2020). *Cola acuminata* commonly known as kola nut is a native of Central African countries (Dah-Nouvlessounon *et al.,* 2015). Kola nuts are widely chewing by many communities in Africa particularly in Nigeria because of their therapeutic properties. The plant has socio-economic value in West Africa traditional ceremonies especially in Nigeria where it is offer to show respect to guests and chiefs on specific occasions; such as traditional weddings and infant naming ceremonies (Ashaye *et al.,* 2021). Kola nuts have been used for the treatment of coughs, intoxication, pain, gastric ulcer, diarrhea, and dysentery (Muhammad and Fatima, 2014; Kuete and Efferth, 2010; Odebunmi *et al.,* 2009). However, the plant is used in foods and pharmaceutical and industries for the production of vital products (Unya, 2021; Ogwu *et al.,* 2024). In Nigeria, *Cola acuminata* is locally called Ọjị in Igbo, Goro in Hausa, and Obi in Yoruba. Kola nut is the second most significant cash crop cultivated in Nigeria (Asogwa *et al.,* 2012). Local herbalists in Northern

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and Southern Nigeria have been claiming uses of *Cola acuminata* for the treatment of infectious diseases. This study aimed at evaluating the phytochemicals constituents and anti-bacterial effect of aqueous leaves extract of *Cola acuminata*.

MATERIALS AND METHODS

Drugs and Chemicals

Amoxicillin manufactured by Reyoung Pharmaceutucal Company Limited (Yiyuan, Shandong Province, China) was purchased from the Nichben Pharm. Industries, Owerri, Imo, Nigeria. All the chemicals used in this study were of analytical grade.

Plant Material

The leaves sample of *Cola acuminata* were collected from the Akabo village, in Ikeduru LGA, Imo State, Nigeria. The plant sample was authenticated by Botany Unit, Centre for Agricultural Research and Extension, Federal University of Technology, Owerri, Imo, Nigeria.

Extracts Preparation

The extract was prepared according to the method described by Aliyu *et al.* (2025). The plant samples were washed with distilled water and then dried at 25ºC for 3 weeks. The dried samples were mashed into fine powder using grinding machine. Five hundred grams of the powdered material was soaked in two litres of distilled water for three days. The extract was filtered using Whatman filter paper and then dried in oven. The weight (27 g) and percentage yield (5.4 %) of the extract was obtained.

Qualitative Phytochemicals Analysis

Alkaloids Test

The qualitative analysis of alkaloids in the extract was carried out using Wagner's test as described by Trease and Evans (1989) and Abubakar *et al.* (2020b). The extract (2 mL) was treated with 3 mL of 1 % HCl solution. The mixture was incubated at 60 ºC for 20 min and then allowed to cool. The Wagner's reagent was added into the mixture in drops. Formation of reddish-brown colour indicated the presence of alkaloids.

Flavonoids Test

Flavonoids presence in the extract was detected using NaOH test as described by Mosa *et al.* (2012) and Ibrahim *et al.* (2024). One mile of 10 % NaOH solution was added into a test tube containing 2 mL of the extract. A yellow colour was formed and then disappeared after addition of HCl solution which indicated the presence of flavonoids.

Glycosides Test

Salkowski's test was employed for the detection of glycosides in the extract using the method of Mosa *et al.* (2012) and Ibrahim *et al.* (2024). The extract (5 mL) was treated with 5 mL of 1% H_2SO_4 solution. The content was boiled for 15 min and then allowed to cool. The mixture was treated with 10% NaOH solution followed by addition of 5 mL of Fehling's solution A and B. formation of brick red precipitate indicated the presence of glycosides.

Tannins Test

Qualitative analysis of tannins was done using Ferric chloride test described by Trease and Evans (1989) and Ibrahim *et al.* (2024). Two miles of the extract was treated with 2 mL of 5% $FeCl_2$ solution. The observed blue-green colour indicated the presence of tannins.

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Saponins Test
Saponins presence in the extract was detected using Froth test described by Mosa *et al.* (2012) and Trease and Evans (1989). Two miles of the extract was diluted with 2 mL of distilled water, mixed and then allowed to stand for 30 minutes. A stable persistent froth was observed indicating the presence of saponins.

