International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

https://ijpsat.org/

Vol. 57 No. 2 June 2026, pp. 63-82

Phytochemical Profiling, Antioxidant And Antimicrobial Potentials Of Ethanol And Ethyl Acetate Extracts Of Kigelia africana Stem Bark

[1]Adepoju A. J., [*2]Omodara N.B., [1]Esan A. O., [3]Olawoore I. T., [1]Adeoye A. O; [1]Obisesan K. M., [1]Haastrup B. S., [1]Abegunrin A. I., [1]Ayodele E.T., [1]Ishola K.T.

[1]Department of Pure and Applied Chemistry Ladoke Akintola University of Technology Ogbomosho, Nigeria
[2]Department of Chemistry, Adeyemi Federal University of Education, Ondo, Nigeria
[3]Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.

*Corresponding Authors: omodaranb@afued.edu.ng

Abstract: The research examines the phytochemical composition, antioxidant potential, and antibacterial properties of ethyl acetate (Kafr-EtOAc) and ethanolic (Kafr-EtOH) extracts from *Kigelia africana* (Kafr) that was harvested in Ogbomosho, Oyo state, Nigeria. Compared to Kafr-EtOAc, the Kafr-EtOH extract showed greater levels of total phenolic (33.67±0.17 GAE/mg/g), tannins (12.18±0.04 GAE/mg/g), flavonoids (27.89±0.05 QE/mg/g), alkaloids (7.22±0.09 %), and saponins (2.34±0.06 %). Important phenolic and sterol components were detected by GC-MS, such as 2, 3-dihydro-benzofuran (31.41 %), alpha-tocopherol (8.22 %), 2,6-hydroxycholesterol (18.68 %), and eugenol (5.13 %). The presence of distinctive functional groups was validated by FT-IR analysis. Strong DPPH scavenging capabilities were found in antioxidant studies; Kafr-EtOH exhibited the best findings (IC_{50} = 1.88 mg/mL). Furthermore, Kafr-EtOH demonstrated significant antibacterial effectiveness against the fungus *Candida albicans* as well as Gram-negative and Gram-positive bacteria. These results highlight the potential of ethanol extraction for pharmacological and nutraceutical uses by suggesting that it is a more effective method of separating bioactive components from Kafr.

Keywords: *Kigelia africana*; FT-IR; antioxidant activity; antimicrobials; GC-MS analysis

INTRODUCTION

Growing worries about the negative effects of synthetic medications and rising antibiotic resistance have accelerated the quest for natural bioactive compounds in recent years (Vaou et al., 2021; Muteeb et al., 2023; Chaachouay & Zidane, 2024). With their abundance of bioactive compounds, medicinal plants have long been a mainstay of conventional medicine. Today, they are being thoroughly studied for possible uses in contemporary pharmaceuticals, nutraceuticals, and dentistry, among other areas (Rathor, 2021; Dar et al., 2023). *Kigelia africana* (Kafr) (Fig.1), also called the sausage tree, is a member of the Bignoniaceae family. This plant family, which contains over 800 species of trees, shrubs, and vines, is also referred to as the bignonias or the trumpet creeper (Bello et al., 2016). The African continent, which stretches from Sudan and Ethiopia in the north to South Africa in the south, is home to the unusual and intriguing Kafr tree species (Nabatanzi et al., 2020). It is a deciduous tree with a maximum height of 30 meters (Ali and Patnaik, 2019).

Its huge, pendulous fruit, which can reach up to 60 centimeters in length and 30 cm in diameter, is the tree's most remarkable feature. When unripe, the fruit is green; as it ripens, it turns yellow or orange. The fruit's pulp is fibrous and has a large number of

black seeds, while its skin is thick and leathery (Assanti et al., 2022). For decades, traditional African medicine has utilized Kafr, sometimes referred to as the sausage tree, to treat a variety of illnesses (Assanti et al., 2022). Remedies for various ailments are made from the plant's bark, leaves, fruits, and roots (Nabatanzi et al., 2020). Skin disorders like dermatitis, acne, and eczema are treated with the bark and leaves (Fakudze et al., 2023). In order to promote healing and avoid infection, the stem bark is also used to treat cuts, wounds, and injuries (Karatay et al., 2023). Fever and malaria are treated with the root bark (Assanti et al., 2022). The bark and leaves are used to treat respiratory and digestive disorders (Nabatanzi et al., 2020). Breast, cervical, and prostate cancers are among the cancers that are treated in traditional medicine (Ahrens et al., 2022).

The extraction techniques and solvents employed have a significant impact on how effective plant-based extracts are. In order to isolate bioactive chemicals from plant sources, solvent extraction is a crucial step (Stephane et al., 2021). The yield and content of the extracted chemicals can be greatly impacted by the solvent selection (Gil-Martín et al., 2021; Bitwell et al., 2023). Because they can dissolve a variety of bioactive compounds, ethanol and ethyl acetate are frequently employed solvents in phytochemical research (Nawaz et al., 2020; Gil-Martín et al., 2021; Akullo et al., 2023; Kozhantayeva et al., 2024). Ethanol and ethyl acetate were chosen because of their distinct polarity, which enable thorough extraction of both polar and non-polar substances. Because of its polarity and capacity to effectively permeate plant tissues, ethanol is especially useful for extracting phenolic chemicals. Meanwhile, ethyl acetate targets less polar components, guaranteeing a varied phytochemical profile from Kafr (Kumar et al., 2023; Kozhantayeva et al., 2024).

Given this plant species' pharmacological potential, it is essential to look into Kafr biological activities and phytochemical makeup in order to study its possible uses in contemporary medicine. This study aims to bridge the knowledge gap by evaluating the phytochemical profile, antioxidant capability, and antibacterial qualities of ethanol and ethyl acetate extracts from Kafr collected in southwest Nigeria. Gas Chromatography-Mass Spectometry, Fourier-transform infrared spectroscopy (FT-IR), and a variety of spectrophotometric assays are used in this work to provide a thorough analysis of the chemical compositions and bioactivities of the extracts.

International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

https://ijpsat.org/
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Figure 1. Wild Kafr various part (leaves, flower, stem and fruit respectively)

MATERIALS AND METHODS

Reagents and Chemicals

The following reagents were used: Mayer's reagent, Wagner's reagent, chloroform, anhydrous acetic acid, sulfuric acid, hydrochloric acid, sodium hydroxide, iron (III) chloride, ascorbic acid (98%), Iron chloride (97%), potassium ferricyanide (99%), sodium carbonate (99.5%), aluminum chloride anhydrous (99.99%), and 2, 2-diphenyl-1-picrylidrazyl (98%). All of the chemicals were supplied by Sigma Aldrich, which is based in Burlington, Massachusetts, USA. Methanol, ethanol, n-hexane, ethyl acetate, and dimethyl sulfoxide are examples of analytical-grade organic solvents that were purchased from authorized local suppliers.

