

Phytochemical Screening by FTIR Spectroscopic Analysis and Anti-Bacterial Activity of Methanolic Extract of Selected Medicinal Plant of *Anethum Graveolens* and *Plantago Major*

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ABSTRACT

The objectives of this study were analysis of the secondary metabolite products of of *Anethum graveolens* and *Plantago major* and evaluation of anti-bacterial activity. The FTIR analysis of *Anethum graveolens* proved the presence of functional assignment Alkenes, Alkyl halides, Amide, and Alkane with Peak (Wave number cm^{-1}) 667.3 Bending (Strong =C-H Bond), 758.0 Bending (Strong =C-H Bond), 1026.1 Stretch (Strong C-F Bond), 1238.6 Stretch (Strong C-F Bond), 1317.2 Stretch (Strong C-F Bond), 1375.2 Stretch (Strong C-F Bond), 1608.5 Stretch (Strong N-H Bond), 1732.1 Stretch (Strong C=O Bond), 2850.4 Stretch (Strong C-H Bond), 2920.9 Stretch (Strong C-H Bond), The FTIR analysis of *Plantago major* proved the presence of functional assignment Alkenes, Alkyl halides, Amide, and Alkane with Peak (Wave number cm^{-1}) 1001.06 Stretch (Strong C-F Bond), 1049.28 Stretch (Strong C-F Bond), 1238.30 Stretch (Strong C-F Bond), 1386.82 Stretch (Strong C-F Bond), 1394.53 Stretch (Strong C-F Bond), 1517.98 Stretch (Medium C=C Bond), 1635.64 Stretch (Bending N-H Bond), 2854.65 Stretch (Strong C-H Bond), 2926.01 Stretch (Strong C-H Bond). Zone of inhibition (mm) of test bacterial strains to *Anethum graveolens* bioactive compounds and standard antibiotics were (5.001 ± 0.31), (4.006 ± 0.23), (4.961 ± 0.21), and (3.996 ± 0.20) uses *Anethum graveolens* bioactive compounds, and (2.103 ± 0.11), (2.431 ± 0.11), (2.011 ± 0.11), and (1.027 ± 0.01) uses Rifambin, and (1.732 ± 0.10), (2.009 ± 0.11), (1.417 ± 0.10), and (1.915 ± 0.11) uses Kanamycin for *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia* respectively. Zone of inhibition (mm) of test bacterial strains to *Plantago major* bioactive compounds and standard antibiotics were (4.591 ± 0.21), (5.014 ± 0.30), (4.992 ± 0.30), and (4.087 ± 0.21) uses *Plantago major* bioactive compounds, and (3.811 ± 0.21), (3.001 ± 0.19), (1.961 ± 0.12), and (1.063 ± 0.10) uses Rifambin, and (2.007 ± 0.10), (2.038 ± 0.11), (2.001 ± 0.10), and (2.075 ± 0.11) uses Kanamycin for *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia* respectively.

Keywords: FTIR spectroscopic analysis, Anti-Bacterial activity, *Anethum graveolens*, *Plantago major*

INTRODUCTION

A metabolite is end product of metabolism[1]. Metabolite is Sometimes used for small molecules. Metabolites have various functions, including signaling, stimulatory, fuel, structure, and inhibitory effects on catalytic activity of their own (usually as a cofactor to an enzyme), and interactions with pigments, odorants, and pheromones) [2,3]. A primary metabolite is directly involved in normal "growth", development, and reproduction. Ethylene exemplifies a primary metabolite produced large-scale by industrial microbiology. It does not involve secondary metabolism directly in those processes, but it usually has an important environmental function. Examples are antibiotics and colorants like resins, terpenes etc. Secondary compounds also [4] called specialized metabolic by-products or natural products are organic compounds produced by bacteria, fungi, or plants that do not participate directly in the normal growth, development, or reproduction of an organism. Unlike primary metabolism, the absence of secondary compounds does not lead to immediate death, but rather in the long-term impairment of the organism to viability, fertility, or aesthetic, or perhaps in no change mentioned at all. Secondary compounds specific to a narrow group of species within the phylogenetic group are often restricted. Secondary compounds often play an important role in plant defense against the defenses of herbivores and among other species..