Steroids Test
The identification test for steroids was conducted using the method of Trease and Evans (1989) and Ibrahim *et al.* (2024). The extract (1 mL) was treated with 5 mL of chloroform and sulphuric acid solution. The violet colour which changed to blue-green showed the presence of steroids.

Quantitative Phytochemicals Analysis

Alkaloids Test
Quantitative analysis of alkaloids was done using the method of Trease and Evans (1989) and Ibrahim *et al.* (2024). The extract (2 mL) was treated with 2 mM sulphuric acid, shaken and then partitioned with ether. The ammonia solution (2 mL) was introduced to the top liquid layer and then extracted with chloroform. The extract was concentrated to dryness, the alkaloid residue was weighed and the alkaloids content was obtained.

Flavonoids Test
Estimation of flavonoids content was carried out according to the method described by Harborne (1973) and Ibrahim *et al.* (2024). The extract (5 mg) was treated with 50 mL of 2 M HCl, boiled for half hour and then allowed to cool. The content was filtered and ethylacetate (50 mL) was added to the filtrate. The mixture was filtered and the filtrate was dried. The flavonoids residue was weighed to obtain the flavonoids content.

Glycosides Test
Spectrophotometric technique was used for the quantitative analysis of glycosides. The extract (10 mL) was treated with 50 mL of chloroform. The mixture was shaken, filtered, and the filtrate was treated with 10 mL of pyridine and 2 mL of 2% sodium nitroprusside. The mixture was shaken for 10 min followed by addition of 3 mL of 20% sodium hydroxide. Absorbance was read using spectrophotometer at 510 nm and the glycosides content was calculated (Ibrahim *et al.,* 2024).

Tannins Test
Tannins content was estimated using AOAC (1999) method. The extract was treated with 2 mL of Folin-Denis reagent and 1 mL of sodium carbonate solution. The content was allowed to stand for 30 min and then the absorbance was measured spectrophotometerically at 760 nm. The tannins content was obtained from the prepared standard curve.

Saponins Test
The quantitative determionation of saponins was done using the method of El-Olemyl *et al.* (1994). The extract (5 g) was treated with 150 mL of 50% ethanol, boiled for 30 min, cooled and then filtered. Equal amount of charcoal was added to the filtrate, heated for 30 min, and then filtered. The filtrate was treated with 150 mL of acetone, filtered, and the filter paper was taken into the desiccator containing anhydrous calcium chloride. The saponins residue was weighed and saponins content was calculated.

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Steroids Test

Steroids content in the extract was estimated according to the method of Trease and Evans (1989) and Ibrahim *et al.* (2024). The extract (1 mL) was treated with 2 mL of H_2SO_4, $FeCl_2$, and potassium hexacynoferrate (III). The mixture was heated at 70 °C for 30 min and the absorbance was read spectrophotometerically at 780 nm. The steroids content was calculated.

Bacterial Isolates

The bacterial isolates (*Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus*) were obtained from Microbiology Laboratory, Federal Medical Center Owerri, Imo, Nigeria. The isolates in the original plates were sub-cultured and then taken to MacConkey agar and Selenite F broth media plates. Gram staining technique and biochemical tests were used for the identification and confirmation of the isolates.

Antibacterial Activity Test

The plant extract was tested for the antibacterial activity using agar diffusion method of Kirby-Bauer (1996) and amoxicillin (30 µg) (Udensi *et al.,* 2025) as standard control. A loopful colony of each bacterial isolate was transferred into nutrient agar plates. The plates were incubated at 37°C for 24 hours and the turbidity developed was made to 0.5 Mac Farland standard value. The agar discs (6 mm) were aseptically dipped in two miles of the extract (25, 50, 75 and 100 mg/ml) for 1 minute. The discs were taken over the nutrient agar plates containing the isolates. The plates were incubated at 25°C for 30 min and then at 37°C for 24 hours. The formation of zone of inhibition in the plates was observed and then measured in millimetre using meter rule. The zone of inhibition was measured three times and the average value was obtained.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of the extract was evaluated using broth microdilution method of CLSI (2012). A serial dilution of the extract (100, 75, 50, and 25 mg/ml) was prepared. Suspension of the bacterial isolates (50µl) was added to each well containing one mile of the extract. The wells were incubated at 37°C for 24 h and then observed for the presence of bacterial suspension using microplate reader. The lowest concentration of the extract with disappearance of the bacterial suspension was considered as the MIC value of the extract.

