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Bioactivity Guided Isolation of Gallic Acid and Kaempferol-7-O-Glucoside from Ethyl Acetate Fraction of *Entada africana* Guill. & Perr. Stembark Crude Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Medicinal plants have historically played a crucial role in human healthcare due to their phytochemical compounds which provides protection against various pathogens and environmental challenges. The aim of the study was the isolation of bioactive compounds from Entada africana; a

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plant recognized for its medicinal properties in Africa. The stembark extract of Entada africana was obtained using maceration and fractionated. The fractions were evaluated for antibacterial activities using agar disc diffusion method and the antioxidant activities of the most active fraction (ethyl acetate) was evaluated using ferric reducing antioxidant power (FRAP), ferric ion chelating activity (FICA) and hydrogen peroxide assay (H_2O_2). Gradient elution on column chromatography was used to obtain pure isolates. The isolates were characterized using FT-IR, NMR and in comparison with literature. The extraction process yielded 165g of extract from which 11.4 g was obtained from the ethyl acetate fraction. The ethyl acetate extract had inhibition zones of 13.53 mm for Salmonella typhi, 11.00 mm for Enterococcus faecalis, and 10.67 mm for Klebsiella pneumoniae. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the ethyl acetate extract suggests moderate non-bactericidal activity against Salmonella typhi, and weak on other pathogens in the study. Antioxidant assays revealed IC_{50} values of 7.78 µg/ml for FICA, 33.73 µg/ml for FRAP assay, and 16.60 µg/ml for H_2O_2 scavenging activity; all indicating extract's strong ability to scavenge free radicals. The characterization of P43 and P17 based on spectroscopic data and literature comparison suggests P43 as gallic acid while P17 as kaempferol-7-O-glucoside. While gallic acid had been previously reported, this is the first time kaempferol-7-O-glucoside is being reported from the stem bark extract of Entada africana. Both compounds had in previous studies demonstrated remarkable antibacterial and antioxidant properties. The study therefore supports the traditional medicinal uses of Entada africana.

Keywords: Entada africana; antibacterial; antioxidant; Isolation; gallic acid; kaempferol-7-O-glucoside, Characterization.

1. INTRODUCTION

Since ancient times, medicinal plants or herbs, had been identified and employed in traditional medication procedures [1]. Even in contemporary times, plants are still employed traditionally to treat or cure several ailments and diseases due to their unique ability to synthesize phytochemical compounds or secondary metabolites [2,3]. These phytocompounds are used in defense against pathogenic microbes, dangerous insects and to withstand severe environmental changes. These compounds can also confer protection on human beings and animals against infectious and non-infectious diseases. Based on differences in chemical structure, several classes of phytochemicals have been identified such as phytosterols, flavonoids, terpenoids, saponins, alkaloids, carotenoids, aromatic acids, organic acids, essential oils [4]. These metabolites can act as a direct or indirect defense mechanism against infections or hazardous substances since they possess properties such as antibacterial, anti-inflammatory, anthelmintic, anticarcinogenic, anti-genotoxicity, and antioxidant properties [5].

Entada is a genus in the Fabaceae family belonging to the Caesalpinioideae of the subfamily's Mimosoideae with over 30 different species of tropical lianas, shrubs, and trees. Six species are found in Asia and about 21 from Africa. Their seed pods can grow up to 1.5 meters long and possess compound leaves [6]. *Entada africana* has brown-grey to black rough bark that is transversely striped, scaly, peeling in long fibrous strips which is red or yellow-brown in color. Its height ranges from 4-10 meters, and 90 cm in girth. The root decoction is taken as a stimulant and tonic, while the bark has abortifacient properties. The emetic qualities of *Entada africana* is believed to serve as antidote against a variety of hazardous substances. Drinks made from the leaves, bark, roots, and shoots are claimed to relieve fever. The leaf or bark infusion are also consumed as a tonic and for stomachache cure in northern Nigeria and Ghana. The leaves are also used in wound dressing to prevent suppuration [7].

Miko et al. [8] evaluated the antibacterial activity of *Entada africana* root extract from methanol, ethyl acetate, and hexane against six wound isolates. The methanol extract showed the strongest antibacterial activity, inhibiting four out of six bacteria, with MIC of 1.5 mg/ml against *Staphylococcus aureus* and 6.25 mg/ml against *Streptococcus pyogenes* and *Klebsiella pneumoniae*. The ethyl acetate extract had mild activity against *Pseudomonas florescence* at 100 mg/ml, while hexane and ethyl acetate extracts inhibited only three organisms each. Kwaji et al. [9] also reported the antibacterial activities of n-hexane, dichloromethane, acetone, and methanol extracts from *Entada africana* stem bark extract. The acetone extract was particularly

effective against *Enterococcus faecalis, E. coli*, and *S. aureus*, with inhibition zones between 14.00 ± 1.00 to 17.00 ± 1.73 mm; however, *S. aureus* was resistant with an MIC of 13.88 mg/ml. Tchana et al. [10] also reported on the CH_2Cl_2/MeOH extract's effectiveness against multi-drug-resistant Gram-negative *E. coli* with an MIC of 64 µg/ml. These results highlight the potency of *E. africana* in addressing antibiotic-resistant infections. Ifemeje et al. [11] also found that the extract inhibited the growth of both *Salmonella typhi* and *Bacillus subtilis* with inhibition zones of 12.00 ± 0.02 mm for *S. typhi* and 8.02±0.05 mm and MIC/MBC values of 5 and 10 mg/ml respectively. Further research by Mbatchou et al. [12] showed that various fractions from the ethanol stem bark extract were effective against *S. typhi*, with the ethanol soluble fraction achieving a mean inhibition zone between 9.2 ± 0.3 to 26.7 ± 2.0 mm.

Antioxidant activity refers to the capacity of compounds to inhibit oxidation, through neutralization of free radicals that can cause cellular damage [13]. Tibiri et al. [14] reported the free radical scavenging activity of *Entada africana* extracts and found that both aqueous and methanol extracts exhibited significant effects, with IC_{50} values of 5.7 and 5.3 µg/ml, respectively. In another report, Tibiri et al. [15] reported that the aqueous root extract showed comparable activity to standard antioxidants like quercetin and ascorbic acid. Njayou et al. [16] investigated various stem bark extracts using assays such as DPPH and found that methylene chloride-methanol (MCME) and water extracts significantly inhibited lipid peroxidation, with IC_{50} values of 0.50 ± 0.07 and 3.50 ± 0.11µg/ml. In a follow-up study, Njayou et al. [17] noted that antioxidant activity showed positive correlation with total polyphenolic content.

