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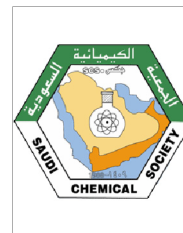

ORIGINAL ARTICLE

Gas Chromatography-Mass Spectroscopic, high performance liquid chromatographic and In-silico characterization of antimicrobial and antioxidant constituents of *Rhus longipes* (Engl)

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Abstract Rhus longipes is one of those underutilized plant species with inherent values. Therefore, we conducted a phytochemical study and investigated the antioxidant and antimicrobial potentials of the plant extracts. Aqueous and ethanol extract was subjected to phytochemical analysis. Gas chromatography-mass spectroscopy was performed to identify the volatile compounds while high performance liquid chromatography was done to identify the phenolic and flavonoids in the ethanol extract. Bioactivity and molecular docking analysis was also done for the identification of the bioactive constituents. Tannins, flavonoids, phenolics, terpenoids, steroids, alkaloids, and saponins were identified both in the ethanol and in aqueous extracts of R. longipes. The extracts and ascorbic acid exhibited radical inhibition in a concentration dependent manner. The IC_{50} values; 3.23, 4.13, 70.75 μg/mL (ABTS), 200.82, 103.63, 390.83 μg/mL (DPPH), 10.06, 93.46, 253.26 μg/mL ($O_2^{\cdot}$), and 99.77, 109.23, 446.34 μg/mL ($NO^{\cdot}$) for ascorbic acid, ethanol and aqueous extract respectively showed that ethanol extract exhibited better radical inhibition than the aqueous extract. 4-hydroxybenzoic and 4-hydroxycoumarin were the most abundant phenolics in the extract. The ethanol extract of R. longipes demonstrated broad-spectrum antibacterial activity with inhibition zone of 25.5 mm against S. aureus, 27.5 mm against E. coli, and 20.5 mm against P. aeruginosa. The identified phytochemicals demonstrated inhibitory potentials against bacterial glucosamine

Abbreviations: GLM synthase, glucosamine 6-phosphate synthase; PBP, penicillin binding protein 3; HPLC, High Performance Liquid Chromatography; GC-MS, Gas Chromatography Mass Spectroscopy

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6-phosphate synthase, penicillin-binding protein 3, DNA gyrase and β-lactamase. It is evident that R. longipes have some antioxidant and antibacterial properties and the plant contain important phytochemicals with ability to retard food oxidation and deterioration and thus could be annexed for various industrial and medicinal purposes.

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1. Introduction

In the recent years, studies on traditionally useful plants have attracted a considerable interest within the scientific community (Seebaluck-Sandoram et al., 2019). The wide commercial application in medicine, food, and cosmetics contributes to the rigorous research involving medicinal plants as source of beneficial ingredients to human (Orphanides et al., 2016). Several research efforts have led to the uncovering of significant and unique findings, which could be beneficial to humankind (Sarker and Oba, 2019a; 2020a; 2021). Despite the tremendous studies into plants, many beneficial plant are yet unexploited. Out of the underutilized plant species, are those with economic value that can play advance role in medicinal, food and industrial breakthroughs (Sarker and Oba, 2019b; Singha et al., 2021). Translation of traditional belief and knowledge of medical plants into adaptable practice through scientific research is imperative for the validation and identification of effective plant species (Sarker et al., 2018a, 2018b; Sarker and Oba, 2018a, 2018b, 2018c).

Rhus longipes also known as *Searsia longipee* is an underutilized plant species with inherent values. The plant belongs to the Anacardiaceae family and is acclaimed with some traditional usefulness (Olorunnisola et al., 2017). According to Oladele et al. (2011), *R. longipes* known as Ewe Orijin in Yoruba is popularly cultivated in one of the states in the South Western part of Nigeria because of it broad usage among traditional medicinal practitioners. From the literature, there are very few to no reports on the biological properties and phytochemical characteristics of the plant. Therefore, we conducted a phytochemical study, identified some compounds, and investigated the antioxidant and antimicrobial potentials of the plant extracts. We further implicated some of the identified constituents with the antioxidant and antimicrobial properties of *R. longipes*.

Plant leaves are major source of antioxidants. Natural antioxidant provides defencess against several diseases, such as cardiovascular diseases, cancer, cataracts, atherosclerosis, retinopathy, arthritis, emphysema, and neuro-degenerative diseases (Sarker and Oba, 2019a). Natural antioxidant compounds are not only significant to the food industry because of their health-promoting effects, but also as natural preservatives of food products (Repo-Carrasco-Valencia et al., 2010; Venskutonis and Kraujalis, 2013). Plant antioxidants such as anthocyanins, carotenoids, betacyanins, betaxanthins, betalains, β-carotene, garlic acid, catechin, apigrnin, caffeic acid have attracted consumers and researchers (Sarker and Oba, 2019a) because of the kin interest in the aesthetic, nutritional, and safety aspects of foods and as such increased the demand from natural source (Sarker and Oba, 2021).

Due to antibiotic resistance and because current antibiotics have become progressively moribund, infectious diseases are highly vexatious. Pathogenic bacterial are known causative agents for urinary tract infections, persistent lung infection, and endocarditic, that is mostly responsible for unexpected mortality (Muscedere et al., 2010). Concerted efforts are therefore put in place for new drug development (Swain and Padhy, 2015). Investigation into medicinal plants have yielded a handful of plants that proved effective in the control of pathogenic bacterial such as *Enterococcus faecalis, S. aureus, E. coli, K. pneumoniae, P. aeruginosa* and a few more (Mallik et al., 2012; Mishra and Padhy, 2013; Rout et al., 2014; Rath and Padhy, 2015; Sahu et al., 2015). Premise on these, we investigated the antioxidant and antimicrobial efficacy and performed some phytochemical studies on the leave extracts of *R. longipes*.

2. Materials and methods

2.1. Plant collection

Fresh leave of *Rhus longipes* was purchased from a traditionalist at Oja Odan, Ogun state, Nigeria in the month of May 2018. The plant sample was identified and authenticated by a Taxonomist at the College of Natural Sciences, Department of Pure and Applied Botany, Federal university of Agriculture, Abeokuta. Ogun State. The leaves were washed and drained prior to air-drying under room temperature (37 °C) until constant weight was reached. The air-dried sample was pulverized with an electric blender for solvent extraction.