Plant Material

Kigelia africana stem bark (Figure 1) was gathered in September, 2023. It was recognized and identified by Professor A.T.J. Ogunkunle of the Department of Pure & Applied Biology, LAUTECH, Ogbomoso, Oyo state, Nigeria. Following collection, distilled water was used to carefully clean and wash the plant material. A shaded, well-ventilated place was used to air-dry it until it was entirely dehydrated. A 110 V, 1400 rpm/min electric microplant grinding machine was then used to grind it into a fine powder. Prior to being prepared for analysis, all samples were stored at -20°C.

Extraction

A sequential maceration approach was used to extract five hundred and fifty grams of dried plant material. Before soaking in 2.5 L of ethanol and ethyl acetate solvents to take out any polar extract, the sample was soaked in 2.5 L of n-hexane to remove any non-polar components from the plant material. Each extraction procedure lasted for seventy-two hours. Following the collection of the filtrates from this procedure using Whatman filter papers, the solvents were eliminated by evaporating them at 40 °C under reduced pressure using a rotary evaporator (IKA RV10 auto V-C, Staufen, Germany). Finally, the semisolid product became a solidified mass when exposed to ambient air. The extract contents (W, %) were quantified using equation (1), which displays the percentage ratio of the final dry mass of extracts (M_1) to the initial dry mass of the extracted plant material (M_2).

$$W\ (\%) = \frac{M1}{M2} \times 100$$

Phytochemical Analysis

Determination of total alkaloid contents

The Van Tans (2018) method was used to determine the alkaloid content. In a 250 mL beaker, 200 mL of 10% acetic acid in ethanol was mixed with a 5 g sample. After four hours of filtering, the extract was concentrated in a water bath to about a quarter of its original volume. Ammonium hydroxide concentrate was progressively added till precipitation happened. After that, the precipitate was filtered and treated with a diluted ammonium hydroxide solution. The end result was an alkaloid that had been dried and weighed.

Determination of total saponin contents

The same methods outlined by Adepoju et al. (2024) was adhered to. In a conical flask, a 20g sample of each was macerated with 100cm³ of 20% aqueous ethanol and heated using a hot water bath for four hours at 55°C. 200 milliliters of 20% ethanol were added to the mixture after it had been filtered in order to further eliminate any remaining residue. After reducing the combined extracts to 40 mL in a water bath set at 90°C, 20 mL of diethyl ether was added. Ten milliliters of 5% aqueous sodium chloride were used to separate and wash the aqueous layer twice. The amount of saponin was determined after heating the leftover solution to a constant weight.

Determination of total phenolic content

A technique for utilizing spectrophotometric analysis with the Folin-Ciocalteu test to ascertain the total phenolic content in plant extracts was presented by Siddiqui et al. (2017). In a 25 mL volumetric flask, 1 mL of plant extract, 1 mL of Folin-Ciocalteu reagent, and 9 mL of distilled water are combined to create a reaction mixture. After the reaction runs for five minutes, ten milliliters of a 7% sodium carbonate solution is added. Gallic acid standards (20–100 µg/mL) are prepared using the same procedure. Following a 90-minute incubation period at room temperature, the absorbance of the test and standard solutions is then measured at 550 nm using a UV/Visible spectrophotometer. Milligrams of gallic acid equivalent (GAE) per gram of extract is the unit of measurement for the total phenol concentration.

Determination of total tannin Content

The Folin-Ciocalteu method, developed by Adepoju et al. in 2024, was employed to ascertain the tannin concentration. A volumetric flask (10 mL) was filled with 0.1 mL of the sample extract, 7.5 mL of distilled water, 0.5 mL of the Folin-Ciocalteu reagent, and 1 mL of 35% Na_2CO_3. The combination remained for half an hour after stirring. Standard solutions of gallic acid (20–100 µg/mL) go through a similar procedure. The absorbance at 725 nm was measured using a UV/visible spectrophotometer. The tannin concentration of each gram of extract was expressed in milligrams of GAE.

Determination total flavonoid content

The aluminum chloride colorimetric method was used to quantify the total flavonoid concentration (Shraim et al., 2021). 1 mL of extract and 4 mL of distilled water were combined to create a solution in a 10 mL volumetric flask. Following a 5-minute reaction period, 0.3 mL of 10% aluminum chloride and 0.30 mL of previously treated 5% sodium nitrite were added. After another 5 minutes of reaction time, 2 mL of 1M sodium hydroxide was added, and distilled water was used to dilute the solution to 10 mL. For different standard quercetin solutions (20–100 µg/mL), this procedure was repeated. The absorbance of the test and standard solutions at 510 nm was then measured using a UV/Visible spectrophotometer, with the reagent blank serving as a reference.

Analysis by Gas Chromatography Mass Spectrometry (GC–MS)

Agilent Technologies' GC equipment was used to conduct a GC-MS study of the extract's bioactive components. In particular, an HP-5MS column (30 m length, 250 µm diameter, 0.25 µm film thickness) was utilized in conjunction with the GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA). In GC-MS spectroscopic detection, 70-eV electrons were employed as the electron ionization technique. The carrier in this study was 99.995% pure helium gas. The flow rate of carrier gas was 1 mL/min. The temperature was raised by 3 °C every minute after initially being set between 50 and 150 °C. For ten minutes, this temperature was maintained. To reach 300 °C, the temperature increased by 10 °C every minute. One microliter of the generated 1% extracts was splitlessly added to the system together with a suitable solvent that had been diluted. The chromatogram peak area was used to determine the percentage of each extract's chemical components.

FTIR Analysis

Utilizing the Buck Scientific M530 USA, FTIR analysis was performed. They employed a potassium bromide beam splitter and a deuterated triglycine sulphate detector. After thoroughly mixing a 1.0 g sample with 0.5 ml of nujol, it was placed on top of the salt pellet. For the measurement, Fourier Transform Infrared (FTIR) spectra were obtained between 4,000 and 500 cm-1.