Some phytocompounds or secondary metabolites previously isolated from *Entada africana* extracts include Betulin [18], Stigmasta-7,22-dien-3-ol and alkylferulate [19]. Gallic acid and dihydrokaempferol-7-O-β-D-glucoside among several other anti-inflammatory polyphenolic compounds were also reported by Codo-Toafode et al. [20]. Similarly, Hassan et al. [21] reported the isolation and characterization of β-sitosterol from *Entada africana* ethylacetate stembark extract.

The present study focuses on the isolation of bioactive compounds from the relatively active ethyl acetate extract fraction of *Entada africana* stem bark through evaluation of antibacterial and antioxidant potentials of crude extract fractions: The antioxidant activities were assessed using three methods namely; Ferrous Ion Chelating Activity (FICA), Ferric Reducing Antioxidant Power (FRAP), and Hydrogen Peroxide Scavenging Activity (H_2O_2); while the antibacterial activity was assessed using agar disc diffusion assay. Isolation of compounds was achieved on a thin layer chromatography (TLC) monitored glass column.

While previous studies had reported the isolation of gallic acid [20], this is the first report on the isolation of Kaempferol-7-O-glucoside from the ethylacetate fraction of *Entada africana* based on available literature.

2. MATERIALS AND METHODS

2.1 Sample Collections, Identification and Preparation

The *Entada africana* stem bark sample was obtained from Akko Local Government Area of Gombe State, Nigeria, and subsequently identified by a Botanist in the Department of Botany Gombe State University of Nigeria. The stem bark sample was air dried and milled to powder. This was kept in dry polythene bag until required for use.

2.2 Extraction and Fractionations

Entada africana stem bark sample (1.5 Kg) was soaked in 15 liters of methanol for six days with intermittent shaking for proper penetration of sample matrix and dissolution of phytocompounds. The soaked sample was filtered over cotton wool in a funnel and clarified with Whatman No. 1 filter paper. The filtrate was subsequently concentrated on a rotary evaporator at 45°C. The concentrated extract was dried under shade to a constant weight as described by Abubakar and Haque, [22]. The percentage yield was calculated according to equation (1).

$$\%\ yield = \frac{mass\ of\ extract\ in\ grams}{mass\ of\ dry\ plant\ in\ grams} x100 \qquad (1)$$

Three distinct solvents n-hexane, dichloromethane and ethyl acetate were used to fractionate the *Entada africana* crude methanol extract as described by Abubakar and Haque, [22].

2.3 Antibacterial Activity

2.3.1 Source of organisms

The microorganisms utilized in this study were clinical isolates sourced from the Research Laboratory of the Department of Medical Microbiology and Parasitology at the Federal Teaching Hospital of Gombe State, Nigeria and confirmed at Pharmaceutical Microbiology Research Laboratory, Faculty of Pharmaceutical Sciences, Gombe State University. The isolates include Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhi*, and Gram-positive bacteria; *Staphylococcus aureus*, *Enterococcus faecalis*, and *Bacillus subtilis*. To ensure the purity of the cultures, these microorganisms were maintained and sub-cultured on nutrient agar to obtain pure colonies.

2.3.2 Preparation of discs

The solution of the fractions from the stem-bark extract were prepared by dissolving 2 mg of extract each in 4 ml of 10 % aqueous dimethyl sulfoxide (aq. DMSO) to give final concentration of 500 µg/ml. Sterile filter paper discs (6 mm) were immersed in a 500 µg/ml extract solution for 10 seconds and placed on the inoculated agar plate. Similarly, the negative control was prepared in the same manner while the commercially available antibiotic gentamicin at 10 µg/ml from Glaxo-welcome served as the positive control for all bacterial strains. All test samples and control were prepared according to the method reported by Dibala et al*.,* [23] with slight modification.

2.3.3 Disc-diffusion assay

The antibacterial activity of *Entada africana* ethyl acetate crude fraction was evaluated using the disc diffusion assay as reported by Dibala et al. [23]**.** Sterile petri dishes (90 mm in diameter) were prepared by pouring 20 ml of molten Mueller Hinton agar and allowed to solidify in a sterile chamber. Each petri dish was inoculated with 10 µl of each bacterial suspension (10^6cfu/ml). After drying in a sterile hood, 6 mm diameter discs at 500 µg/ml of the different extracts were placed on the medium. A disc containing gentamicin (10 µg/ml) was used as a positive control and 10 % DMSO as a negative control. The plates were incubated for 18 - 24 hours at 37 °C. The diameters of the inhibition zones were measured in millimeters with a transparent meter rule. All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition in diameters (mm) produced.

2.3.4 Minimum Inhibitory Concentration (MIC)

The MIC of the extract was determined using a modified standard broth dilution method described by Yohanna et al. [24]. Nine serial two-fold dilutions of the extract were prepared from the stock solution, resulting in a final concentration range from 500 µg/ml to 1.95 µg/ml. The tenth test tube (No. 10) acted as a negative control (broth + inoculum), the eleventh test tube (No. 11) served as a neutral control (broth only), and the twelfth test tube (No. 12) functioned as a positive control (broth + inoculum + standard drug). For each bacterial species, three sets of nine test tubes were utilized. Each test tube received 2ml of culture medium, while the first nine tubes were supplemented with 10 µl of the test organism at McFarland standard. The tenth and twelfth test tubes also received 10 µl of the test organism, with the twelfth tube additionally containing 2 ml of gentamicin at a concentration of 10 µg/ml. The test tubes were covered and incubated at 37°C for 24 hrs. The turbidity or growth was monitored by visual inspection. The lowest concentration that showed no turbidity or growth among the test tubes was recorded as the MIC.

2.3.5 Minimal Bactericidal Concentration (MBC)

The MBC was determined using the method reported by Yohanna et al. [24]. This was assessed by transferring the contents of test tubes that exhibited no turbidity or visible growth from the MIC tests onto neutral sterile Mueller Hinton Agar plates, which were labeled according to the specific test bacterium. The bacterial cultures were uniformly spread across the agar surface using a swab stick. The plates were incubated at 37°C for another 18 to 24 hrs, after which colony growth was examined. The MBC was identified as the lowest concentration of the plant extract at which no bacterial colonies appeared on the agar plate.

2.4 Antioxidant Activity

The antioxidant activities were evaluated using previously described methods by Kwaji et al*.* [25] for FRAP and H_2O_2 assays while the method reported by Yohanna et al*.* [24] was adopted for the FICA assay.