2.2. Preparation of plant extract

The pulverized leaf of R. *longipes* was subjected to water and ethanol extraction using cold maceration method (1:10, plant weight to extracting solvent ratio) (Dieudonne et al., 2010). 180 g of the pulverized sample was soaked differently in 1.8 L of distilled water and 95% ethanol respectively for 72 h (Azadi et al., 2019) with intermittent shaking to aid extraction. The aqueous mixture was stored at 4 °C throughout the maceration period to prevent microbial growth. At the end of the 72 h, the mixture was filtered separately through No 1 Whatman's filter paper. The filtrate was concentrated using rotary evaporator model (RE 300) at 40 °C while the water extract was freeze dried with a freeze drier. The concentrated extract was kept in a refrigerator at 4 °C until required

2.3. Qualitative phytochemical screening

Phytochemical analysis of *R. longipes extract* was carried out using the method described by Abdullahi et al. (2013); Emmanuel et al. (2013) for the detection of saponins, tannins, phenolics, alkaloids, steroids, triterpenes, phlobatannins, glycosides, and flavonoids.

2.3.1. Determination of alkaloids

1 % HCL was mixed with extracts in 1:3 ratio. The mixture was heated for 20 min, cooled, and filtered. Thereafter, 2 drops of Wagner's reagent (2 g of iodine and 6 g of potassium iodide in 100 mL distilled water) was added to 1 mL of the filtrate. The appearance of a reddish brown precipitate indicates the presence of alkaloids.

2.3.2. Determination of tannin

When 1:1 of freshly prepared 1 % KOH and extract were mixed together, a dirty white precipitate indicates the presence of tannins.

2.3.3. Determination of phenolic

2 drops of 5 % $FeCl_3$ was added to 1 mL of the extracts in a test tube. A greenish precipitate indicates the presence of phenolic.

2.3.4. Determination of glycosides

10:1 of 50 % H_2SO4 and extracts, were mixed together, the mixture was heated in boiling water for 15 min, followed by the addition of 10 mL of Fehling's solution (7 g copper (II) sulphate in 100 mL distilled water (Fehling's A) and 12 g NaOH in 100 mL distilled water + 35 g potassium sodium tartrate (Fehling's B)) and then boiling. Appearance of a brick red precipitate indicates the presence of glycosides.

2.3.5. Determination of saponin

Saponin was determined by Frothing test. Dissolved extract was vigorously shaken in test tubes for 2 min. The appearance of foam indicates the present of saponin.

2.3.6. Determination of flavonoid

1:3 of 10 % NaOH and extracts were mixed together and then observed for the presence of a yellow colouration as indicator for flavonoids.

2.3.7. Determination of Steroid

The Salakowsti test was adopted for the determination of steroids. Briefly, 5 drops of concentrated H_2SO4 was added to 1 mL of the extracts. Red colouration indicates the presence of steroids.

2.3.8. Determination of phlobatannins

When 1 mL of the extracts was added to 1% of HCl, red precipitate indicates the presence of phlobatannins.

2.3.9. Determination of triterpene

5 drops of acetic anhydride was added to 1 mL of the extract, followed by a drop of concentrated H_2SO4 and steaming for 1 h. After steaming, the mixture was neutralized with 0.3 M NaOH followed by addition of chloroform. A blue green color indicates the presence of triterpenes.

2.3.10. Determination of terpenoids

5 mL of the sample was mixed with 2 mL of $CHCl_3$ in a test tube. Thereafter, 3 mL of concentrated H_2SO4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration indicates the presence of terpenoids.

2.4. Determination of antioxidant activity

2.4.1. ABTS radical assay

ABTS (2, 2′-azino-bis 3-ethylbenzthiazoline-6- sulfonic acid) radical cation scavenging activity of *R. longipes* was determined using the method described by Jeyadevi et al. (2019). The ABTS radical was pre-generated by adding 5 mL of 4.9 mM potassium persulfate solution to 5 mL of 14 mM ABTS solution and kept for 16 h in the dark. This solution was suitably diluted with distilled water to yield an absorbance of 0.70 at 734 nm and then used for antioxidant assay. Different concentrations of extract (10–150 μg/mL) were prepared and 50 μl was added separately to 950 μl ABTS solution while ascorbic acid was used as reference. The mixture was vortexed for 10 secs, and reduction in absorbance was read at 734 nm, using distilled water as a blank. The result was compared with control (only ABTS solution) having absorbance 0.712 ± 0. 032.

2.4.2. DPPH radical assay

DPPH (2, 2′-diphenyl-1-picrylhydrazyl) radical inhibition by *R. longipes* was determined by using the protocol of Jeyadevi et al. (2019). Stock solution of DPPH (8 mg/100 mL) was prepared in ethanol. To take the absorbance of blank solution, 1 mL of DPPH was added to 3.5 mL ethanol and the absorbance was read at 517 nm. 500 μl of different concentration of *R. longipes* extract was missed with 1 mL DPPH solution and kept in the dark for 30 mins to allow scavenging reaction to occur. The percentage of DPPH scavenging activity was calculated as follows:

$$\mathrm{DPPHscavengingactivity}(\%) = \frac{\mathrm{Absorbanceofblank} \times \mathrm{Absorbanceofsample}}{\mathrm{AbsorbanceBlank}} \times 100$$

2.4.3. Superoxide radical assay

Superoxide radical scavenging potential of *R. longipes* was reported in terms of its capacity to inhibit the formazan formation upon photochemical reduction of nitro bluetetrazolium (Chen et al., 2019). In brief, each 3 mL reaction mixture contain (0.01 M phosphate buffer (PH 7.8), 130 mM methionine, 60 μM riboflavin, 0.5 mM EDTA, NBT (0.75 mM) with 0.5 mL extract or CuSO4 solution). These tubes were kept in front of fluorescent light for 6 min and absorbance was taken at 560 nm. The non-enzymatic phenazine methosulfate-nicotinamide adenine di-nucleotide (PMS-NADH) system generates superoxide radicals, which reduce NBT to a purple formazan. The decrease in absorbance at 560 nm with the plant extract and the reference compound ascorbic indicates their abilities to quench superoxide radicals in the reaction mixture. The results were expressed in percent inhibition as compared to control.