Determination of DPPH Free Radical Scavenging Activity

The antioxidant and free radical scavenging capabilities of Kafr stem bark extracts were evaluated using varying extract concentrations in a methanolic solution of DPPH against the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), in compliance with Adepoju et al. (2024). Using vitamin C as a reference substance, the absorbance at 517 nm was measured using a spectrophotometer following a 30-minute incubation period at room temperature and in the dark. The % inhibition for each extract and standard concentration was calculated using the procedure below.

$$\% \text{ Inhibition} = \left(\frac{\text{Ab}}{\text{Ab}}\right) \text{ x } 100$$

Ab stands for the absorbance of the blank solution, whereas As denotes the absorbance of the extracts of Kafr leaves at various dosages. After plotting the dose-response curve, the IC50 values for the extracts and the standard were ascertained.

Antibacterial and Antifungal Activity

Methicillin-resistant *Staphylococcus aureus, Staphylococcus aureus, Escherichia coli, Asprigillus fumigates, Aspergillus flavus,* and *Aspergillus niger* were among the bacterial strains against which the extract's antimicrobial efficacy was assessed. The cultures of bacteria and fungi were adjusted to 0.5 McFarland's standard and 106 3pores per milliliter, respectively. Mueller Hinton Agar plates for bacteria and Saboraud Dextrose Agar plates for bacteria and isolates were used to inoculate the cultures (Adejumo et al., 2020). The MHA and SDA plates were drilled with an 8mm diameter well using a sterile cork borer. Extract dilutions of 25, 50, and 100 mg/mL were added to each well. The negative control was distilled MeOH (50°C). As a positive control, the middle wells contained 1% and 10 ug/ml of ketoconazol and ciprofloxacin. The antibacterial activity of the agar plates was assessed by measuring the diameter of the zones of inhibition during a 24-hour incubation period at 37 °C and 25 °C.

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RESULTS AND DISCUSSION

Extraction Yield Analysis

After the plant material was defatted using n-hexane, both ethanol and ethyl acetate solvents in a sequential maceration extraction process, significant amounts of extract from Kafr stem bark material was obtained. Figure 2 shows that the extraction yields for ethanol and ethyl acetate extracts were 13.15 g (2.38 % w/w) and 5.94 g (1.08 % w/w), respectively, based on the initial dry mass of the plant material. According to the findings, ethanol extraction yielded more extract than ethyl acetate extraction. A larger extraction yield may result from ethanol's ability to more effectively permeate plant cell walls and extract a range of polar bioactive substances, including flavonoids and phenolic acids (Plaskova & Mlcek, 2023). Since ethyl acetate is less polar, it extracts these compounds less effectively, which lowers the yield (Kozhantayeva et al., 2024).

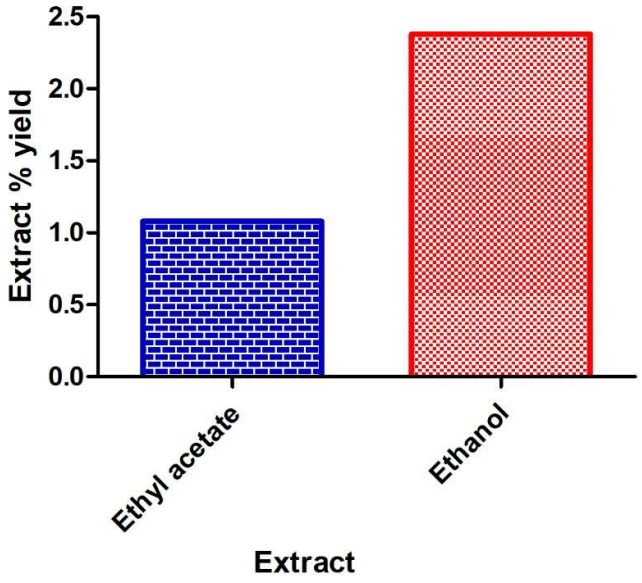


Figure 2: Percentage yield of crude extract of Kafr stem bark

Phytochemical Study

Total Phenolic, Alkaloid, Saponin, Tannin and Flavonoid Content

Quantifying the main bioactive components of Kafr, specifically the phenolic, alkaloid, saponin, tannin, and flavonoid, was the primary aim of this study. The plant was obtained in Ogbomosho, Oyo state, Nigeria. Standard spectrophotometric techniques were used to determine the TPC, TAC, TFC, TTC, and TSC values of Kafr-EtOAc and Kafr-EtOH extracts, as indicated in Table 1. According to the table's data, the ethanol extract of Kafr stem bark has higher amounts of phenols, tannins, flavonoids, alkaloids, and saponins than the ethyl acetate extract. Both the ethanol extract (33.67±0.17 GAE mg/g) and the ethyl acetate extract (27.51±0.19 GAE mg/g) contain phenols. Polyphenols and other phenolic compounds are important and common classes of plant metabolites. Because polyphenols are made up of thousands of different compounds, they are widely distributed. Numerous phenolics have been investigated for their potential as antioxidants and free radical scavengers due to their high levels of antioxidant activity. Additionally, they contribute to resistance to UV rays and infections (Kumar & Goel, 2019).

Flavonoids are abundant secondary metabolite in the stem bark of Kafr (19.87±0.01 QE/mg/g for the ethyl acetate extract and 27.89±0.05 QE/mg/g for the ethanol extract). Flavonoids have a wide range of biological activities, such as anti-inflammatory,

antibacterial, antiviral, anticancer, cytotoxic, and anti-allergic effects (Hasnat et al., 2024). They also decrease capillary permeability, fragility, lipid peroxidation, and platelet aggregation. They also have a vasodilatory impact. Their capacity to chelate divalent cations, function as antioxidants and free radical scavengers, and inhibit a variety of enzymes is responsible for these actions (Ullah et al., 2020). There are higher tannins in the ethanol extract (12.18±0.04 GAE/mg/g) than in the ethyl acetate extract (9.75±0.24 GAE/mg/g). Because of its astringent action and antibacterial qualities, tannins accelerate the healing of wounds and mucosal membranes. Additionally, they might have antiviral and antibacterial properties (Ahmad et al., 2015). The higher concentrations of the secondary metabolites under evaluation in the ethyl acetate extract are caused by the substance's ease of crossing cell membranes and extracting intracellular components from plant materials (Adepoju et al., 2024).