Fourier-transform infrared spectroscopy (FTIR)[5-9] It is a technique used to obtain the infrared spectrum for the absorption or emission of a solid, liquid or gaseous substance. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. This confers a huge advantage over dispersion spectroscopy, which measures intensities over a narrow range of wavelengths simultaneously. The goal of absorption spectroscopy techniques (FTIR, UV-visible ("UV-Vis") spectroscopy, etc.) is to measure the amount of light a sample absorbs at each wavelength. The most straightforward way to do this, the "dispersion and spectroscopy" technique, is to shine a monochromatic light beam on a sample and measure the amount of light absorbed, and repeat for each [10-13] different wavelengths. (This is how some UV-vis spectrometers work, for example.) Fourier-transform spectroscopy It is a less intuitive way to get the same information. Instead of shining a beam of monochromatic light (a beam consisting only of one wavelength) into the sample, this technique illuminates a beam

containing many frequencies of light simultaneously and measures how much of the beam is absorbed by the sample. Next, a beam containing a different set of frequencies is modified, giving a second data point. This process is quickly repeated many times over a short period of time. Then, a computer takes all this data and works with previous versions to deduce what absorption is at each wavelength. As mentioned, computer processing is required to convert the raw data (light absorption per mirror position) into the desired result (light absorption per wavelength). The required processing turns out to be a common algorithm called Fourier transform [14]. The Fourier transform transforms a single field (in this case the displacement of the mirror in cm) in its inverse field (the wave number in cm^{-1}).

Materials and Methods

Extraction and purification of the antibacterial agent:

Antibacterial compounds were recovered from *Anethum graveolens* and *Plantago major* by solvent extraction with methanol in a ratio of 1:1 (v/v) and shaken well for 1 h. The methanol phase was separated and evaporated to dryness in water bath at 80 - 90°C. Residue was weighed and re-dissolved with little methanol.

Preparation of sample

About 20 grams of the plant sample (*Anethum graveolens* and *Plantago major*) powdered were soaked in 100 ml methanol for 16 hours in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of *Anethum graveolens* and *Plantago major* respectively. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture.

Fourier transform infrared spectrophotometer (FTIR)

The powdered sample of *Anethum graveolens* and *Plantago major* was treated for FTIR spectroscopy (Shimadzu, IR Affinity, Japan). The sample was run at infrared region between 400 nm and 4000 nm [15-19].

Determination of antimicrobial activity of crude bioactive compounds of *Anethum graveolens* and *Plantago major*

The test pathogens were swabbed in Müller-Hinton agar plates. Sixty μL of *Anethum graveolens* and *Plantago major* extract was loaded on the bored wells. Antibacterial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent

control. The antibacterial activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