2.4.4. Nitric oxide radical scavenging assay

Nitric oxide generated from aqueous sodium nitroprusside (SNP) solution interacts with oxygen to produce nitrite ions at physiological pH, and may be quantified according to GriessIllosvoy reaction (Atere et al., 2018). The reaction mixture contained 10 mM SNP in 0.5 M phosphate buffer (pH 7.4) and various concentrations (10–150 μg/mL) of *R. longipes* in

a final volume of 3 mL. After incubation for 60 min at 37 °C, Griess reagent (0.1% α-napthyl-ethylenediamine in water and 1% sulphanilic acid in 5% H_3PO_4 was added. The pink chromophore generated during diazotization of nitrite ions with sulfanilamide and subsequent coupling with α-napthylethylenediamine were measured spectrophotometrically at 540 nm. Ascorbic acid was used as a positive control. Nitric oxide scavenging ability (%) was calculated using;

$$\text{NOscavengingactivity}(\%) = \frac{\text{Absorbanceofblank} \times \text{Absorbanceofsample}}{\text{AbsorbanceBlank}} \times 100$$

2.5. Antibacterial activity

2.5.1. Determination of bacterial sensitivity using the Agar-well diffusion method

The antimicrobial activity of *R. longipes* was tested against three bacteria viz Escherichia *coli, Staphylococcus aureus* and *Pseudomonas aureginosa*. The selected bacterial strains were cultured separately in Mueller Hinton broth prepared with distilled-deionized water for 24 h. The microbial strains were standardized aseptically by serial dilution to the McFarland standard of 0.5 absorbance at 625 nm and were further adjusted to 0.37 at 625 nm to have 10^9 cfu mL^{-1} for the susceptibility test. Aliquot of 0.1 mL of the test organism suspension was aseptically inoculated on the Mueller Hinton agar surface with aid of hockey stick (spreader). The plates were allowed to absorb the organism suspension at room temperature and a sterile cork-borer of diameter 5 mm was punched on the agar surface to make wells. Subsequently each well was filled with 100 µl of the plant extract prepared in ethanol (1000 mg/mL). Control well containing the same volume (100 µl) ethanol was made. The plates were incubated at 37 °C for 24 h. The antibiogram plates were observed for zone of inhibition (area of no growth around the wells). Bacterial strains that were resistant to antimicrobial agent grew up to the edge of the well as against the sensitive strains, which are inhibited at a distance from the well. The zone of inhibition around each well was measured with the aid of a transparent metric ruler in millimetres (mm) (Azadi et al., 2019; Amalraj et al., 2020).

2.5.2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

A serial dilution of extract was performed into a sterile nutrient broth (31.25, 62.5, 125, 250, 500, and 1000 mg/mL) respectively. To each of the serial diluted extract, 100 µl of the McFarland standardized inoculum of the bacterial strain was inoculated aseptically in a sterilized environment with three of the test tubes serving as control in which one was inoculated with the extract alone, ethanol and the third with the bacterial inoculum alone. This was performed for the extract against the individual bacterium of the three microbial strains. The inoculated broth was then incubated at 37 °C for 24 h. The assessment of antimicrobial activity was observed by turbidity in the tubes when compared with the control tubes. The lowest concentration at which no detectable bactericidal growth occurred was considered as minimum inhibitory concentration (MIC) (Azadi et al., 2019).

The minimum bactericidal concentration of the ethanol extract of *R. longipes* was determined by selecting tubes that showed no growth during MIC determination. A loopful was taken from the test tubes and inoculated on sterile Mueller Hinton agar. The plates were incubated at 37 °C for 24 h. The lowest concentration of the extracts that showed no colony growth on the solid medium was regarded as minimum bactericidal concentration.

2.6. Gas Chromatography-Mass spectroscopic (GC–MS) analysis of R. Longipes

The GC–MS analysis was performed on Agilent Technologies 789OA coupled with mass spectrophotometer with triple axis detector (VL5675C) equipped with an auto injector (10 µl syringe). Chromatographic separation was performed on capillary column (30 m × 250 µm × 0.25 µm) using Helium as the carrier gas at a constant flow rate of 1.5 mL/min. The sample injection volume was 1 µl in a split mode with split ratio of 1:50. The column temperature started at 35 °C for 5 min at a rate of 4 °C/min to 150 °C and was raised to 250 °C at the rate of 20 °C/min with a holding time of 5 min. The compounds were identified by comparing the spectrum of the separated components with the standard mass spectra from the National Institute of Standards and Technology Library (NIST), Maryland, USA.

2.7. High performance liquid chromatography (HPLC)

2.7.1. HPLC analysis of flavonoids

High performance liquid chromatography UV detector was used to analyse the content and amount of flavonoids in the extracted sample using N200 chromatography software. The HPLC system (Hangzhou LC-8518, column size 150 × 4.6 m m) with a low-pressure gradient, solvent delivery pump, high-pressure switching valves, and a high-sensitivity ultraviolet (UV) detector with Acetonitrile/Water/Formic acid (25:74:1) as mobile phase. The wavelength was 254 nm, column temperature of 35° centigrade, sample injection volume of 40 micro liter and 25 min run time. Separate stock solutions of standards were made by weighing 0.001 g of reference standards into a test tube and dissolving each standard with 10 mL of 70 % methanol or ethanol. Each of the standard was then agitated for 10 min using vortex mixer and then filtered using a cosmonice filter or micron filter into the sample bottle.

2.7.2. HPLC analysis of phenolics

High performance liquid chromatography UV detector was used to analyze the content and amount of phenolics in the extracted sample using N200 chromatography software. The HPLC system (Hangzhou LC-8518, column size 150 × 4.6 m m) with a low-pressure gradient, solvent delivery pump, high-pressure switching valves, and a high-sensitivity ultraviolet (UV) detector with Acetonitrile/Water/Acetic acid (19:80:1 respectively) as mobile phase. The wavelength was 272 nm, column temperature of 40°centigrade, sample injection volume of 40 Âµl and 25 min run time. Separate stock solutions of standards were made by weighing 0.001 g of reference standards into a test tube and dissolving each standard with 10 mL of 70% methanol or ethanol. Each of the standard was then agitated for 10 min using vortex mixer and then filtered using a cosmonice filter or micron filter into the sample bottle.

2.8. In silico bioactivity prediction and molecular docking

The Simplified Molecular Input Line Entry System (SMILES) notations of the GC–MS and HPLC identified constituents of *R. longipes* were fed into the PASS (Prediction of Activity Spectra of Substance) online software to predict broad spectrum of bioactivity. Guided molecular docking of the small molecule inhibitors with the proteins (DNA gyrase, β-lactamase, penicillin binding protein, and glucosamine 6-phosphate synthase) was performed using Auto Dock Vina 1.5.6. On the Autodock tool, polar-H-atoms were first added to the proteins followed by Gasteiger charges calculation. The protein file was saved as pdbqt file and the grid dimensions were set as 48 × 58 × 44 for 1KZN, 46 × 78 × 94 for 1XFF, 64 × 64 × 50 for 3VSL, and 68 × 52 × 66 for 6VHS. Docking calculations (Binding Affinity (DG) were then performed by using Vina folder (Trott and Olson, 2010). Interactions between the ligand and protein were visualized using discovery studio 2019. For details on the ligand and protein preparation, check Adegbola et al. (2021a, 2021b).