Another class of secondary metabolites, alkaloids, offer defense against long-term illnesses. By blocking DNA topoisomerase, for instance, alkaloids have been shown to have antibacterial properties (Khare et al., 2021; Othman et al., 2019). Kafr stem bark was discovered to contain the fourth most abundant amount of alkaloids, with 7.22±0.09 percent in the ethanol extract and 3.77±0.32 percent in the ethyl acetate extract. The ethanol extract contains more saponins (12.18±0.09%) than the ethyl acetate extract (9.75±0.09%). Saponins exhibit a wide range of biological functions, such as lytic activity, attraction for phospholipids in cell membranes, and the ability to form insoluble complexes with proteins and sterols, all of which are based on their unique chemical properties (Timilsena et al., 2023).

Table 1: Quantitative phytochemical composition of Kafr stem bark extract

Parameters	Kafr-EtOAc	Kafr-EtOH
% Saponins	1.19±0.11	2.34±0.06
% Alkaloid	3.77±0.32	7.22±0.09
Flavonoids (QE/mg/g)	19.87±0.01	27.89±0.05
Phenols (GAE/mg/g)	27.51±0.19	33.67±0.17
Tannins (GAE/mg/g)	9.75±0.24	12.18±0.04

Data expressed as mean ±SD (n=3). GAE and QE are gallic acid and quercetin equivalent respectively.

GC-MS Analysis

The constituents of the ethanol extract from Kafr stem bark was thoroughly revealed by the GC-MS analysis (Table 2), which is especially important for the therapeutic effects this study examined. Retention indices and a comparison with the Willey and NIST libraries included with the GC-MS system were used to identify compounds. Several bioactive compounds were identified through retention time and mass spectra comparison, revealing the presence of alkaloids, phenolic compounds, saponins, and sterols in the extract. Among the most abundant compounds found were glucosides (2.49%), 3,4,5-trimethoxyphenol (3.05%), 3-fluoro-o-xylene (3.14%), coumarin (3.58%), eugenol (5.13%), alpha-tocopherol (8.22%), 2-methoxy-4-vinylphenol (14.29%), 26-hydroxycholesterol (18.68%), and benzofuran, 2,3-dihydro- (31.41%). These compounds are known for their significant pharmacological properties (Yao et al., 2022). Figure 3 show secondary metabolites found in Kafr extracts and their chemical structures.

The alkaloids identified, such as 3-methylpyridazine and 2,3,6-trimethylhept-6-en-1-ol, indicate the presence of bioactive nitrogen-containing compounds, which are often associated with medicinal activities like anti-inflammatory and analgesic effects (Heinrich et al., 2021). Benzofuran, 2,3-dihydro-, the major phenolic compound detected, is renowned for its strong antioxidant and antimicrobial properties, making it an important compound in therapeutic applications (Miao et al., 2019). Additionally, other phenolic compounds such as 2-methoxy-4-vinylphenol and eugenol were found to possess notable medicinal value, particularly for their antioxidant and antimicrobial activities (Rubab et al., 2020). Eugenol is widely recognized for its use in traditional

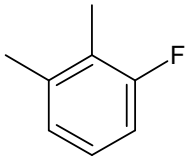
medicine and its strong antifungal and antibacterial properties, further emphasizing the therapeutic potential of the extract (Jeong & Jeong, 2010; Rubab et al., 2020). Alpha-tocopherol, also known as Vitamin E, enhances the antioxidant capacity of the extract, contributing to cell protection and skin health (Rizvi et al., 2014).

The presence of 2,6-hydroxycholesterol, a sterol known for its role in regulating cholesterol metabolism, further strengthens the extract's potential health benefits, particularly for cardiovascular health (Griffiths & Wang, 2021; Kopylov et al., 2021). The combination of these bioactive compounds, along with other minor constituents, demonstrates the extract diverse therapeutic applications. This analysis not only sheds light on the antioxidant and antimicrobial properties of Kafr but also highlights the potential synergistic effects of its various compounds. The findings provide valuable information that reinforces the medicinal relevance of the extract and suggests further investigation into its broader therapeutic applications in treating various health conditions.

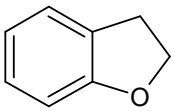
Table 2. GC-MS analysis of the components in the ethanol extracts from Kafr stem bark

Peak No.	Retention Time Rt (min)	$[M+H]^-$ (m/z)	Identified Metabolites	Class	Molecular Formula	Quantity Kafr-EtOH
1	3.407	94	3-methylpyridazine	Alkaloids	$C_5H_6N_2$	0.83
2	3.551	43	Tricyclo[3.3.0.0(2,8)]octan-3-one, 7-hydroxy-4-methyl-4-(propan-2-on-1-yl)-	Phenolic	$C_{12}H_{16}O_3$	0.34
3	4.186	67	7-Pentadecyne	-	$C_{15}H_{28}$	0.25
4	4.741	109	3-Fluoro-o-xylene	-	C_8H_9F	3.14
5	5.834	60	Octanoic acid	-	$C_8H_{16}O_2$	0.58
6	6.005	70	2,3,6-Trimethylhept-6-en-1-ol	Alkaloids	$C_{10}H_{20}O$	0.65
7	6.360	41	Isopulegol	Phenolic	$C_{10}H_{18}O$	0.30
8	6.434	120	Benzofuran, 2,3-dihydro-	Phenolic	C_8H_8O	31.41
9	7.476	135	2-Methoxy-4-vinylphenol	Phenolic	C_8H_9F	14.29
10	7.939	164	Eugenol	Phenolic	$C_{10}H_{12}O_2$	5.13
11	8.208	135	4-Hydroxy-2-methylacetophenone	Phenolic	$C_9H_{10}O_2$	0.24
12	8.346	88	Heptanoic acid, ethyl ester	Alkaloids	$C_9H_{18}O_2$	1.08
13	8.409	57	Dodecyl nonyl ether	Saponins	$C_{21}H_{44}O$	0.25
14	8.729	93	1-Methylene-2-vinylcyclopentane	Alkaloids	C_8H_{12}	0.99
15	8.929	43	7-Oxabicyclo [4.1.0] heptane, 1-methyl-4-(2-methyloxiranyl)-	Phenolic	$C_{10}H_{16}O_2$	1.58
16	8.981	146	Coumarin	Phenolic	$C_9H_6O_2$	3.58