2.5 Column Chromatography Procedure

The column chromatography purification of the crude ethylacetate fraction was performed in accordance with the procedure outlined by Kwaji et al. [18]. The extract was dissolved in sufficient solvent, pre-adsorbed onto silica gel, and allowed to dry to yield free flowing powder. The column was packed using the wet slurry method. Gradient elution was conducted with hexane/ethyl acetate and dichloromethane/ethyl acetate with polarity increase at 5% change in volume of eluting solvents (100:00, 95:05, 90:10, 85:15, 80:20, 75:25, etc.) to produce several fractions. These fractions were monitored using thin layer chromatography (TLC). Fractions with the same Rf values and contained a single were combined together and labelled P17. Same procedure was employed for P43. Finally, the compounds obtained (P17 and P43) were purified by recrystallization after washing off soluble impurities with appropriate solvents.

2.6 Spectroscopic Analysis of Isolated Compounds

The characterization and identification of the isolated compounds was performed using Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance Spectroscopy (^{1}H and ^{13}C NMR) data. The ^{1}H and ^{13}C NMR spectra were recorded on 600 MHz with the sample initially dissolved in deuterated chloroform ($CDCl_3$). Chemical shift values (δ) were reported in parts per million (ppm) relative to tetramethylsilane (TMS) standard.

3. RESULTS AND DISCUS1ON

3.1 Antibacterial Activity

The ethyl acetate extract had the highest inhibition zone against *Salmonella typhi, Enterococcus faecalis* and *Klebsiella pneumonia* with 13.53 mm, 11.00 mm and 10.67 mm respectively. This indicates that the Ethyl acetate extract possess significant antibacterial activities relative to the gentamicin standard drug. On the other hand, n-hexane and dichloromethane extracts presented minimal activity. Hexane extract (non-polar solvent) showed inhibition zones of 8.00 mm and 8.33 mm against *Escherichia coli* and *Salmonella typhi,* respectively. Similarly, the dichloromethane extract with moderate polarity displayed a weak effect with inhibition zone of 7.53 mm against *E. coli*. The n-hexane and dichloromethane extracts showed the least activity. This is consistent with previous literature report [9].

Table 2 shows the MIC, MBC and MBC/MIC ratio of the ethyl acetate extract of *Entada africana* stem bark for *Salmonella typhi, Escherichia coli, Enterococcus faecalis* and *Klebsiella pneumoniae.* If the ratio MBC/MIC <4, the effect is considered as bactericidal but if the ratio MBC/MIC > 4, the effect is considered as bacteriostatic [26].

Table 1. Shows the results of Agar disc diffusion test of Entada africana crude extract inhibition zones (mm) at 500 µg/m

Bacterial	Hexane	Dichloromethane	Ethyl acetate	Gentamicin (10µg/ml)
Escherichia coli	8.00±1.00	7.53±1.55	12.67±1.73	26.33±1.58
Bacillus cereus	NA	NA	NA	20.00±0.00
Salmonella typhi	8.33±1.53	NA	13.53±1.33	20.13±1.63
Proteus vulgaris	NA	NA	NA	20.00±1.00
Enterococcus faecalis	NA	NA	11.00±1.00	19.55±1.31
Staphylococcus aureus	NA	NA	NA	17.67±1.15
Pseudomonas aeruginosa	NA	NA	NA	18.17±1.76
Klebsiella pneumoniare	NA	NA	10.67±1.73	13.52±1.22

NA= no activity.

Table 2. MIC and MBC (µg/ml) of Ethyl acetate extract against Bacterial Strains

Bacteria	MIC	MBC	MBC/MIC
Escherichia coli	7.8125 ± 0.00	31.25	4.03
Salmonella typhi	5.208± 0.25	15.625	3.00
Enterococcus faecalis	13.02± 0.51	62.5	4.73
Klebsiella pneumoniare	20.63±0.62	125	6.06

The MIC and MBC results for the various bacterial strains gave important insights into the efficacy of the crude extract fraction antibacterial activity. The ethylacetate crude extract is bacteriostatic to all organisms under test except on *Salmonella typhi* where the effect is bactericidal.

3.2 Antioxidant Activity

3.2.1 Ferric Reducing Antioxidant Power (FRAP)

Figs. 1 and 2 revealed the %FRAP IC_{50} value of ethyl acetate fraction and the reference compound Rutin as 33.73 µg/ml and 10.23 µg/ml. This indicates that the concentration of ethyl acetate crude fraction required to reduce ferric ions to half its initial concentration. This means both ethyl acetate crude extract fraction and Rutin at concentrations of 33.73 µg/ml and 10.23 µg/ml respectively neutralizes or scavenge equal amount of free radicals. The results from the present study revealed greater potency than that reported by Kwaji et al. [9] for the methanol stembark extract of *Entada africana* and acetone fractions, with an IC_{50} of 0.261 mg/ml, and IC_{50} of 0.595 mg/ml respectively while that of ascorbic acid standard was 0.443 mg/ml.

3.2.2 Ferrous Ion Chelating Activity (FICA)

Figs. 3 and 4 showed the chelating activity of crude ethyl acetate fraction of *Entada africana* stem bark. The IC_{50} value for this fraction was found to be 7.78 µg/ml. In contrast, the EDTA, a well-known chelating agent had an IC_{50} value of 3.524 µg/ml. This shows that the ethyl acetate fraction has strong chelation capacity for ferrous ions relative to the EDTA, a standard chelating agent. These results are in line with the study of Baidoo et al. [27] on the antioxidant potential of *Erythrina africana* extracts using different antioxidant assays. The fractions of extract showed potent antioxidant activity but more pronounced in the ethyl acetate fraction. The stem bark extract in particular demonstrated the best radical scavenging activity, with an IC_{50} value of 18.63 ± 2.11 µg/ml using the DPPH assay as compared to the leaf extracts which had an IC_{50} 92.25 ±9.87 µg/ml. Additionally, the stem bark showed high levels of phenolic content and flavonoid content.

3.2.3 Hydrogen peroxide (H_2O_2) radical Scavenging Activity

The antioxidant activity of Rutin on the hydroxyl ions of H_2O_2 revealed IC_{50} value of 8.77 µg/ml (Fig. 6). The potency of reference compound (IC_{50} 8.77 µg/ml) is twice that of the crude extract (Fig. 5; IC_{50} = 16.60 µg/ml). This is similar to the report of Tibiri et al. [15] for the aqueous extracts of leaves and roots as well as methanol extract from the root. Of these, the ethyl acetate fraction in particular exhibited the most striking result. It showed high radical scavenging activity with an EC_{50} value of between 3.39 and 16.72 µg/ml. These values are similar to the findings in the present study (IC_{50} = 7.78 and 16.60 µg/ml.).