2.8.1. Active site

The active site of each protein has been previously defined; therefore, this study did not attempt defining the active site using the CASTp predictor. The active sites were previously defined by Durand-Reville et al. (2020); Isupov et al. (1996); Lafitte et al. (2002); Yoshida et al. (2012).

Residues Val 43, Asn 46, Ala 47, Val 71, Asp 73, Ile 78, Met 95, Val 120, Arg 136 and Val 167; Cys 1, Arg 26, Asp 29, Arg 73, Trp 74, Thr 76, Asn 98, Gly 99 and Asp 123 represent the active site amino acids of DNA gyrase (PDB ID: IKZN), and glucosamine-6-phosphate synthase (PDB ID: 1XFF) respectively. Ser 392, Ser 448, Ser 449, Asn 450, Gln 524, Thr 603, Lys 618, Thr 619, Gly 620, Thr 621, Glu 623, and Pro 660; Ser 70, Lys 73, Tyr 103, Asn 104, Tyr 105, Ser 130, Asn 132, Glu 166, Thr 216, Lys 234, Thr 235 and Ser 237 represent the active site amino acids of penicillin binding protein (PDB ID: 3VSL), and β-lactamase (PDB ID: 6VHS) respectively.

3. Results

3.1. Phytochemical analysis

The qualitative identification of phytochemicals in the aqueous and ethanol leaf extract of *R. longipes* is reported in Table 1. The identified phytochemicals included tannin, flavonoids, phenolics, terpenoids alkaloids, saponin and steroids. In the ethanol extract, all the tested phytochemicals except for phlobatanin were present whereas, triterpene and glycosides detected in the ethanol extract and phlobatanin were absent in the aqueous extract of *R. longipes*.

Table 1 Qualitative phytochemical screening of *R. longipes* leaf extracts.

S/N	Phytochemicals	Ethanol Extract	Aqueous extract
	Tannin	+	+
	Flavonoid	+	+
	Phenolics	+	+
	Terpenoid	+	+
	Triterpene	–	–
	Glycosides	+	–
	Phlobatanin	–	–
	Steroid	+	+
	Alkaloid	+	+
	Saponin	+	+

3.2. Antioxidant activity

The antioxidant capacity of the aqueous and ethanol leaf extract of *R. longipes* was evaluated using the 2, 2′-azino-bis 3-ethylbenzthiazoline-6- sulfonic acid (ABTS), 2, 2′-diphenyl-1 –picrylhydrazyl (DPPH), superoxide radical ($O_2^{\cdot}$), and nitric oxide (NO) scavenging activities. The result reported in Table 2 showed that the extracts and ascorbic acid exhibited radical inhibition in a concentration dependent manner, whereas the antioxidant potency was evaluated from the concentration of the extracts that inhibited 50 % (IC_{50}) of each radical. The result of the IC_{50} values is reported in Table 3. Overall, the ethanol extract exhibited better efficacy than the aqueous extract in scavenging all the radicals tested. The ethanol extract of *R. longipes* demonstrated comparable ABTS radical scavenging activity with ascorbic acid used as standard. Ascorbic acid had the least IC_{50} value of 3.23 μg/mL while the ethanol extract had IC_{50} value of 4.13 μg/mL. Similarly, the ethanol extract of *R. longipes* demonstrated better inhibition of DPPH radical than the aqueous extract. The activity of the ethanol extract was comparable to that of ascorbic acid. The concentration of the extract and standard that inhibited 50 % of the DPPH radical was 200.82, 103.63, and 390.83 μg/mL for the ethanol extract, ascorbic acid, and aqueous extract respectively. The superoxide radical scavenging activity ranged from 52.54 to 90.81%, 34.21 to 59.73%, and 19.34 to 34.85% respectively for ascorbic acid, ethanol, and aqueous leaf extracts of *R. longipes*. The ethanol extract had an IC_{50} value of 93.46 μg/mL while the aqueous extract had an IC_{50} value of 253.26 μg/mL, whereas ascorbic acid had an IC_{50} value of 10.06 μg/mL. The nitric oxide radical scavenging activity observed for the ethanol extract of *R. longipes* (IC_{50} value of 109.23 μg/mL) was in tandem with that observed for ascorbic acid (IC_{50} value of 99.77 μg/mL) that was used as standard, while the aqueous extract on the contrary, had higher IC_{50} value of 446.34 μg/mL.

3.3. Antibacterial activity

The sensitivity of *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa* to the ethanol extract of *R. longipes* and streptomycin is reported in Table 4 and the representative of the petri-dishes with the inhibition zone are shown in Fig. 1. The ethanol extract of *R. longipes* demonstrated broad-spectrum antibacterial activity with inhibition zone of 25.5 mm against *S. aureus,* 27.5 mm against *E. coli*, and 20.5 mm against *P. aeruginosa*. Streptomycin on the other hand demonstrated antibacterial activity with inhibition zone of 34.0 mm against *S. aureus* 32.0 mm against *E. coli* and 30.0 mm against *P. aeruginosa*.

Table 2 Antioxidant activity of aqueous and ethanol leaf extracts of *R. longipes*.

Concentration (µg/mL)	Antioxidant activity of *R. longipes* (%)											
	ABTS Scavenging activity			DPPH Scavenging activity			$O_2^{\bullet-}$ Scavenging activity			$NO^{\bullet}$ Scavenging Activity		
	Ethanol	Aqueous	Ascorbic Acid	Ethanol	Aqueous	Ascorbic Acid	Ethanol	Aqueous	Ascorbic Acid	Ethanol	Aqueous	Ascorbic Acid
10	71.77	16.66	81.17	12.66	2.23	19.56	34.21	19.34	52.54	36.73	27.81	36.86
20	81.02	24.85	83.01	21.23	7.12	29.30	38.51	19.47	71.18	41.39	29.72	44.32
50	80.53	50.13	83.78	25.63	18.21	35.24	42.47	23.00	82.60	44.90	31.57	46.68
100	83.99	71.98	84.14	46.96	18.62	48.57	51.16	34.26	86.35	48.60	34.18	50.64
150	84.27	76.09	84.35	47.36	19.82	63.17	59.73	34.85	90.81	54.27	34.89	57.08

Table 3 IC_{50} values for the antioxidant activity of *R. longipes* leaf extracts.

Radicals	IC_{50} (µg/mL)		
	Ethanol Extract	Aqueous Extract	Ascorbic Acid
ABTS	4.13	70.75	3.23
DPPH	200.83	390.83	103.63
$O_2^{\bullet-}$	93.46	253.26	10.06
$NO^{\bullet}$	109.23	446.34	99.77

Table 4 Sensitivity of tested bacteria strains to the ethanol extract of *R. longipes*.