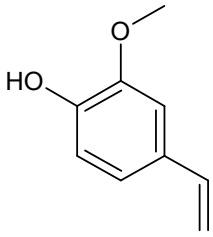
17	9.553	60	Glucosides	Glycosides	$C_6H_{10}O_5$	2.49
18	9.673	164	3-Allyl-6-methoxyphenol	Phenolic	$C_{10}H_{12}O_2$	1.59
19	10.428	137	Alpha-Tocopherol	Phenolic	$C_{29}H_{50}O_2$	1.33
20	10.634	169	3,4,5-Trimethoxyphenol	Phenolic	$C_9H_{12}O_4$	3.05
21	12.694	137	Alpha-Tocopherol	Phenolic	$C_{29}H_{50}O_2$	8.22
22	15.561	55	26-Hydroxycholesterol	Sterols	$C_{27}H_{46}O_2$	18.68



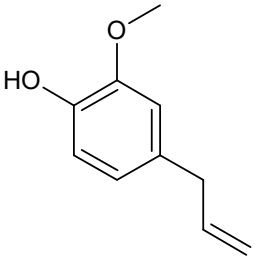
3-Fluoro-o-xylene



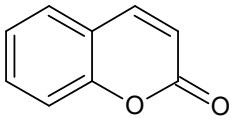
2,3-Dihydrobenzofuran



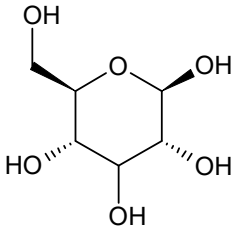
2-Methoxy-4-vinylphenol



Eugenol



Coumarin



Glucosides

International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

https://ijpsat.org/
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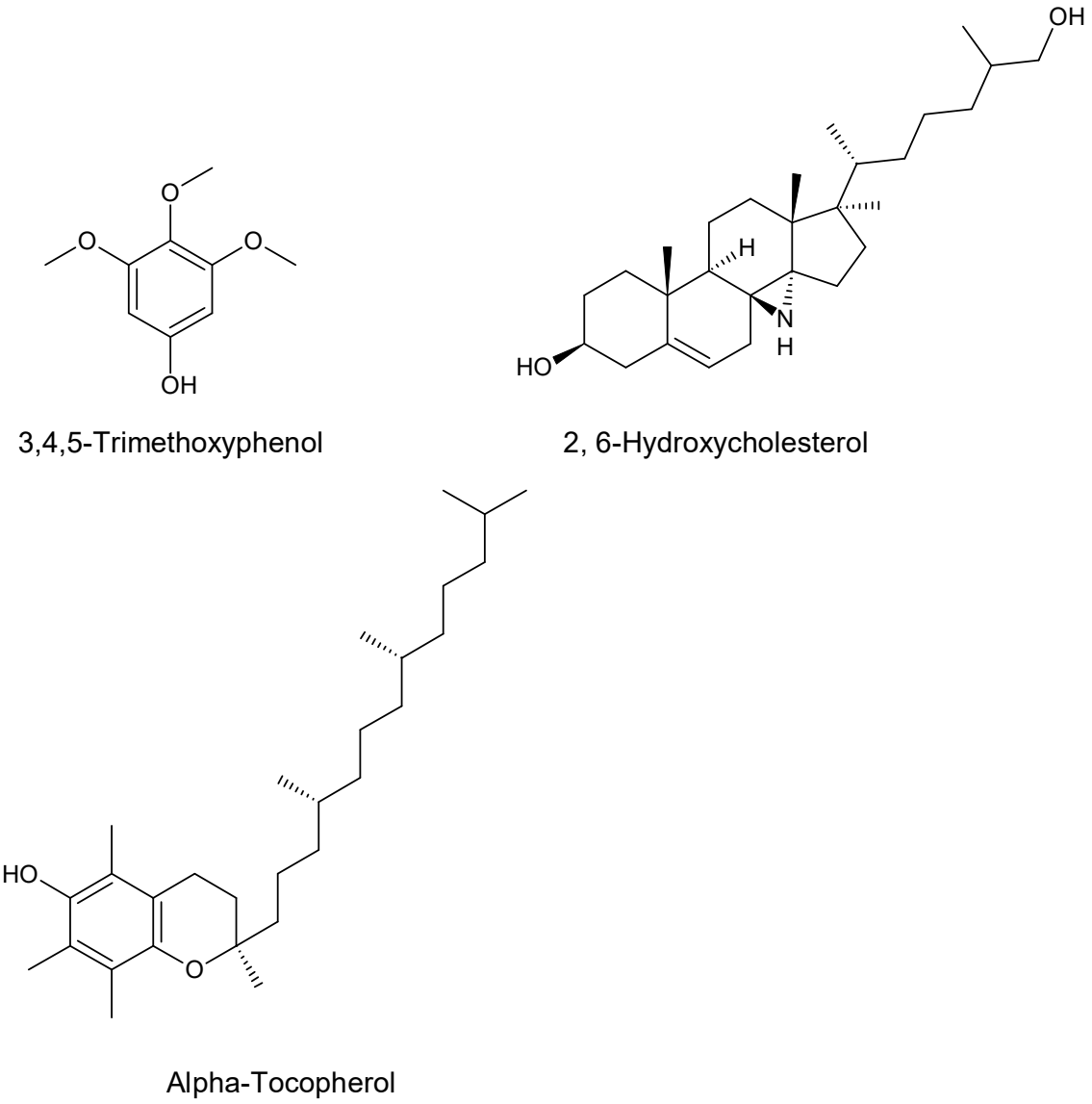

Figure 3: Secondary metabolites found in Kafr extracts and their chemical structures.

FT-IR Analysis

The FT-IR technique, which is commonly used to elucidate the unique fingerprints of functional groups in secondary metabolites present in plant extracts, was used to study Kafr extracts in order to gain a better understanding of the compound profile (Kozhantayeva et al., 2024; Wongsa et al., 2022; Nkogo et al., 2024). The FT-IR spectra of Kafr crude extracts obtained with ethanol and ethyl acetate solvents are shown in Figure 4a-b. These spectra show discrete signals at particular wavenumbers that correspond to individual molecules.

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The Kafr-EtOH and Kafr-EtOAc extracts' FT-IR study results. The FT-IR spectra of Kafr extracts have been recorded using ethanol and ethyl acetate solvents, and they display a range of functional groups, each having unique peak values. The prominent, broad, and well-defined peaks seen at 3384.89 cm^{-1} and 3326.40 cm^{-1} in the two extracts suggest the presence of stretching vibrations associated with O-H bonds, which are commonly found in the hydroxyl groups of alcohols and phenols (Oliveira et al., 2016). Additionally, the double bonds at 2921.92/2853.20 cm^{-1} and 2977.00/2930.42 cm^{-1} are caused by C-H stretching vibrations in aliphatic hydrocarbons, which are suggestive of methyl and methylene groups (Al-Marri et al., 2023). Either the presence of O-H functional groups from flavonoid glycosides and phenolic compounds or the attachment of alkyl and aromatic rings to the C-H stretching functional group are demonstrated by these peaks.