The evaluation of the antioxidant activities of the ethyl acetate extract fraction of *Entada africana*, using three different assays (FICA, FRAP and H_2O_2 assays) showed that the ethyl acetate fraction demonstrated strong antioxidant activity relative to the reference standard antioxidants in the study. The highest antioxidant activity (IC_{50}) value for ethyl acetate extract of *Entada africana* was recorded in the H_2O_2 assay and while the lowest was observed in the FICA assay. Such variations underscore the fact that no one method is sufficient to evaluate the antioxidant potential of a given substance.

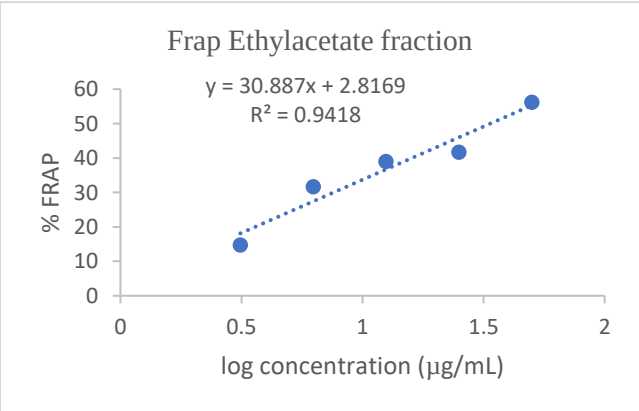


Fig. 1. % FRAP Ethyl acetate (IC_{50} = 33.73 µg/ml)

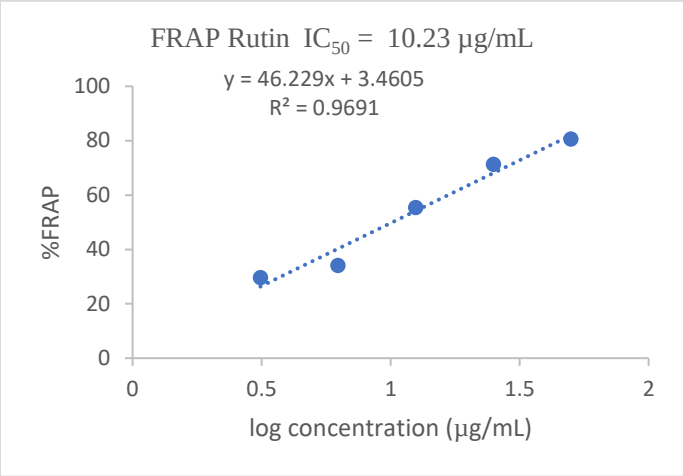


Fig. 2. % FRAP of the Rutin (IC_{50} = 10.23µg/ml)

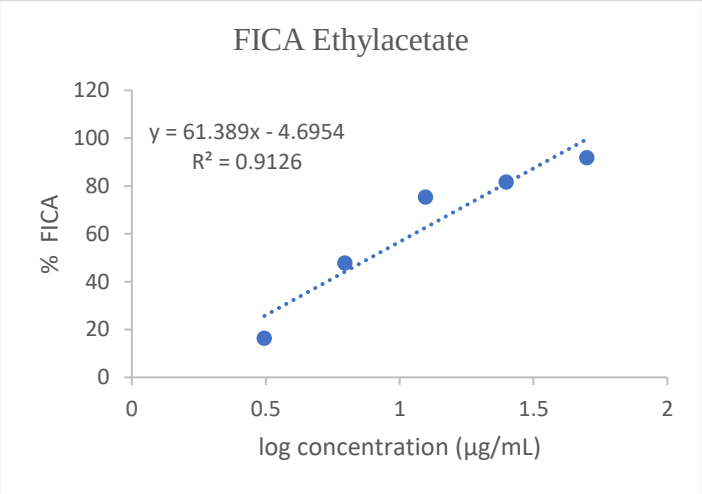


Fig. 3. Ethyl acetate fraction Chelating power. IC_{50} = 7.78 µg/ml

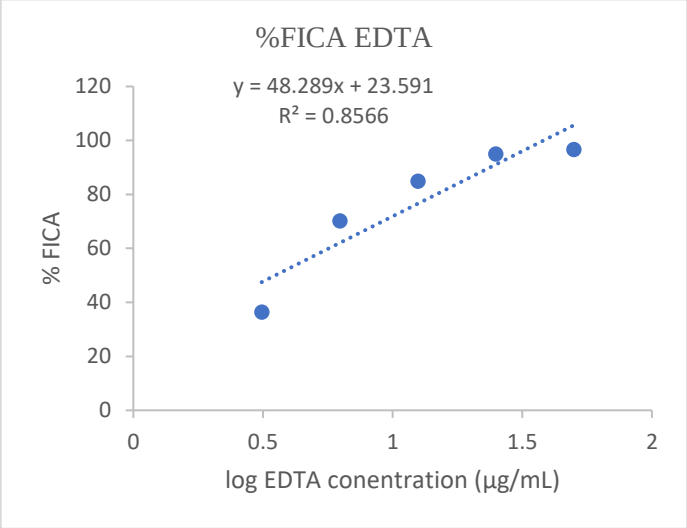


Fig. 4. Chelating power of EDTA IC_{50} = 3.524 µg/ml

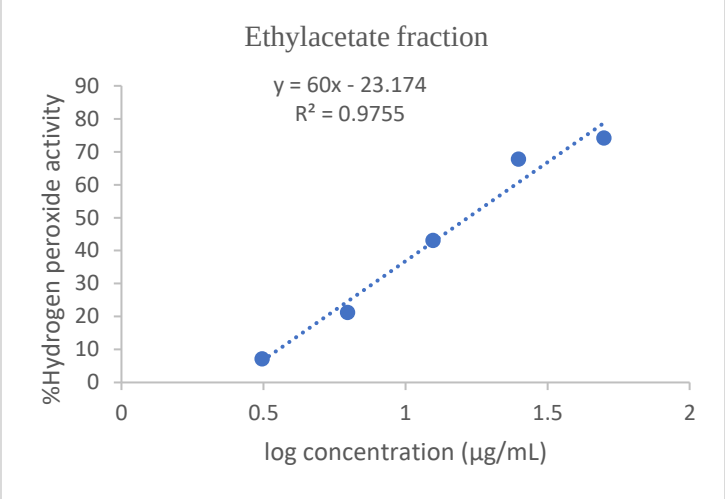


Fig. 5. *Ethylacetate fraction* H_2O_2 activity (IC_{50} = 16.60 µg/ml)