Mean zone of inhibition of ethanol extract of *R. longipes* leaf (mm)		
Test Organisms	**Ethanol extract**	**Streptomycin**
Staphylococcus aureus	25.5	34.0
Escherichia coli	27.5	32.0
Pseudomonas aureginosa	20.5	30.0

3.3.1. Minimum inhibitory concentration (MIC) and Maximum bactericidal concentration (MBC)

From the result in Table 5, the ethanol extract inhibited *S. aureus*, *E. coli* and *P. aureginosa* at minimum concentration of 31.25, 125 and 62.5 mg/mL respectively. Bactericidal activity was demonstrated against *S. aureus*, *E. coli* and *P. aureginosa* at concentration of 250, 125, 125 mg/mL respectively. The highest MIC value (125 mg/mL) was recorded for *E. coli* while the least (MIC) value (31.25 mg/mL) was recorded for *S. aureus*. *S. aureus* had the highest MBC value at 250 mg/mL. While *E.coli* and *P. aureginosa* had the least MBC value at 125 mg/mL.

3.4. Gas chromatograohy mass spectrometric GC–MS) analysis

Identification of compounds was done by comapring the retention time and molecular weight with refrence compounds in the NIST library. The GC–MS analysis of the extract identified several volatile compounds with significant biological activities. In Table 6, the retention time, amount and names of the identifed compounds were listed. In Fig. 2, the GC–MS spectra of the compounds was shown while fragmentation patern of some of the identifed compounds was reported in Fig. 3. The most abundant and the order of abundance of the some of the identified compounds included, Neophytadiene (0.72%), .psi.,.psi.-Carotene, 7,7′,8,8′,11 270,519 000540–04-5 10,11′,12,12′-octahydro- (0.46 %), Z-28-Heptatriaconten-2-one (0.44 %). Other compounds are, Silane, [[(3.beta.,6.beta.)-cholest-4-ene-3,6-diyl]bis(oxy)]bis[trimethyl- (0.43%), Tricyclo[5.4.0.0(2,8)]undec-9-ene,2,6,6,9-tetramethyl-, (1R,2S,7R,8,R)-), (0.42 %), Aziridine, 1-(2-buten-2-yl)- (0.40%),

3.5. HPLC profile of flavonoids and phenolic constituents of R. Longipes

For the identification of flavonoid and phenolic constituent of the leaf extract of *R. longipes* , the extract was subjected to quantitative HPLC analysis and the chromatogram is reposted in Figs. 4 and 5 respectively. Two major flavonoids, namely catechin and apigenin were identified with catechin being the most abundant (124.82 µg/100 g) (Table 7). The name of the identified phenolics viz garlic acid, ferulic acid, salicyclic acid, 4-hydroxycoumarin, vanillic acid, 4-hydroxybenzoic acid and caffeic acid, amount and their retention time is shown in Table 8. 4-hydroxybenzoic was the most abundant followed by 4-hydroxycoumarin. Because the HPLC was not coupled to spectrometer, some of the peaks could not be identified,

Table 5 Minimum Inhibitory Concentration (MIC) and Maximum Bactericidal Concentration (MBC) of ethanol leaf extract of *R. longipes*.

Test Organisms	Ethanol Extract (mg/mL)						MIC (mg/mL)	MBC (mg/mL)
	1000	**500**	**250**	**125**	**62.5**	**31.25**		
Staphylococcus aureus	–	–	–	+	+	+	31.25	250
Escherichia coli	–	–	–	–	+	+	125	125
Pseudomonas aeruginosa	–	–	–	–	+	+	62.5	125

+: Growth –:No Growth.

Table 6 GC–MS profile of compounds identified in the ethanol extracts of *R. longipes*.

Retention time	Compound	Percentage (%)
3.441	Cholest-2-eno[2,3-b]quinoxaline,',8′-dichloro-	0.12
5.791	1-(3-(Cyclohexylamino)propyl)guanidine	0.12
5.893	Aziridine, 1-(2-buten-2-yl)-	0.40
6.428	.pi.-Cyclopentadienyl-dicarbonyl-ethylisonitril-trichlorgermyl-tungsten	0.35
7.135	1,5-Methano-1H,7H,11*H*-furo[3,4-g]pyrano[3,2-b]xanthene-7,15-dione, 3 ,3a,4,5-tetrahydro-8-hydroxy-1-(4-hydroxy-3-methyl-2-butenyl)-3,3,11,11-tetramethyl-13-(3-methyl-2-butenyl)-, [1R-[1.alpha.,1(Z),3a.beta.,5. alpha.,14as*]]-	0.20
7.868	N-Heptafluorobutyrylmorpholine	0.23
7.895	3-Pyridinecarboxylic acid, 1,4,5,6-tetrahydro-1,2-dimethyl-6-oxo-, ethyl ester	0.16
8.771	2,2-Dibromocholestanone	0.22
9.800	Oleanan-29-oic acid, 3-(acetyloxy)-12,13-epoxy-11-oxo-, methyl ester, (3.beta.,12.beta.,20.beta.)-	0.27
11.185	9,19-Cyclolanost-6-ene-3,7-diol, diacetate	0.09
11.735	4a,7a-Epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecen-4(1H)-one, 2,7,10,11-tetrakis(acetyloxy)-1a,2,3,6,7,10,11,11a-octahydro-1,1,3,6,9-entamethyl-, [1ar-(1ar*,2R*,3S*,4ar*,6S*,7S*,7as*,8E,10R*,11R*,11as*)]-	0.09
11.839	Cholestan-6-en-3-ol, O-acetyl-24-methyl-5,8-[tetrahydrofuran-2,5-dione-3,4-diyl)-	0.08
14.224	Acetic acid, 17-(4-chloro-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1-phenanthryl-	0.11
14.486	Tricyclo[5.4.0.0(2,8)]undec-9-ene,2,6,6,9-tetramethyl-, (1R,2S,7R,8,R)-	0.42
16.666	3-Methyl-6-(6-methylhept-5-en-2-yl)cyclohex-2-enone	1.48
16.992	Neophytadiene	0.72
17.206	Silane, [[(3.beta.,6.beta.)-cholest-4-ene-3,6-diyl]bis(oxy)]bis[trimethyl-	0.43
18.118	3.beta.,4.beta.-Bis(trimethylsiloxy)cholest-5-ene	0.18
18.617	.psi.,.psi.-Carotene, 7,7′,8,8′,11 270,519 000540–04-5 10 ,11′,12,12′-octahydro-	0.46
19.608	3-Diethoxyphosphonyl-demethylthiocolchicine	0.22
19.661	9-Octadecenoic acid, (E)-, TMS derivative	0.16
20.508	Z-28-Heptatriaconten-2-one	0.44
25.508	*cis*-4-Trimethylsilyloxy-cyclohexyl (trimethylsilyl)carboxylate	0.11
26.253	Estriol, 3TMS derivative	0.13
26.347	1′,1′-Dicarboethoxy-1.beta.,2.beta.-dihydro-3′H-cycloprop[1,2]cholesta-1,4,6-trien-3-one	0.12

Table 7 Flavonoid profile of the ethanol leaf extract of *R. longipes*.