Determination of Minimum Bactericidal Concentration

A sub-culturing method of CLSI (2012) was used for the determination of minimum bactericidal concentration (MBC) of the extract. The broth wells without bacterial growth during MIC analysis were sub-cultured on nutrient agar plates. The plates were incubated at 37 °C for 24 h and then observed for bacterial growth. The lowest concentration of extract without growth was considered as MBC of the extract.

Statistical Analysis

The experiments were conducted in triplicate and the results were expressed as mean ± SEM. Statistical Package for Social Sciences (SPSS) (version 22 software) was used for the data analysis. Significant differences among the average values were computed at 95 % confidence level by One-way analysis of variance (ANOVA). Two-tailed ($p < 0.05$) value was considered significance.

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RESULTS

Phytochemicals Composition of Aqueous Leaves Extract of *Cola acuminata*

The qualitative phytochemicals screening of the aqueous leaves extract of *Cola acuminata* is shown in Table 1. High amount of flavonoids, alkaloids, tannins, glycosides and steroids was observed in the aqueous leaves extract of *Cola acuminata*. Saponins and cardiac glycosides were moderately present in the extract (Table 1).

Table 1: Qualitative Phytochemicals Screening of Aqueous Leaves Extract of *Cola acuminata*

Phytochemical	Ca Extract
Flavonoids	+++
Alkaloids	+++
Tannins	+++
Saponins	++
Glycosides	+++
Cardiac Glycosides	++
Steroids	+++

+++ (High amount), ++ (Moderate amount), + (Low amount)

Table 2 shows the quantitative phytochemicals composition of the aqueous leaves extract of *Cola acuminata*. The results showed that the extract contains 49.09%, 33.11% 20.41%, 12.08%, 34.13%, 7.74%, and 1.81% of flavonoids, alkaloids, tannins, steroids, glycosides, cardiac glycosides, and saponins, respectively.

Table 2: Quantitative Phytochemicals Composition of Aqueous Leaves Extract of *Cola acuminata*

Phytochemical	Composition (%)
Flavonoids	49.09 ± 0.08
Alkaloids	33.11 ± 0.66
Tannins	20.41 ± 0.47
Steroids	12.08 ± 0.09
Glycosides	34.13 ± 0.17
Cardiac Glycosides	7.74 ± 0.28
Saponins	1.81 ±0.13

Values are expressed as mean ± SD (n = 3)

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Effect of Aqueous Leaves Extract of *Cola acuminata* on the Bacterial Growth

Figure 1 shows the effect of aqueous leaves extract of *Cola acuminata* on the growth of *Staphylococcus aureus*. The extract significantly inhibited the growth of *S. aureus* in dose dependent manner. The extract showed a significant ($p < 0.05$) inhibition of 9.77 mm, 17.93 mm, 25.75 mm, and 32.02 mm at 25 mg/mL, 50 mg/mL, 75 mg/mL, and 100 mg/mL, respectively. The extract (100 mg/mL) showed a highest zone inhibition (32.02 mm) more than the amoxicillin (30.83 mm) (Figure 1).