Additionally, bands at roughly 1740.91 cm^{-1} in the Kafr-EtOAc and 1693.40 cm^{-1} in the Kafr-EtOH correspond to the carbonyl group's stretching vibration (C=O), this can indicate the existence of carboxyl groups in phenolic compounds and keto groups in flavonoids (Semenescu et al., 2023). The C=O stretching vibrations of conjugated carboxylic acids are associated with the Kafr-EtOAc signal at 1689.73 cm^{-1}. Moreover, a band observed in the Kafr-EtOH at 1631.37 cm^{-1} might be caused by the stretching vibration of the C=C groups of conjugated alkene systems found in phenolic acids. The peaks at 1637.70/1602.20 cm^{-1} and 1604.10/1517.42 cm^{-1} that might be C=C stretching vibrations validate the presence of the aromatic ring system in both samples (Hosseini et al., 2016).

Additionally, compounds with alcohol and carboxylic acid groups have O-H bending vibrations, as indicated by the bands at 1459.13 cm^{-1} and 1374.69/1373.36 cm^{-1}. Moreover, the stretching of phenolic C-O bonds observed at about 1200 cm^{-1} was caused by the C–O bonds of pyran, which are characteristic of flavonoid C-rings. These frequency groups are closely associated with the presence of aromatic compounds. According to Kozhantayeva et al. (2024), residual peaks may be linked to additional C=C bending bonds of alkene and C-H bending bonds of alkane, as well as several aromatic out-of-plane C–H (670–900 cm^{-1}) and in-plane (950–1225 cm^{-1}) bending bonds.

Remarkably, both extracts displayed a broad, high peak at around 1040.04 cm^{-1}, which may be associated with functional groups of sulfoxides. This implies the existence of compounds with distinct molecular structures that call for more investigation and identification. These findings are consistent with the flavonoids and phenolic compounds found in both extracts. The presence of these chemicals in the extract's functional group is confirmed by the peaks seen in the FT-IR spectra, which support their distinctive vibrations.

International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

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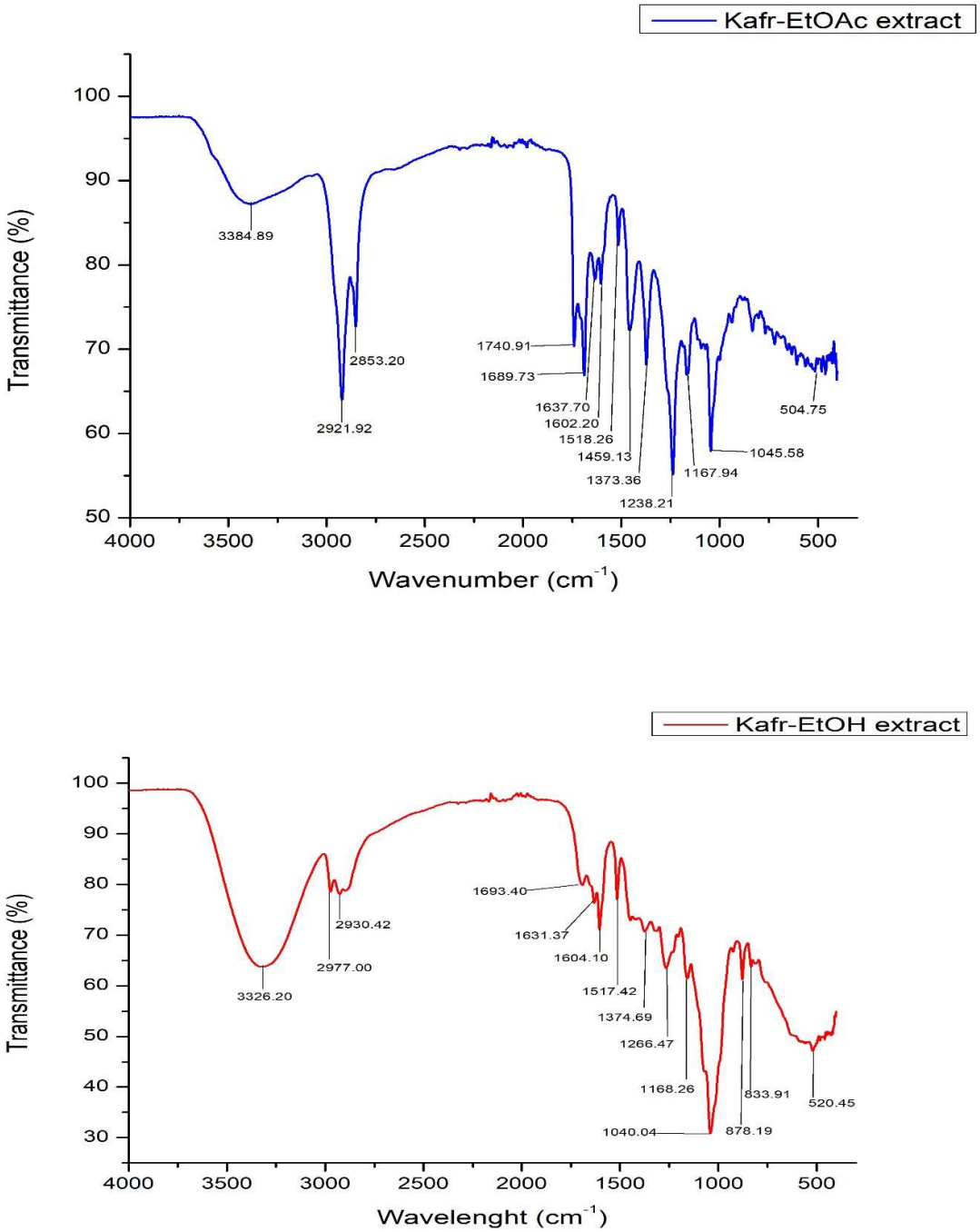

Figure 4. FT-IR spectra of (a) ethyl acetate and (b) ethanol extracts of Kafr.