Peak ID	Retention Time	Conc. (μg/100 g)
Catechin	8.88	124.82
Apigenin	23.50	5.65
Caffeic acid	15.16	14.22

Table 8 Phenolic profile of the ethanol leaf extract of *R. longipes*.

Peak ID	Retention Time	Conc. (μg/100 g)
Gallic acid	1.273	2.846
Ferulic acid	1.432	4.931
Hydroxybenzoic acid	2.048	12.472
Salicyclic acid	2.457	6.968
4-hydroxycoumarin	3.290	21.272
Vanillic acid	4.398	15.091
4-hydroxybenzoic acid	5.39	170.32

therefore preventing the complete identification of components in the leaf extract.

3.6. Bioactivity prediction and molecular docking

The bioactivity prediction of the compounds identified in the *R. longipes* extract by the PASS software was used to screen those with antioxidant, anti-fungal, antimycobacterial and antibacterial potentials. The PASS software predicts bioactivity of a compound based on the structure similarity to the large data set of already known active compounds. The bioactivity score in form of Pa and Pi values are probability score of which compound with Pa score ≥ 0.5 have higher chances of being active. The bioactivity score of the constituents that are likely responsible for the observed antibacterial and antioxidant activity in this study is reported in Table 9. The predicted score for the antioxidant activity ranged between 0.318 and 0.810. While the predicted score for the antibacterial constituents of the plants ranged from 0.302 to 0.421. Cholestan-6-en-3-ol,O-acetyl-24-methyl-5,8-[tetrahydrofuran-2,5-dione-3,4-diyl)- had the highest antibacterial potential and Z-28-Heptatriaconten-2-one, and 9,19-Cyclolanost-6-ene-3,7-diol, diacetate had the least potential. Catechin on the other hand had the highest antioxidant potential and Salicyclic acid had the least antioxidant potential.

Study of the interaction between the ligands and bacterial glucosamine 6-phosphate synthase

(PDB ID: 1XFF), penicillin binding protein 3 (PDB ID: 3VSL), DNA gyrase (PDB ID: IKZN) and β-lactamase (PDB ID: 6VHS) is reported in Table 10 and Supplementary Figs. 1–4. The affinity of each ligands for the respective

Table 9 *In silico* bioactivity of the chemical constituents of *R. longipes* extract.

S/N	Compounds	Antioxidant		Antifungal		Antimycobacterial		Antibacterial	
		Pa	**Pi**	**Pa**	**Pi**	**Pa**	**Pi**	**Pa**	**Pi**
	1,5-Methano-1H,7H,11*H*-furo[3,4-g]pyrano[3,2-b]xanthene-7,15-dione, 3 ,3a,4,5-tetrahydro-8-hydroxy-1-(4- hydroxy-3-methyl-2-butenyl)-3,3,11,11-tetramethyl-13-(3-methyl-2-butenyl)-, [1R-[1.alpha.,1 (Z),3a.beta.,5.alpha.,14as*]]-	0.454	0.009						
	Cholestan-6-en-3-ol,O-acetyl-24-methyl-5,8-[tetrahydrofuran-2,5-dione-3,4-diyl)-			0.624	0.016			0.421	0.026
	9,19-Cyclolanost-6-ene-3,7-diol, diacetate			0.605	0.018			0.323	0.052
	Acetic acid, 17-(4-chloro-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1-phenanthryl-			0.639	0.014	0.014		0.389	0.033
	Neophytadiene	0.374	0.014	0.579	0.021	0.319	0.070	0.363	0.040
	Z-28-Heptatriaconten-2-one			0.496	0.031	0.306	0.077	0.302	0.060
	Catechin	0.810	0.003	0.552	0.023			0.320	0.053
	Apigenin	0.732	0.002	0.524	0.027	0.507	0.018	0.391	0.032
	Ferulic acid	0.540	0.005	0.430	0.044	0.491	0.019	0.333	0.048
	Caffeic acid	0.603	0.005	0.450	0.039	0.486	0.021	0.358	0.041
	4-hydroxycoumarin	0.455	0.009	0.490	0.032	0.431	0.032	0.380	0.035
	Gallic acid	0.520	0.006	0.398	0.050	0.432	0.032	0.418	0.020
	Vannilic acid	0.374	0.014	0.360	0.060	0.449	0.028	0.376	0.036
	Salicyclic acid	0.318	0.020	0.395	0.051	0.476	0.023	0.404	0.029
	4-Hydroxybenzoic acid	0.320	0.020	0.384	0.053	0.405	0.038	0.384	0.034

Table 10 Binding affinity of the ligands with the selected bacterial accessory proteins.

S/N	Name of Compound (Ligand)	Biding Energy (Kcal/mol)			
		DNA Gyrase	**GLM synthase**	**PBP**	**β-Lactamase**
	4-hydroxybenzoic acid	−5.7	−5.8	−5.4	−5.4
	4-hydroxycoumarin	−6.6	−6.3	−6.8	−6.0
	9,19-Cyclolanost-6-ene-3,7-diol, diacetate	−7.4	−7.3	−6.4	−6.0
	Acetic acid, 17-(4-chloro-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1-phenanthryl-	−6.7	−7.2	−8.4	−6.6
	Apigenin	−8.5	−7.5	−7.4	−7.5
	Caffeic acid	−6.2	−6.2	−6.7	−6.3
	Catechin	−8.6	−7.4	−7.2	−8.0
	Cholestan-6-en-3-ol, O-acetyl-24-methyl-5,8-[tetrahydrofuran-2,5-dione-3,4-diyl)-	−7.8	−7.8	−8.2	−6.2
	Ferulic acid	−5.9	−6.0	−5.8	−6.4
	Gallic acid	−5.8	−6.3	−6.0	−5.8
	Neophytadiene	−3.7	−4.1	−4.0	−3.5
	Salicylic acid	−5.7	−6.1	−5.6	−6.2
	Streptomycin	−6.7	−6.7	−7.7	−7.1
	Vanillic acid	−6.0	−5.9	−5.9	−5.5
	Z-28-Heptatriaconten-2-one	−2.5	−3.7	−4.3	−3.4