RESULTS AND DISCUSSION

The FTIR analysis of *Anethum graveolens* proved the presence of functional assignment Alkenes, Alkyl halides, Amide, and Alkane with Peak (Wave number cm^{-1}) 667.3 Bending (Strong $=\text{C}-\text{H}$ Bond), 758.0 Bending (Strong $=\text{C}-\text{H}$ Bond), 1026.1 Stretch (Strong C-F Bond), 1238.6 Stretch (Strong C-F Bond), 1317.2 Stretch (Strong C-F Bond), 1375.2 Stretch (Strong C-F Bond), 1608.5 Stretch (Strong N-H Bond), 1732.1 Stretch (Strong $\text{C}=\text{O}$ Bond), 2850.4 Stretch (Strong C-H Bond), 2920.9 Stretch (Strong C-H Bond), The FTIR analysis of *Plantago major* proved the presence of functional assignment Alkenes, Alkyl halides, Amide, and Alkane with Peak (Wave number cm^{-1}) 1001.06 Stretch (Strong C-F Bond), 1049.28 Stretch (Strong C-F Bond), 1238.30 Stretch (Strong C-F Bond), 1386.82 Stretch (Strong C-F Bond), 1394.53 Stretch (Strong C-F Bond), 1517.98 Stretch (Medium $\text{C}=\text{C}$ Bond), 1635.64 Stretch (Bending N-H Bond), 2854.65 Stretch (Strong C-H Bond), 2926.01 Stretch (Strong C-H Bond). Zone of inhibition (mm) of test bacterial strains to *Anethum graveolens* bioactive compounds and standard antibiotics were (5.001 ± 0.31) , (4.006 ± 0.23) , (4.961 ± 0.21) , and (3.996 ± 0.20) uses *Anethum graveolens* bioactive compounds, and (2.103 ± 0.11) , (2.431 ± 0.11) , (2.011 ± 0.11) , and (1.027 ± 0.01) uses Rifambin, and (1.732 ± 0.10) , (2.009 ± 0.11) , (1.417 ± 0.10) , and (1.915 ± 0.11) uses Kanamycin for *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia* respectively. Zone of inhibition (mm) of test bacterial strains to *Plantago major* bioactive compounds and standard antibiotics were (4.591 ± 0.21) , (5.014 ± 0.30) , (4.992 ± 0.30) , and (4.087 ± 0.21) uses *Plantago major* bioactive compounds, and (3.811 ± 0.21) , (3.001 ± 0.19) , (1.961 ± 0.12) , and (1.063 ± 0.10) uses Rifambin, and (2.007 ± 0.10) , (2.038 ± 0.11) , (2.001 ± 0.10) , and (2.075 ± 0.11) uses Kanamycin for *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia* respectively. There are three principal advantages for an FT spectrometer compared to a scanning (dispersive) spectrometer.[1] The multiplex or Fellgett's advantage. This stems from the fact that information from all wavelengths is simultaneously collected. It leads to a higher signal-to-noise ratio for a given-time scan of the notes of limited input to constant detector noise (usually in the thermal infrared spectral region where the optical detector is limited due to noise generation recombination). For a spectrum with m resolution elements, this increase is equal to the square root of m . Instead, it allows for a shorter examination time for a given decision. In practice multiple scans are often averaged, which increases the signal-to-noise ratio by the square root of the number of scans. The throughput or Jacquinot's advantage. This results from the fact that in the dispersion instrument,

the monochromator has an entrance and exit slits that limit the amount of light that passes through it. The throughput interference is determined only by the diameter of the parallel beam coming from the source. Although no slits are required, the FTIR spectrum does not require an aperture to reduce the convergence of a parallel beam at overlap. This is because the modulated rays converged at different frequencies as the differential path. Such an aperture is called a Jacquinot stop.[20-25] For a specific resolution and wavelength this circular aperture allows more light through the aperture, resulting in a higher signal-to-noise ratio. The wavelength accuracy or Connes' advantage. The wavelength scale is calibrated by a laser beam of known wavelength that passes through the interferometer. This is more stable and accurate than the dispersion instruments as the scale relies on the mechanical movement of the diffraction gratings [26]. In practice, the resolution is limited by the difference of beams in the interference which depends on the resolution. Another minor feature that is less sensitive to stray light, is the radiation of one wavelength appearing at another wavelength in the spectrum. In the dispersion instruments [27-34], this is the result of defects in the diffraction gratings and unintended reflections. In FT instruments there is no direct equivalent as the apparent wavelength is determined by the frequency modulation in the overlap.

Table 1. FT-IR peak values of solid analysis of *Anethum graveolens*

No.	Peak (Wave number cm ⁻¹)	Intensity	Corr. Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	667.3	72.695	1.895	Strong	=C-H	Bending	Alkenes	650-1000
2.	758.0	77.926	0.633	Strong	=C-H	Bending	Alkenes	650-1000
3.	1026.1	64.543	16.005	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1238.6	81.859	0.339	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1317.2	81.685	1.786	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1375.2	81.635	1.701	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1608.5	79.778	0.565	Bending	N-H	Stretch	Amide	1550-1640
8.	1732.1	87.925	3.027	Strong	C=O	Stretch	Aldehyde	1720-1740
9.	2850.4	87.077	4.293	Strong	C-H	Stretch	Alkane	2850-3000
10.	2920.9	82.847	6.132	Strong	C-H	Stretch	Alkane	2850-3000