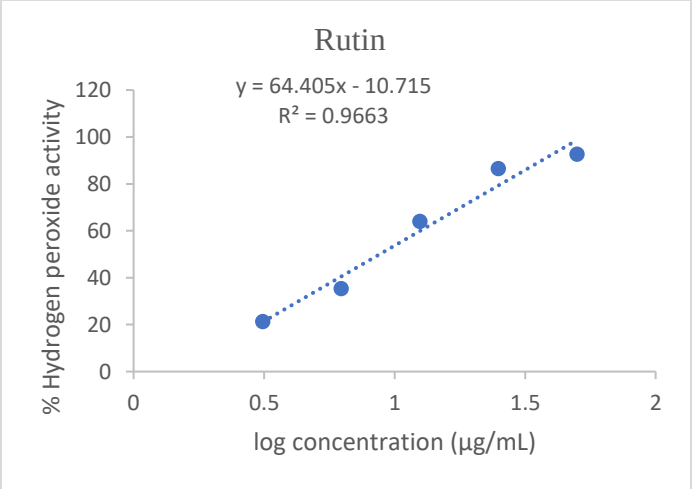


Fig. 6. Rutin H_2O_2 activity IC_{50} = 8.77 µg/ml

3.3 Spectroscopic Characterization

3.3.1 FT-IR Spectrum of P43

The FT-IR Spectrum analysis of compound P43 revealed several key peaks that indicate its functional groups. The peak at 3361.43 cm^{-1} corresponds to O-H stretching vibrations, suggests the presence of hydroxyl groups due to hydrogen bonding. At 2983.46 cm^{-1}, C-H stretching vibrations are observed. The peak at 1716.48 cm^{-1} signifies C=O stretching vibrations, characteristic of carbonyl groups found in carboxylic acids or ketones. Additionally, the peak at 1558.55 cm^{-1} suggests bending vibrations associated with aromatic structures. Finally, the peak at 1255.13 cm^{-1} indicates C-O stretching vibrations from both hydroxyl and carbonyl groups. These findings are consistent with previous studies conducted by Genwali et al. [28], Shen et al. [29], Kamatham et al. [30], Abri et al. [31] and Roheem et al. [32].

Table 3. FT-IR Spectra of P43

Wave number (cm^{-1})	Functional group
3361.43	O-H Stretching
2983.46	C-H Stretching
17I6.48	C=O Stretching
1558.55	C=C Aromatic Bending
1255.13	C-O Stretching

3.3.2 NMR analysis of P43

From the ^1H NMR spectrum (Fig. 8), the chemical shifts at δ 8.94 (2H, d, J = 2.7 Hz) and

δ 6.91 (2H, d, J = 5.8 Hz) indicate the presence of aromatic protons. These shifts suggest that the molecule contains a symmetrical aromatic ring structure, which is characteristic of Gallic acid. The doublets reveal that these protons are coupled with neighboring protons, further confirming their positions on an aromatic system. The signal at δ 9.20 (1H, d, J = 6.1 Hz) suggests a highly deshielded proton, possibly of a hydroxyl or an aldehyde group. The most deshielded signal at δ 12.19 (1H, d, J = 7.1 Hz) indicates an acidic proton, from the carboxylic acid group of gallic acid. The assignments are consistent with literature [29,33,34].