proteins range from low to good. Against each proteins, the potency of the ligands range from −2.5 to −8.6 Kcal/mol (DNA gyrase), −3.7 to −7.8 Kcal/mol (glucosamine 6-phosphate synthase), −4.0 to −8.4 Kcal/mol (penicillin binding protein 3) and −3.4 to −8.0 Kcal/mol (β-lactamase). The affinity of the best poses ligand for DNA gyrase ranged in the order Catechin > Apigenin > Cholestan-6-en-3-ol,O-acetyl-24-methyl-5,8-[tetrahydrofuran-2,5-dione-3,4-diyl)- > 9,19-Cyclolanost-6-ene-3,7-diol,diacetate > Streptomycin > 4-hydroxycoumarin. 4-hydroxycoumarin, 9,19-Cyclolanost-6-ene-3,7-diol, diacetate, Apigenin, Caffeic acid, catechin, Cholestan-6-en-3-ol, O-acetyl-24-methyl-5,8-[tetrahydrofuran-2,5-dione-3,4-diyl)-, and Streptomycin all showed strong interaction with all the four bacterial proteins. Although, Acetic acid, 17-(4-chloro-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1-phenanthryl- showed good negative free binding energy with the proteins, there was no interaction with any of the amino acids in the active site of the proteins. The ligands bind with one or more amino acid residue within the active site of the proteins via hydrogen bond, van der waals, alkyl, π-alkyl, π-anion, π- π T-shape and π-σ interactions. More hydrogen bond interactions with active site residues of the proteins as revealed in Supplementary Figs. 1–4 was observed for api-

genin, catechin, streptomycin, salicyclic acid, 4-hydroxybenzoic acid, and ferulic acid.

4. Discussion

R. longpee is an under exploited plant species with few to no report on its chemical constituents and biological properties. We have previously reported the protective effects against acetaminophen induced oxidative stress in rats and the in-vitro anti-inflammatory potentials in our laboratory (Olorunnisola et al., 2017). Detailed studies on the phytochemical class and constituents in the plant are not well reported, therefore our study focused on chemical characterization and biological properties of *R. longipes* leaf extract.

Phytochemicals constitute the active principle in plants and different classes of this principle have unique biological functions. From our study, *R. longipes* leaf is obviously rich in important phytochemicals that have been attributed to specific biological functions. For instance, glycosides have sedative and digestives properties (Galvano et al., 2004; Güçlü-Üstündağ and Mazza, 2007), anticancer (Zhou et al., 2013) and expectorant (Kabera et al., 2014). Tannins on the other hand possess wound healing effect and helps inflamed mucosal membranes (Yadav et al., 2014). Plants rich in tannins act as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours, and as anti-inflammatory, antiseptic, and haemostatics (Khanbabaee and vanRee, 2001). Alkaloids are known for their sedative, antimalarial, and anticancer properties (Swain and Padhy, 2015) and they have a powerful effect on the nervous system (Renu, 2005). Flavonoids have antioxidant potentials hence could offer protection against oxidative stress related diseases and food oxidation. In other words, *R. longipes* will be an important plant with various biological, pharmacological, and industrial usefulness. The important compounds belonging to the phenolic and flavonoids class identified in our study are useful compounds well reported with economical and medical values.

Plant leaves are the natural sources of abundant antioxidants due to the presence of vitamins, such as vitamin C, A and E (Sarker and Oba, 2020b; 2020c), and wide range of phytochemicals i.e., diverse group of pigments, phenolics and flavonoid compounds (Sarker and Oba, 2020d; Sarker and Oba, 2018b). Previous literature of plant leaves have shown that the leaf pigments such as anthocyanins, carotenoids (Sarker and Oba, 2020e), chlorophyll *a* (Sarker and Oba, 2019c), betacyanins, betaxanthins, betalains, β-carotene (Sarker and Oba, 2018c), xanthophylls, had strong antioxidant activity. Furthermore, phenolics and flavonoid compounds of leaves such as simple phenol, coumarins, different groups of phenolic acids, and different groups of flavonoids, including flavonols, flavones, flavanols, flavanones, anthocyanins, chalcones, and different groups of nonflavonoids, including tannins, lignans and stilbenes (Sarker et al., 2020; Sarker and Oba, 2020f) also have tremendous radical quenching capacity. These antioxidant compounds neutralize/scavenge free oxygen and nitrogen radicals and protect against many diseases in the human body.

Gallic acid, catechin, apigrnin, caffeic acid, hydroxybenzoic acid, salicyclic acid, 4-hydroxycoumarin, vanillic acid, and ferulic acid are important antioxidant phenolic found to be abundant in other plant species (Irondi et al., 2016; Lu et al., 2011; Sokamte et al., 2019). These compounds were observed in different proportion in the leaf extract of *R. longipes and* could have contributed significantly to the antioxidant properties exhibited by *R. longipes*. We used four different in-vitro assay methods to establish the antioxidant activity of the aqueous and ethanol extract. Review of the in-vitro antioxidant methods can be accessed from Adegbola et al. (2020). It was clear from our observation that extraction solvent significantly affected the potency of the extracts. Ethanol is less polar than water and could have extracted both the polar and non-polar components of the plant. More so, we conducted the chromatographic and spectroscopic characterization of chemical constituent on the ethanol extract. Based on this study, it is clear that *R. longipes* will be a useful plant, not just for the prevention, treatment, and management of oxidative stress related diseases but could also be useful in the food industry as it contains useful compounds that could protect against food oxidation.

To further explore, the biological properties of *R. longipes*, we conducted antimicrobial studies against gram (+) positive and negative bacterial. Bacterial are important contributor to food deterioration and several human diseases whereas any agents that could inhibit their growth will play useful role in alleviating their negative impacts. The extracts of *R. lonipee* exhibited comparable activity against the tested microbial strains when compared with a standard antimicrobial agent. No doubt, active principles already identified in our study contributed to the observed activity.

To implicate the specific constituents of the *R. longipes* extracts responsible for the observed function, we carried out GC–MS characterization, Insilco bioactivity studies, and molecular docking analysis on various compounds in the extracts of the plant. Some of the identified volatile compounds in the leaf extracts of *R. longipes* have useful biological function. For instance, some of the identified compounds have equally been identified in other plant species with antimicrobial properties ((Achakzai et al., 2019; Gnanaselvan and Sivaraman, 2020; Halim et al., 2013; Kaushik et al., 2014; Prabhu et al., 2020; Shakiba et al., 2018).