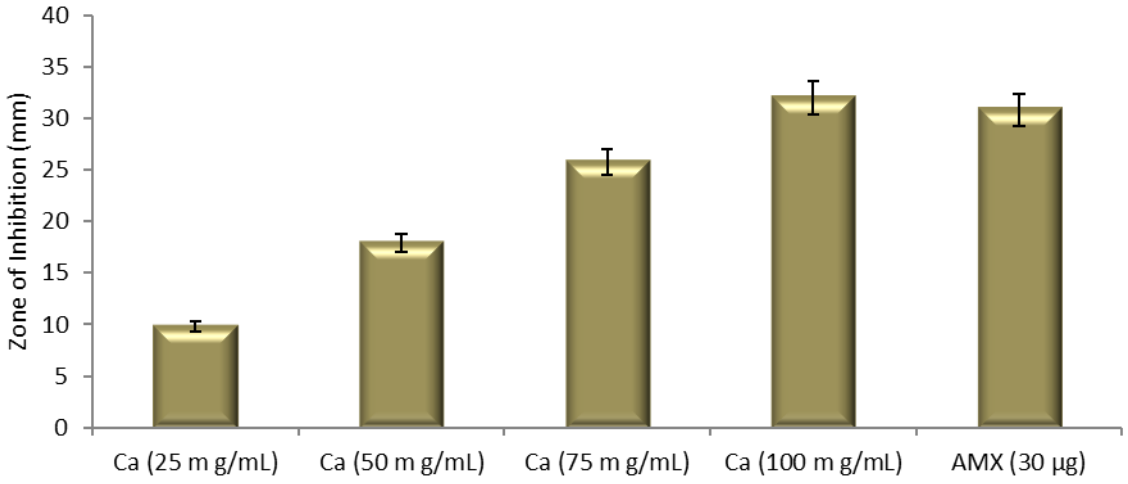

Figure 1: Effect of Aqueous Leaves Extract of Cola acuminata on Staphylococcus aureus Growth

Results are expressed as mean ± SD (n = 3), Ca (*Cola acuminata*), AMX (Amoxicillin)

The effect of aqueous leaves extract of *Cola acuminata* on the growth of *Streptococcus agalactiae* is shown in Figure 2. The extract showed a significant ($p < 0.05$) dose dependent effect on the growth of *Streptococcus agalactiae*. At 25 mg/mL, 50 mg/mL, 75 mg/mL, and 100 mg/mL, the extract showed a significant ($p < 0.05$) zone of inhibition of 7.23 mm, 13.63 mm, 18.75 mm and 24.50 mm, respectively. Amoxicillin, a standard control showed a significant ($p < 0.05$) inhibition of 23.00 mm which is less than that exhibited by the extract (24.50 mm) at 100 mg/mL (Figure 2).

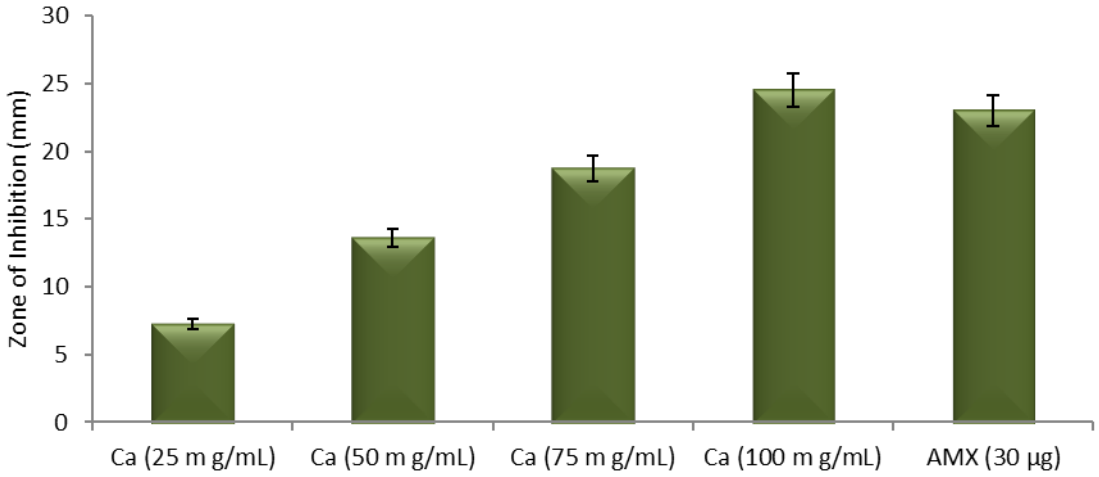

Figure 2: Effect of Aqueous Leaves Extract of Cola acuminata on Streptococcus agalactiae Growth