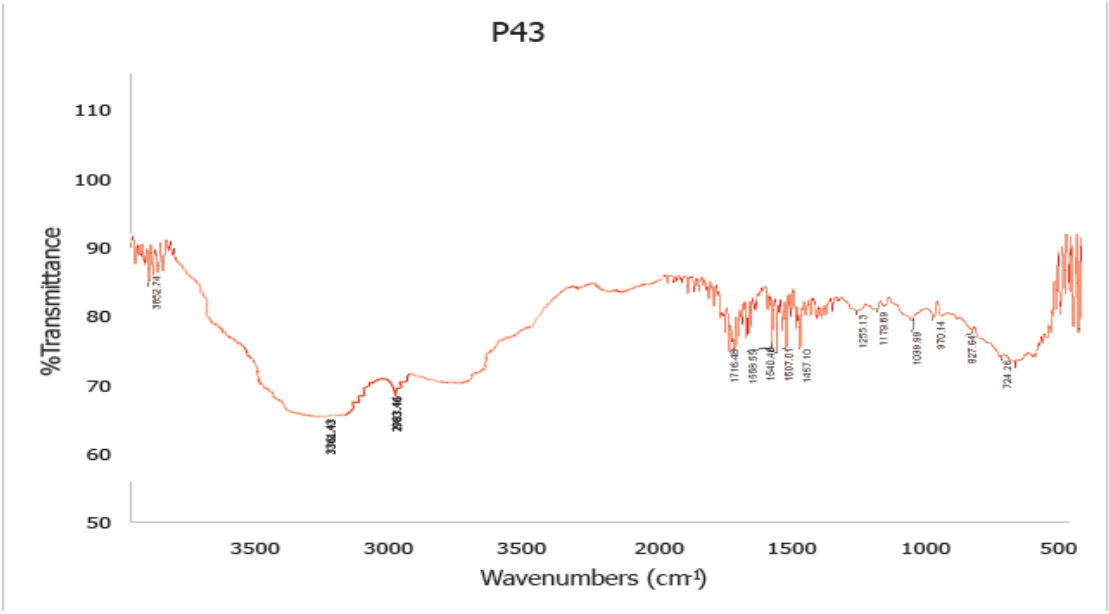


Fig. 7. FT-IR Spectrum of P43

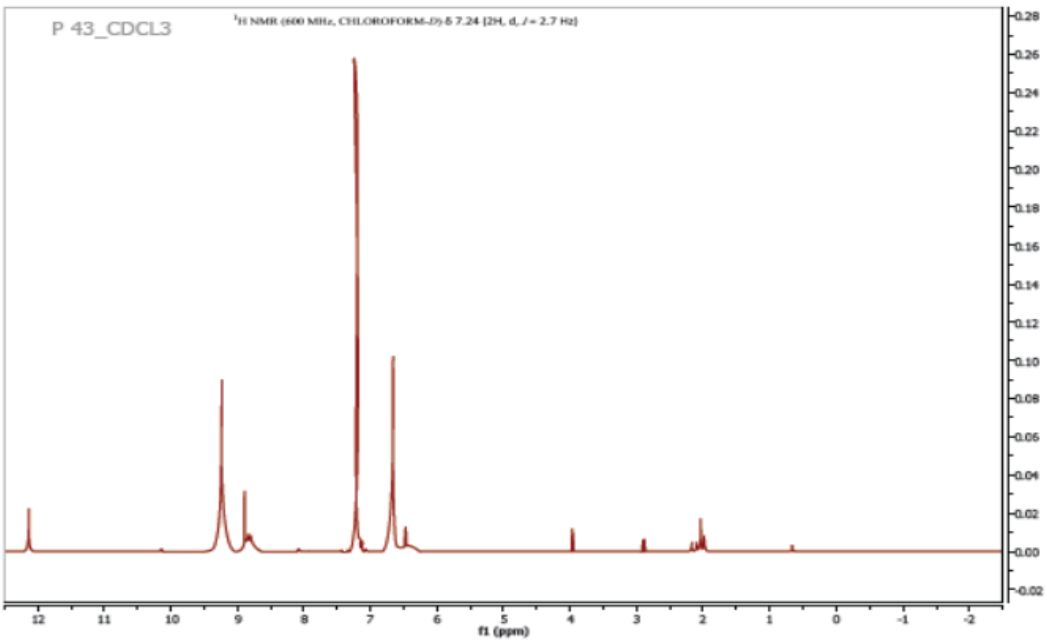


Fig. 8. ^{1}HNMR of P43

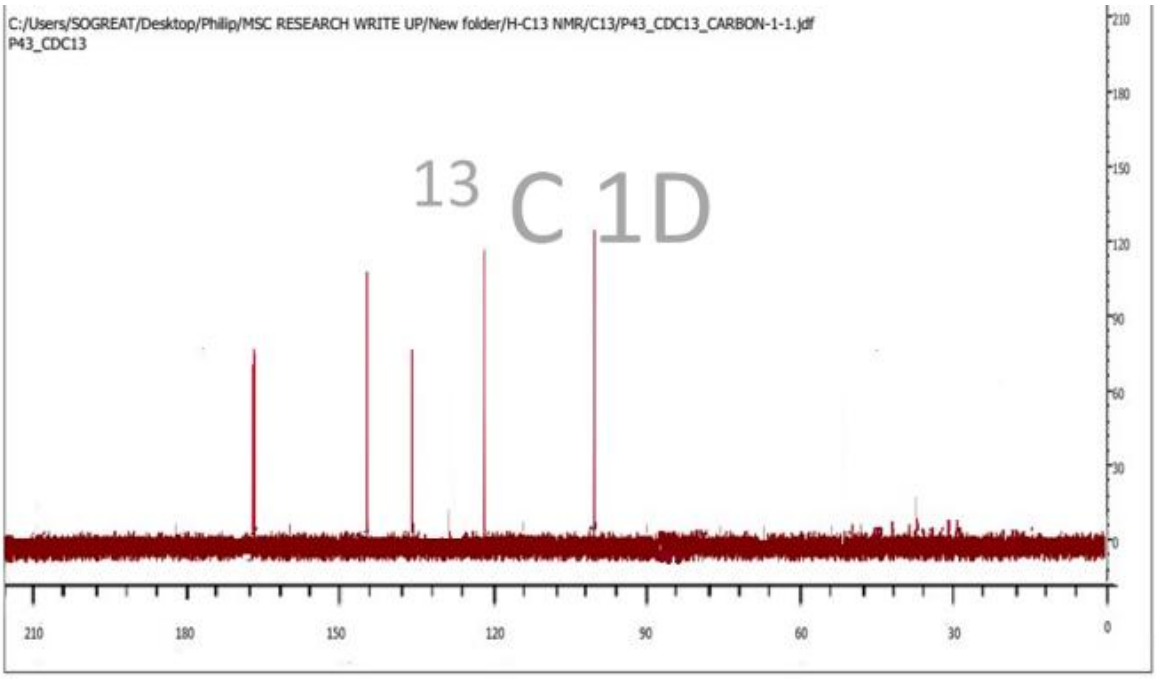
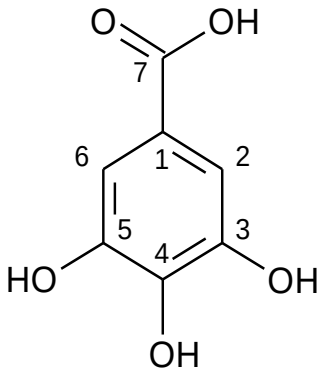


Fig. 9. ^{13}C NMR of P43

The ^{13}C-NMR (600 MHz, CHLOROFORM-D) spectrum of P43 showed peaks at δ 122.4 (1Cs), δ 111.3 (2Cs), δ 145.4 (2Cs), δ 137.9 (1Cs), δ 167.0 (1Cs)

The ^{13}C NMR spectrum (Fig. 9) complements the ^{1}H NMR analysis by providing direct information about the carbon environments within the compound. The signals at δ 111.3 (2C, s), δ 122.4 (1C, s), and δ 137.9 (1C, s) correspond to carbons in the aromatic region, supporting the presence of an aromatic ring structure typical for Gallic acid. The shift at δ 145.4 (2C, s) indicates equivalent carbons that are part of the same structural unit within the aromatic system. Finally, the signal at δ 167.0 (1C, s) corresponds to a carbonyl carbon (C=O), which is indicative of the carboxylic acid functionality present in Gallic acid. These findings correlates with previous reports [29,35,36].



3, 4, 5-trihydroxybenzoic acid (Gallic acid)

3.3.3 FT-IR Spectrum of P17

The FT-IR Spectrum (Fig. 10; Table 5) analysis of compound P17 identified several key peaks indicative of functional groups. The broad absorption band at 3397.3 cm^{-1} corresponds to the hydroxyl group (-OH). The broad peak suggests the presence of bonded hydroxyl groups. The peak at 2968.3 cm^{-1} relates to C-H stretching vibrations. A strong peak at 1652.3 cm^{-1} is characteristic of the carbonyl (C=O) group, prevalent in ketones, aldehydes, and carboxylic acids serving as a definitive marker for these compounds. Additionally, the peak at 1521.3 cm^{-1} corresponds to C=C stretching vibrations in aromatic compounds. These absorption frequencies are consistent with those reported for kaempferol-7-O-glucoside in previous studies by Ibitoye et al. [37], Adhikari-Devkota et al. [38], Nguyen and Dinh, [39], Yang et al. [40] and Mukhtar et al. [41].