In our study, Insilco bioactivity prediction of the components in the plant showed some compounds with antibacterial and antioxidant properties. The method relies on the structural similarity of test compounds to the large database of known active substances. Pa and Pi values (bioactivity score) predict the probability of compound to be active and a compound with a bioactivity score of 0.5 or above has higher chances to be active (Al-Dhahli et al., 2020). In our study, garlic acid, ferulic acid, apigenin, catechin, and caffeic acid possess high antioxidant bioactivity score, therefore suggesting them as the antioxidant constituents of *R. longipes*. In addition, garlic acid, and cholestan-6-en-3-ol,O-acetyl-24-methyl-5,8-[tetrahydrofuran-2,5-dione-3,4-diyl)- had the highest antibacterial bioactivity score and this observation supports the inhibition potentials of the compounds against the bacterial accessory proteins tested in this study. Only compounds with antibacterial potentials were docked against selected bacterial proteins. Antibacterial agents exert their inhibitory effects against bacterial via four basic mechanisms including inhibition of nucleic acid biosynthesis, protein synthesis, cell wall synthesis, and folate synthesis. Consequently, major accessory proteins involved in bacterial multiplication and viability were selected for inhibition studies using Insilco molecular docking.

Penicillin Binding Protein (PBP) is an attractive drug target in the development of antibiotics. The protein is involved in the synthesis of peptidoglycan a principal component of the bacterial cell wall (Sauvage et al., 2008; Vollmer et al., 2008). Peptidoglycans are required for bacterial viability and inhibition of their synthesis will result is bacterial death (Yoshida et al., 2012). From our study, 4-hydroxycoumarin, Apigenin, caffeic acid, catechin, Cholestan-6-en-3-ol,O-acetyl-24-methyl-5,8-[tetrahydrofuran-2,5-dione-3,4-diyl)-, similar to streptomycin showed the highest affinity for penicillin binding protein. However, only streptomycin**,** caffeic acid, catechin, salicyclic acid, 4_hydroxybenzoic acid binds to one or more active site residue of the protein and thus could be considered potential agents for the competitive inhibition of bacterial cell wall formation.

Glucosamine-6-phosphate synthase (GMP-Synthase) is an N-terminal nucleophile family of amidotransferases characterized by a conserved N-terminal catalytic cysteine. The enzyme utilizes the amide nitrogen of glutamine in the biosynthesis of amino sugars, amino acids, and nucleotides. It has a synthase domain that binds the nitrogen acceptor and a glutaminase domain that hydrolyzes the glutamine to glutamate and ammonia (Isupov et al., 1996). In bacterial, inhibition of GMPS can induce morphological changes, agglutination, and lysis (Subbaiah et al., 2017). 4-hydroxycoumarin, 9,19-Cyclolanost-6-ene-3,7-diol, diacetate, Apigenin, Caffeic acid, Catechin, Cholestan-6-en-3-ol,O-acetyl-24-methyl-5,8-[tetrahydrofuran-2,5-dione-3,4-diyl)-, and streptomycin again demonstrated the most significant inhibitory potentials against the GMPS and might provide a strong lead in the development of antibiotics with GMPS inhibition as a major mechanism of activity.

Bacterial DNA gyrase is classified as topoisomerase family of protein involved in DNA replication and transcription. As a type II topoisomerase, it catalyses the negative supercoiling of the closed circular DNA replication and transcription (Lafitte et al., 2002). It is an important target for several classes of antibiotics. The residues Asp 73, Asn 46, Val 71, and Val 43 were the most prominent residue involved in either hydrogen or non-hydrogen bond interactions with the compounds. Among all the compounds however, 4-hydroxycoumarin, 9,19-Cyclolanost-6-ene-3,7-diol,diacetate, Apigenin, Caffeic acid, Catechin, Cholestan-6-en-3-ol,O-acetyl-24-methyl-5,8-[tetrahydrofuran-2,5-dione-3,4-diyl)-, and vanillic acid exhibited the lowest binding energy that ranged between 6.0 and 8.6 Kcal/mol. Overall, our observation revealed the potentials of the compounds to act as potent inhibitor of bacterial DNA gyrase.

Development of resistance to β-lactam class of antibiotics by bacterial especially the gram-negative bacterial is of critical concern. The resistance is due to expression of multiple β-lactamases enzymes, which are capable of hydrolysing and destroying the β-lactams (Bush and Bradford, 2016). β-lactamase inhibitors together with antibiotics can counter the broad resistance to several antibiotics. In this regard, we docked 24 of the plant isolates against the β-lactamase enzyme and observed 4-hydroxycoumarin, 9,19-Cyclolanost-6-ene-3,7-diol, diacetate, Apigenin, Caffeic acid, Catechin, Cholestan-6-en-3-ol,O-acetyl-24-methyl-5,8-[tetrahydrofuran-2,5-dione-3,4-diyl)-, Ferulic acid, and Salicyclic acid to be potential inhibitors. These compounds exhibited binding affinity range of 6.0–8.0 Kcal/mol. Key amino acids; Ser 70, Tyr 105, and Asn 132, involved in the catalytic activity of the protein were the most common residues involved in the interaction with the compounds. Observation in this study indicate that *R. longipes* is a repository of important compounds which could be effective against antibiotic resistant bacteria.

Overall, 4-hydroxycoumarin, 9,19-Cyclolanost-6-ene-3,7-diol,diacetate, Apigenin, Caffeic acid, Catechin, Cholestan-6-en-3-ol,O-acetyl-24-methyl-5,8-[tetrahydrofuran-2,5-dione-3,4-diyl)-, Salicyclic acid, and vanillic acid may have some potentials as inhibitors of important accessory proteins required for bacterial survival and might have contributed either singly or in synergy to the antibacterial properties of *R. longipes*.

5. Conclusion

The result of this showed that *R. longipes* tested positive for the presence of tannins, flavonoids, phenolics, terpenoids, steroids, alkaloids, and saponins. Overall, the ethanol extract exhibited better antioxidant activity than the aqueous extract. The most abundant phenolic in the extract was 4-hydroxybenzoic and 4-hydroxycoumarin. Compounds identified in R. longipes inhibited four major accessory proteins required for bacterial viability and these compounds could have contributed to the observed antimicrobial activity of *R. longipes*. Summarily, it is evident that R. longipes have some antioxidant and antibacterial properties and the plant contain important phytochemicals with ability to retard food oxidation and deterioration and thus could be annexed for various industrial and medicinal purposes. To the best of our knowledge, this is the first report on the identification of bioactive principles of *R. longipes*.

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CRediT authorship contribution statement

Peter Ifeoluwa Adegbola: Conceptualization, Data curation, Formal analysis, Funding, In-vitro investigation, Insilico investigation, Software, Writing - original draft, Writing - review & editing. **Adedoyin Adetutu Olasunkanmi:** Conceptualization, Data curation, Funding, In-vitro investigation. **Olumide Samuel Fadahunsi:** Data curation, Funding, In-vitro investigation.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103601.

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