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Data are given as mean ± SD (n = 3), Ca (*Cola acuminata*), AMX (Amoxicillin)

Figure 3 shows the effect of aqueous leaves extract of *Cola acuminata* on the growth of *Lactobacillus rhamnosus*. The extract exhibited significant ($p < 0.05$) increase in zone of inhibition of *Lactobacillus rhamnosus* in dose dependent manner. The inhibitory effect of the extract (25 mg/mL, 50 mg/mL, 75 mg/mL, and 100 mg/mL) on the growth of *Lactobacillus rhamnosus* is 3.86 mm, 11.73 mm, 17.04 mm, and 21.61 mm, respectively. The extract (100 mg/mL) showed significant ($p < 0.05$) increase in zone of inhibition (21.61 mm) more than the amoxicillin (20.34 mm) (Figure 3).

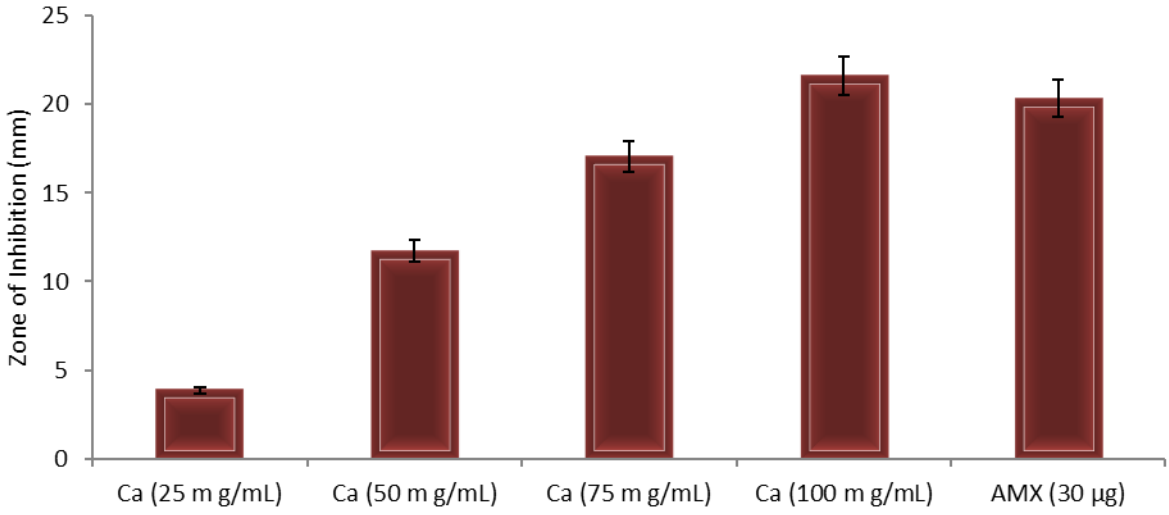

Figure 3: Effect of Aqueous Leaves Extract of Cola acuminata on Lactobacillus rhamnosus Growth

Values are expressed as mean ± SD (n = 3), Ca (*Cola acuminata*), AMX (Amoxicillin)

MIC and MBC Value of the Aqueous Leaves Extract of *Cola acuminata*

Table 3 shows the MIC and MBC value of the aqueous leaves extract of *Cola acuminata* on the growth of *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus*. The extract showed a MIC value of 8.41, 11.04, and 25.23 mg/mL on the growth of *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus*, respectively. However, the observed MBC value of the extract on the growth of *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus* was 15.01, 20.84, and 34.63 mg/mL, respectively.