3.3.4 NMR analysis of P17

The ^{1}H NMR spectrum (Fig. 11) revealed chemical shifts that are indicative of the types of hydrogen atoms present in a molecule. The shift at δ 12.11 indicates a hydroxyl group (–OH), likely involved in hydrogen bonding, which causes deshielding of the proton. The shift at δ

8.66 corresponds to protons on an aromatic ring. Additionally, the shifts at δ 6.97, δ 6.44, and δ 6.31 are also aromatic protons. The shifts at δ 4.31 and δ 3.82 indicate protons on carbon atoms bonded to oxygen, with δ 4.31 likely corresponding to protons in a glucoside moiety, while δ 3.82 reflects protons adjacent to oxygen within a sugar structure. Collectively, these chemical shifts illustrate the functional groups present in kaempferol-7-O-glucoside. This assignments are consistent with previous studies [42,43,44,45].

From ^{13}C NMR chemical shifts (Fig. 12; Table 6), the observed range from approximately δ 60.1 to δ 176.8 ppm, reflect a variety of carbon types within the molecule. The peak at δ 96.3 ppm is indicates an anomeric carbon position or a sp² hybridized carbon. The peaks at δ 101.7 and δ 105.6 ppm further suggest additional sp² hybridized carbons commonly found in aromatic structures. As the spectrum progresses, signals at δ 115.5 and δ 124.0 ppm represent two equivalent carbons in the aromatic ring, while those at δ 128.5 and δ 137.1 ppm further indicate aromatic carbons. Notably, the peaks between δ 145.2 and δ 163.3 ppm correspond to highly deshielded carbons typical for substituted aromatic rings. The peak at δ 176.8 ppm is particularly significant as it represents a carbonyl carbon, which is strongly deshielded due to its sp² hybridization and the presence of a double bond to oxygen. Additionally, the spectrum features aliphatic carbons with shifts at δ 60.1, δ 69.1, δ 72.9, δ76.0 and δ78.4 ppm which are part of the glucosyl moiety attached to the kaempferol backbone.

Both ^{1}H NMR and ^{13}C NMR spectra, IR data and literature comparison aided the structure identification of kaempferol-7-O-glucoside through their respective chemical shifts. The above findings consistent with that of Wan et al. [42], Pereira et al. [43], Behbahani et al. [44] and Benjamin et al. [45].

Table 4. ^{1}H & ^{13}CNMR data of P43 (Gallic acid) compared with other literatures

Chemical Shift (δ)	Experiment (P43)	Shen et al. [29]
H (3,5)	δ 8.94	δ 8.84
OH (2,6)	δ 6.91	δ 6.92
OH (4)	δ 9.20	δ 9.20
OH (7)	δ 12.19	δ 12.24
C-1	δ 122.4	δ 120.9
C-2,6	δ 111.3	δ 109.2
C-3,5	δ 145.4	δ 145.9
C-4	δ 137.9	δ 138.4
C-7	δ 167.0	δ 167.9

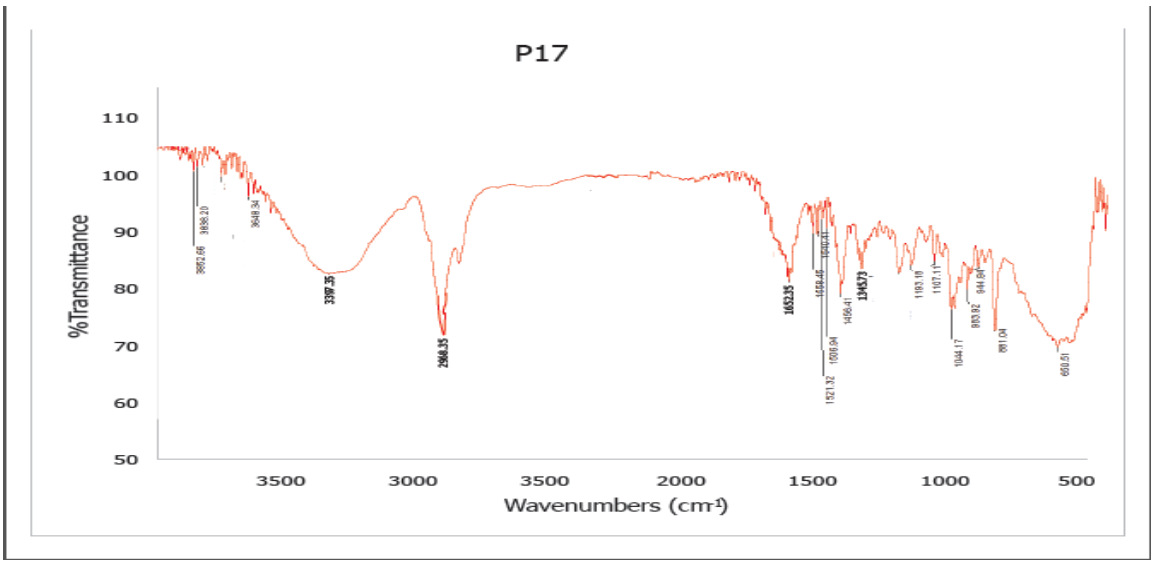


Fig. 10. FT-IR Spectrum of P17

Table 5. FT-IR Spectra of P17

Frequency (cm^{-1})	Functional Group	Type of Vibration
3397.3	Hydroxyl (-OH)	O-H stretching (broad)
2968.3	C-H (sp^3-hybridized carbons)	C-H stretching
1652.3	Carbonyl (C=O)	C=O stretching
1521.3	Aromatic C=C	C=C stretching (aromatic)