International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

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Antioxidant Capacity

The DPPH scavenging activity assay was one of the in vitro methods used to evaluate the antioxidant capacity of extracts from the stem bark of Kafr. By using these methods, the plant extract's antioxidant techniques were intended to be detected. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) test is the most widely used technique to assess the antioxidant activity of plant extracts, specific secondary metabolites, and other medicinal compounds. The DPPH assay relies on the electron contribution from antioxidants to neutralize the DPPH radical. Both of the Kafr extracts showed notable radical scavenging efficacy in the DPPH experiment, as Figure 5a illustrates. As illustrated in Figure 5b, the Kafr-EtOH significantly outperformed the Kafr-EtOAc in terms of DPPH scavenging activity (IC_{50} = 1.88 mg/mL), with an IC_{50} value of 2.65 mg/mL, which was comparable to that of the positive control, ascorbic acid (IC_{50} = 1.00 mg/mL).

This implies that the extract from Kafr-EtOH has potent radical scavenging properties. No previous research has assessed the antioxidant activity of Kafr extracts, specifically in the Ogbomosho population, as far as we are aware. Crucially, our results emphasize how important it is to understand the intricate antioxidant pathways that plant extracts exhibit. The higher total phenolic content and total flavonoid content values of the Kafr-EtOH extract are correlated with its stronger antioxidant activity. These results are further supported by the thorough chemical analysis performed with GC-MS. In comparison to the Kafr-EtOAc extract, the Kafr-EtOH extract had greater quantities of a number of phenolic and flavonoid compounds, including alpha-tocopherol, 2, 6-hydroxycholesterol, eugenol, and 2, 3-dihydro-benzofuran.

The stronger antioxidant capacity seen in the Kafr-EtOH extract is probably due to these chemicals' well-established strong antioxidant properties (Orlo et al., 2023; Gülçin, 2011; Selamat et al., 2018). Further investigation into the synergistic effects of endogenous phenolic components is necessary, as they may cooperate to increase the extract's overall antioxidant activity.

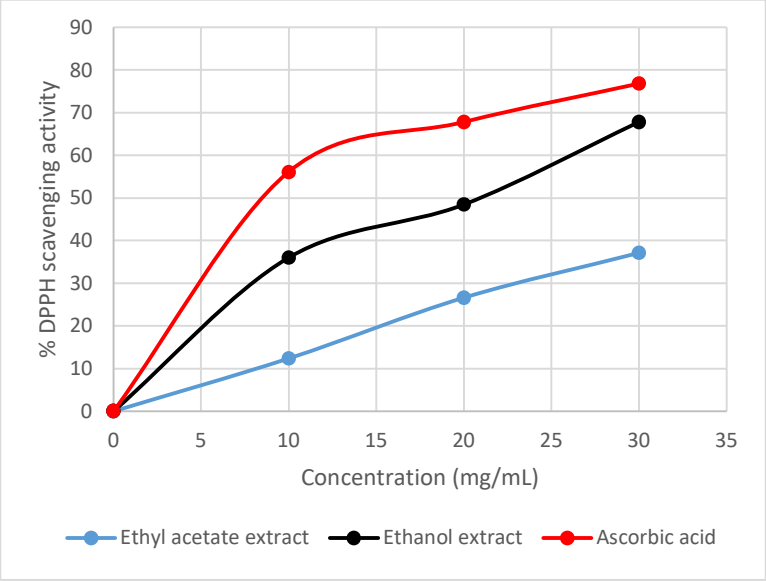

Figure 5a: Determination of (a) DPPH radical scavenging activity

International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

https://ijpsat.org/
Vol. 57 No. 2 June 2026, pp. 63-82

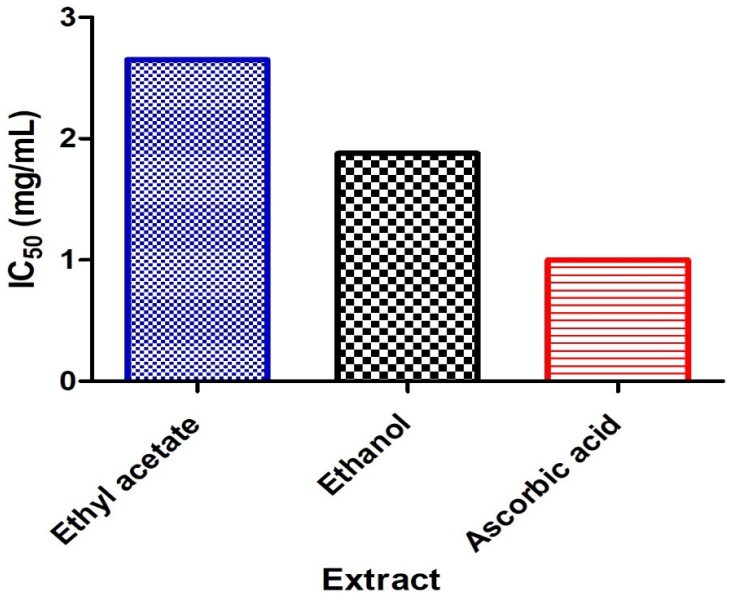

Figure 5b: IC_{50} of the extractive fractions and the standard.

Antibacterial and Antifungal Properties

Variety of fungal and bacterial strains were used to test the antibacterial and antifungal properties of Kafr extracts. The fungal strains were represented by Candida albicans, *Aspergillus niger*, and *Aspergillus flavus*, while the bacterial strains included both Gram-positive (*Staphylococcus aureus* and MRSA) and Gram-negative (*Escherichia coli*) bacteria. The zones of inhibition, which provide a measure of antibacterial activity, were identified using the disc diffusion method. The Kafr.-EtOH extract shown strong antibacterial action against every strain tested, according to the results. In particular, the Kafr-EtOH extract demonstrated inhibition zone diameters (IZD) of 17.00 mm, 18.00 mm, 14.00 mm, 12.00 mm, 13.00 mm, and 16.00 mm against *S. aureus*, MRSA, E. coli, A. niger, A. flavus, and C. albicans, respectively. On the other hand, the Kafr-EtOAc extract shown significant action against A. niger (18.00 mm), A. flavus (16.00 mm), and C. albicans (14.00 mm), but modest activity against *S. aureus* (10.00 mm), MRSA (12.00 mm), and E. coli (16.00 mm) (Table 3).According to quantitative phytochemical research, the Kafr-EtOH extract's rich phenolic and flavonoid constitute is responsible for its strong antibacterial action. Interestingly, compared to the Kafr.-EtOAc extract, the Kafr-EtOH extract might have had higher levels of eugenol, alpha-tocopherol, 2, 6-hydroxycholesterol, and 2, 3-dihydro-benzofuran. The strong antibacterial qualities of these substances have been extensively studied (Nath et al., 2020; Divyadharsini et al., 2023; Zhang et al., 2023; Marchese et al., 2017).