Table 3: MIC and MBC Value of the Aqueous Leaves Extract of *Cola acuminata*

Bacterial Isolate	MIC (mg/mL)	BMC (mg/mL)
Staphylococcus aureus	8.41 ± 0.29	15.01 ± 0.14
Streptococcus agalactiae	11.04 ± 0.21	20.84 ± 0.11
Lactobacillus rhamnosus	25.23 ± 0.25	34.63 ± 0.26

Results are expressed as mean ± SD (n = 3)

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DISCUSSION

In this study, significant amounts of phytochemicals including alkaloids, flavonoids, tannins, saponins, steroids, and glycosides were detected in the aqueous leaves extract of *Cola acuminata*. This finding is in agreement with the similar finding by Omwirhiren *et al.* (2016) who detected the presence of alkaloids, tannins, saponins, flavonoids, steroids, and cardiac glycosides in the aqueous seeds extract of *Cola acuminata*. Study by Otoide and Olanipekun (2018) showed that *Cola acuminata* contained various phytochemicals including tannins and saponins. Alkaloids isolated from the leaves of *Cola acuminata* exhibited therapeutic functions in treatment of infections, stress, and symptoms of depression (Omwirhiren *et al.,* 2016). Saponins from the plants demonstrated many medicinal properties including binding cholesterol and coagulating red blood cells (Omwirhiren *et al.,* 2016; Madhu *et al.,* 2016). Tannins presence in the leaves of *Cola acuminata* could be responsible for the anti-parasitic and antimicrobial activity of the plant (Eleazu *et al.,* 2012). Tannins isolated from the *Cola acuminata* have been used in the management of burn, inflammation, gonorrhea and piles (Navarrete *et al.,* 2013). Cardiac glycosides presences in the leaves of *Cola acuminata* are used for the treatment of cardiovascular diseases and certain forms of arrhythmias (Sura *et al.,* 2020). Plants flavonoids showed antioxidant properties, enzymes modulating activity, vasculoprotective, anti-inflammatory and anti-diabetic activities (Dias *et al.,* 2021; Tungmunnithum *et al.,* 2018).

The present study showed that aqueous leaves extract of *Cola acuminata* significantly inhibited the growth of *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus* isolates. This finding is in consistent with the results of the similar study which indicated that aqueous and methanol extracts of red and white variety of kola nut exhibited antibacterial activity against *Streptococcus anginosus* (Muhammad, 2014). Saravana-kumar *et al.* (2009) found that kola extract demonstrated inhibitory effect against *Proteus mirabilis* with an inhibition zone of 16 mm at 1000 g/ml. Studies by Reid *et al.* (2005) and Adeniyi *et al.* (2004) indicated that crude ethanolic extract of *C. acuminata* showed inhibitory activity against *Staphyloccus albus*. Caroline and Cayla (2020) reported that *Cola acuminata* showed antibacterial activity against gram-negative bacteria. It has observed that methanol and ethanol extracts of cola nut inhibited the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* to a zone of 10 to 15 mm (Attiogbe *et al.,* 2021).

The present finding showed that aqueous leaves extract of *Cola acuminata* exhibited low minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) value on the growth of *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus* Minimum inhibitory concentration (MIC) value is an indicator of inhibitory effect of a substance on the growth of microorganism. Minimum inhibitory concentration (MIC) is the lowest concentration of test substance that inhibits the growth of test microorganism (Balouiri *et al.,* 2016). Plant extracts with The MIC value for a substance with high, moderate, and low antibacterial activity is < 100 µg/mL, 100 to 625 µg/mL, and > 625 µg/mL, respectively (Famuyide *et al.,* 2019; Dzotam and Kuete, 2017). Study showed that *C. acuminata* contains phytochemicals that inhibit the growth of micro-organisms that are resistant to many antibiotics (Caroline *et al.,* 2019). It has been documented that the phytochemicals including alkaloids, flavonoids, saponins, tannins, steroids and triterpenes presence in kola nuts could be responsible for their anti-bacterial activity (Kuete, 2010; Kuete *et al.,* 2008). Thus, the inhibitory effect of the aqueous leaves extract of *Cola acuminata* on the growth of *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus* could be attributed to the phytochemicals identified in the plant extract.

CONCLUSION

The aqueous leaves extract of *Cola acuminata* contains significant amounts of flavonoids, alkaloids, tannins, steroids, glycosides, cardiac glycosides, and saponins. The extract exhibited inhibitory effect on the growth of *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus* with low minimum inhibitory concentration and minimum bactericidal concentration values.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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