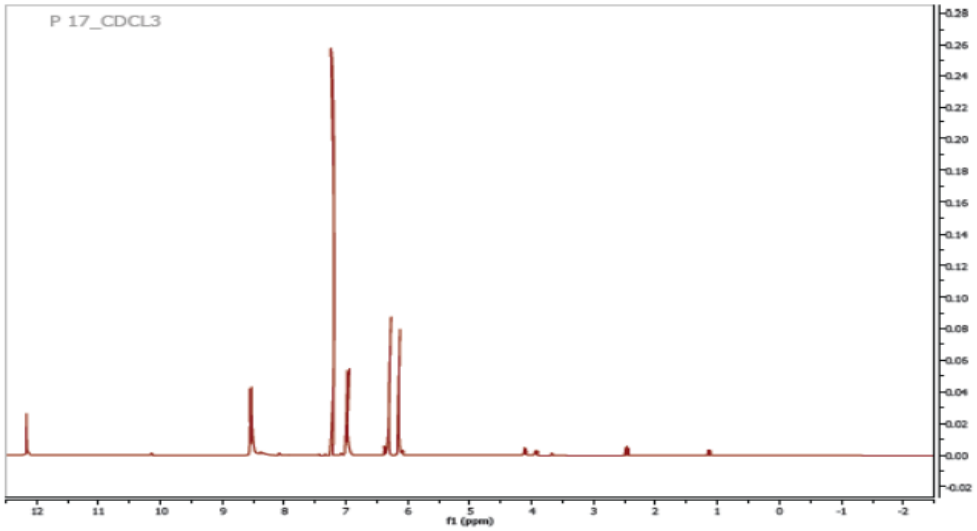


Fig. 11. ^{1}H NMR Spectrum of P17

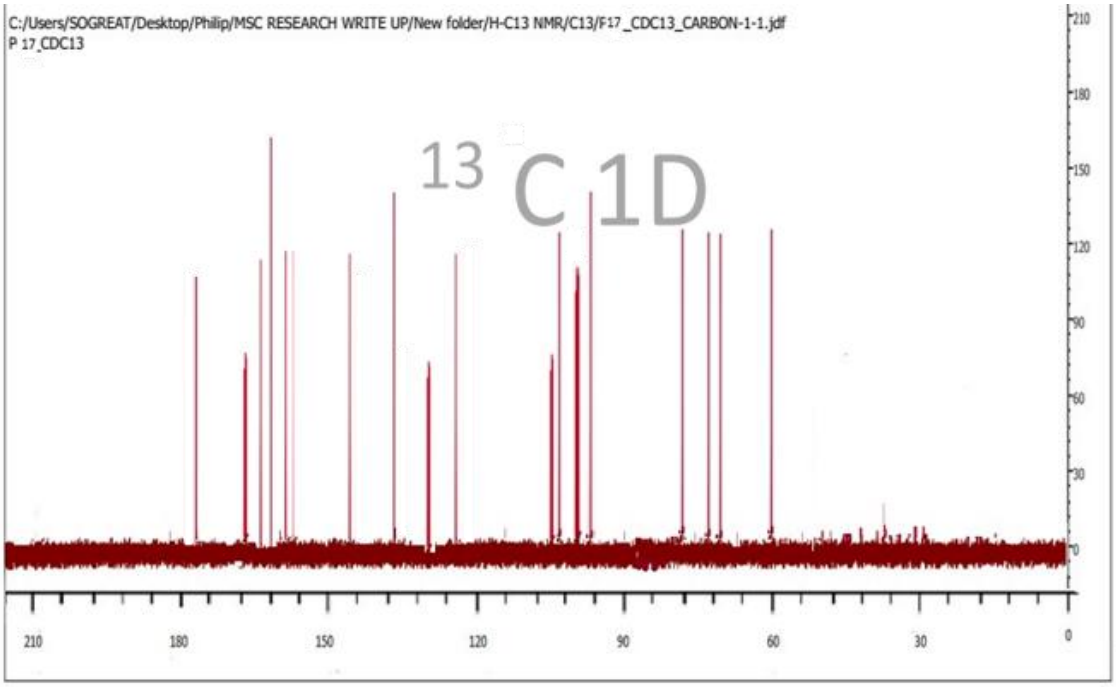


Fig. 12. ^{13}C NMR Spectrum of P17

The ^{13}C-NMR (600 MHz, CHLOROFORM-D) spectrum of P17 showed peaks at176.8 (1Cs), 96.3 (1Cs), 101.7 (2Cs), 105.6 (1Cs), 115.5 (2Cs), 124.0 (1Cs), 128.5 (2Cs), 137.1 (1Cs), 145.2 (1Cs), 157.2 (1Cs), 157.4 (1Cs), 161.0 (1Cs), 163.3 (1Cs), 60.1(1Cs), 69.1(1Cs'), 76.0 (1Cs), 78.4 (1Cs), 72.9 (1Cs)

Table 6. ^{1}H & $^{13}CNMR$ data of P17 (Kaempferol-7-O-glucoside) with literatures

Atom Type	Experiment P17	Benjamin et al. (2022)
C5-OH	δ 12.11	δ 12.22
C6-H	δ 6.97	δ 7.53
C8-H	δ 6.44	δ 5.54
C2' & C6'-H	δ 8.66	δ 7.69
C3' & C5'-H	δ 6.31	δ 6.66
Pyranose-OH	δ 4.31	δ 4.99
Pyranose-H	δ 3.82	δ 4.39
C4	176.8	176.17
C8	96.3	93.56
C6, C1''	101.7	100.92
C10	105.6	105.08
C3', C5'	115.5	115.51
C1'	124.0	122.00
C6', C2'	128.5	129.62
C3	137.1	135.56
C9	145.2	145.33
C2	157.2	159.25
C4'	157.4	
C5	161.0	161.34
C7	163.3	
C6''	60.1	56.00
C4''	69.1	69.66
C3''	76.0	76.21
C5''	78.4	77.24
C2''	72.9	75.78

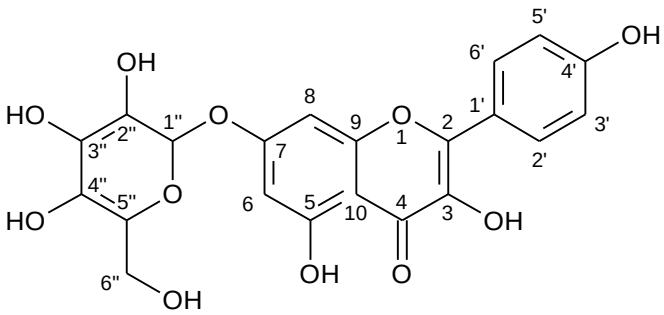


Fig. 13. Kaempferol-7-O-glucoside

To the best of our knowledge, the flavonoid compound kaempferol-7-O-glucoside is isolated from the ethyl acetate fraction of the stem bark of *Entada africana* for the first time, although it has previously been found in other plants. This flavonoid (kaempferol-7-O-glucoside) has been reported to have significant antibacterial and antioxidant activities [46].

4. CONCLUSION

The investigation of *Entada africana* stembark ethylacetate crude extract revealed moderate antibacterial activity and strong antioxidant properties. The successful isolation and characterization of gallic acid and kaempferol-7-O-glucoside indicates the presence of bioactive compounds in the stem bark extract of *Entada africana*. Gallic acid and Kaempferol-7-O-glucoside are well known for their antibacterial, antioxidant and anticancer activities. The study therefore validates the use of *Entada africana* in traditional medicine and may serve as a potential source of therapeutic agents.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image

generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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