Eugenol, for example, has been demonstrated to have potent antibacterial and antifungal properties, which enhance the overall effectiveness of plant extracts (Table 3). (Wang et al., 2024; Hu et al., 2018). The significance of phenolic and flavonoid compounds in antibacterial action has been emphasized in earlier research (Shamsudin et al., 2022). For instance, 2, 3-dihydro-benzofuran have been shown to have strong antibacterial action against both Gram-positive and Gram-negative bacteria by Ravi et al. (2012). Furthermore, it has been established that eugenol, clove oil, and cinnamon can break down the membranes and cell walls of bacteria, increasing cellular permeability and inhibiting vital microbial functions (Devi et al., 2010). These results are consistent with the antibacterial activity found in this investigation. Greater inhibitory zones for the Kafr-EtOH extract against Gram-positive bacteria, like *S. aureus* and MRSA, may be explained by the fact that their stronger peptidoglycan layer renders them less resistant to phenolic compound disruption. However, the Gram-negative bacteria *E. coli*, with its additional outer membrane, showed lower susceptibility, as is often the case in antimicrobial tests of plant extracts (Gonelimali et al., 2018). The

antifungal activity against Candida albicans was extremely noteworthy since the Kafr-EtOH extract's inhibition zone of 16.00 mm was comparable to that of the positive control antibiotic ketoconazole, which had an IZD value of 18.00 mm, suggesting its significant antifungal potential.

This effectiveness is due to the high concentration of flavonoids and phenolic compounds, which are known to inhibit fungal growth through a variety of mechanisms, including disrupting the structure of cell membranes and interfering with fungal enzyme activity (Konuk & Ergüden, 2020; Aboody & Mickymaray, 2020). The Kafr-EtOH extract shown significant antibacterial and antifungal qualities and generally performed better than the Kafr-EtOAc extract. The potent antimicrobial properties of the ethanolic extract, which are directly related to its higher content of bioactive phenolic compounds and flavonoids, demonstrate the potential of Kafr as a natural source of antibacterial agents. More research is required to fully comprehend the mechanisms underlying the antibacterial activity of these medications and look into their synergistic effects.

Table 3: Antibacterial and antifungal activities of Kafr stem bark extract

Bacterials	**Gram Type**	**Extract**		**Positive Control**	
		Kafr-EtOAc, IZD, mm	Kafr-EtOH, IZD, mm	Gentamicin, IZD, mm	Ketoconazole IZD, mm
S. aureus	Gram +	10.00	17.00	20.00	NA
MRSA	Gram +	12.00	18.00	18.00	NA
E. coli	Gram -	16.00	14.00	14.00	NA
A. niger	Fungus	18.00	12.00	20.00	NA
A. flavus	Fungus	16.00	13.00	NA	16.00
C. albican	Fungus	14.00	16.00	NA	18.00

IZD: Inhibition Zone Diameter. NA: no activity.

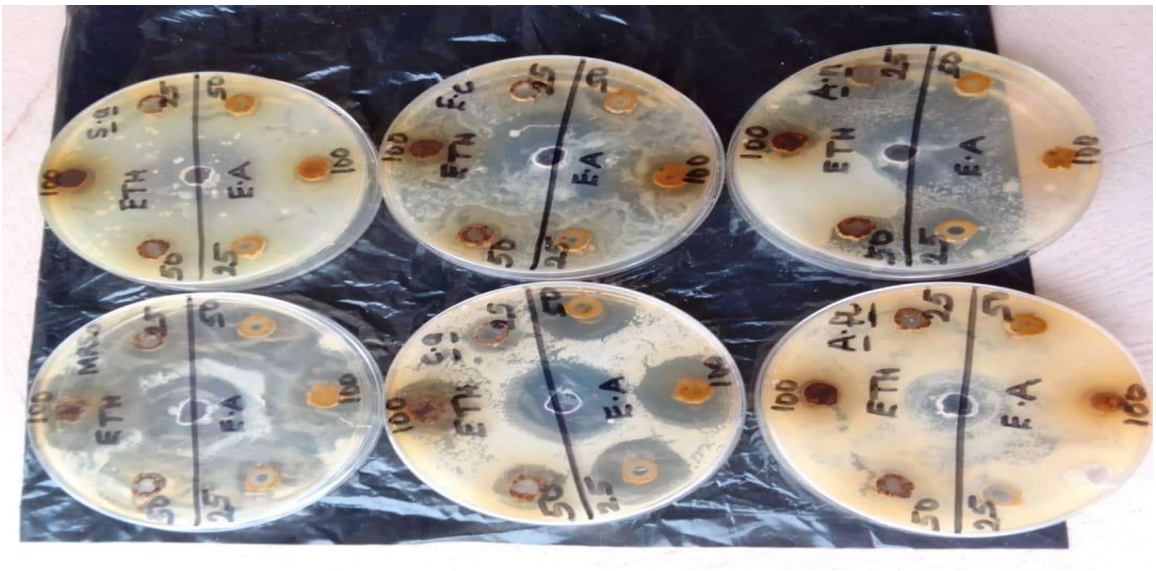

Figure 6: Zone of inhibition of extracts against selected bacterial strains

CONCLUSION

The significant potential of Kafr stem bark ethanol and ethyl acetate extracts as sources of bioactive compounds with notable antibacterial and antioxidant properties is highlighted in the study's conclusion. The ethanol extract demonstrated higher yields of phenolic, tannin, flavonoid, alkaloid, and saponin components in addition to improved antioxidant and antibacterial qualities.

The Kafr-EtOH extract demonstrated high DPPH scavenging activity (IC_{50} = 1.88 mg/mL), demonstrating its potent antioxidant and radical scavenging capabilities. The Kafr-EtOAc extract was marginally less effective than the ethanol extract, although it still showed significant antioxidant activity (IC_{50} = 2.65 mg/mL). These results highlight how crucial solvent selection is to maximizing the extraction of bioactive substances. The ethanol extract is particularly promising for use in pharmaceutical and medical applications, such as a natural antibacterial and antioxidant agent, due to its outstanding properties. The findings give the traditional medical use of Kafr a scientific foundation. Further research on the ethanol extract's potential medical applications, such as its use in therapeutic formulations, as well as the mechanisms of action and synergistic effects of its bioactive components, is necessary to fully realize its therapeutic potential.

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