Table 2. FT-IR peak values of solid analysis of *Plantago major*

No.	Peak (Wave number cm^{-1})	Intensity	Corr. Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	1001.06	71.703	1.860	Strong	C-F	Stretch	alkyl halides	1000-1400
2.	1049.28	66.405	8.020	Strong	C-F	Stretch	alkyl halides	1000-1400
3.	1238.30	81.325	3.466	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1386.82	80.732	0.218	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1394.53	80.345	0.743	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1517.98	76.852	0.526	Medium	C=C	Stretch	Aromatic	1400-1600
7.	1635.64	70.901	1.581	Bending	N-H	Stretch	Amide	1550-1640
8.	2854.65	87.336	2.458	Strong	C-H	Stretch	Alkane	2850-3000
9.	2926.01	82.720	4.003	Strong	C-H	Stretch	Alkane	2850-3000

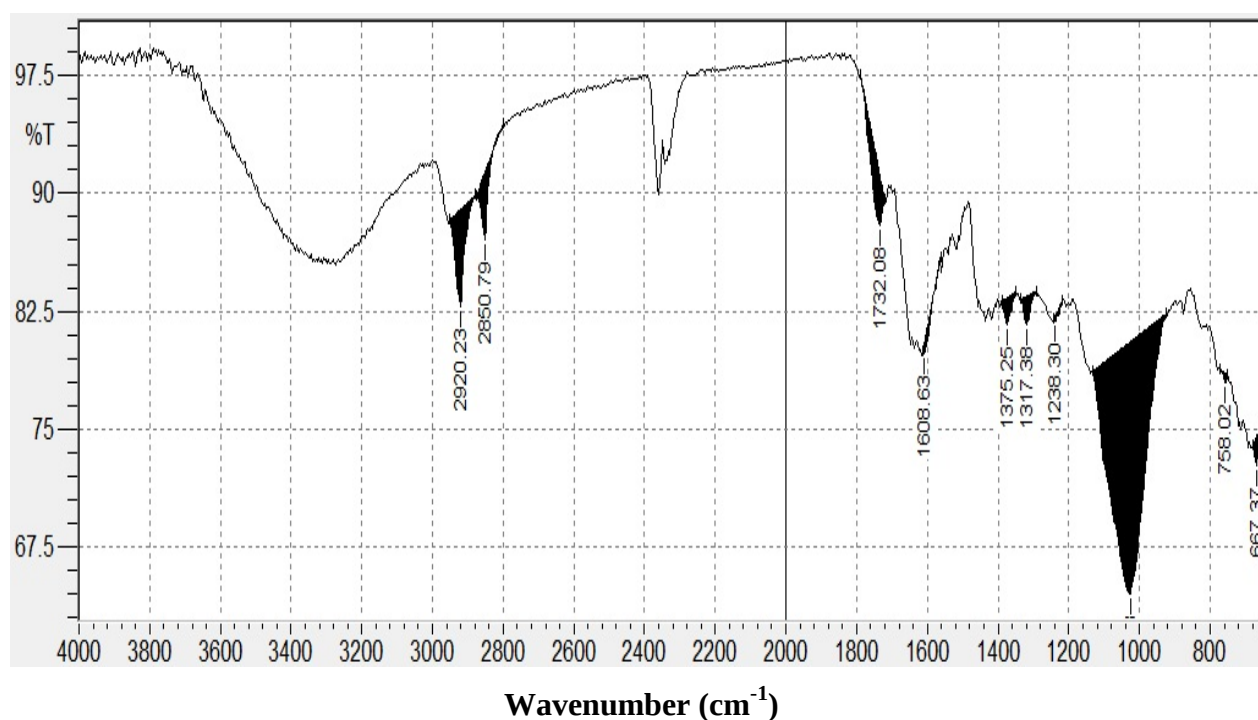


Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Anethum graveolens*

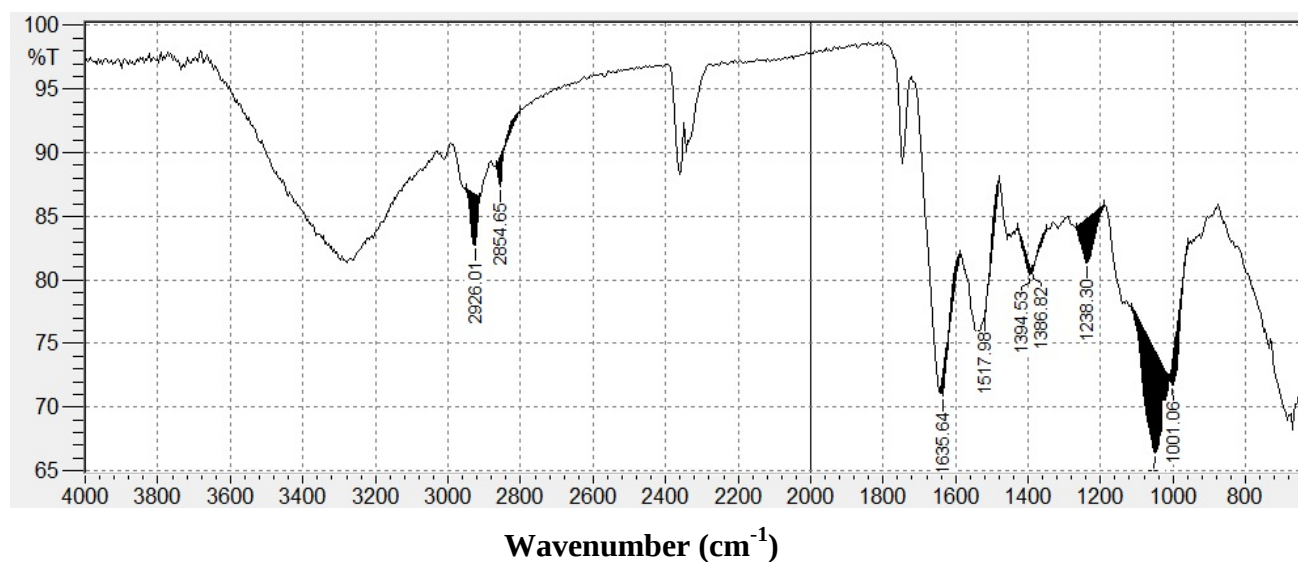


Figure 2. Fourier-transform infrared spectroscopic profile solid analysis of *Plantago major*

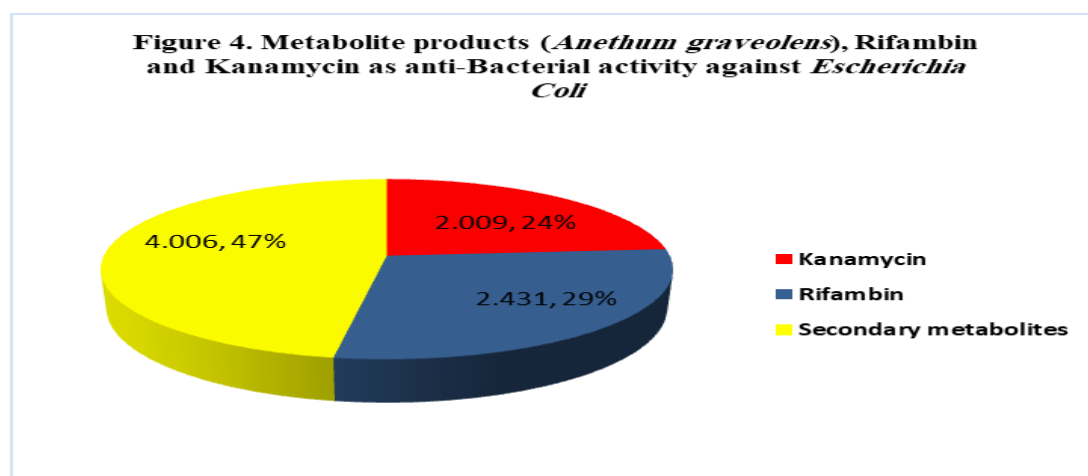
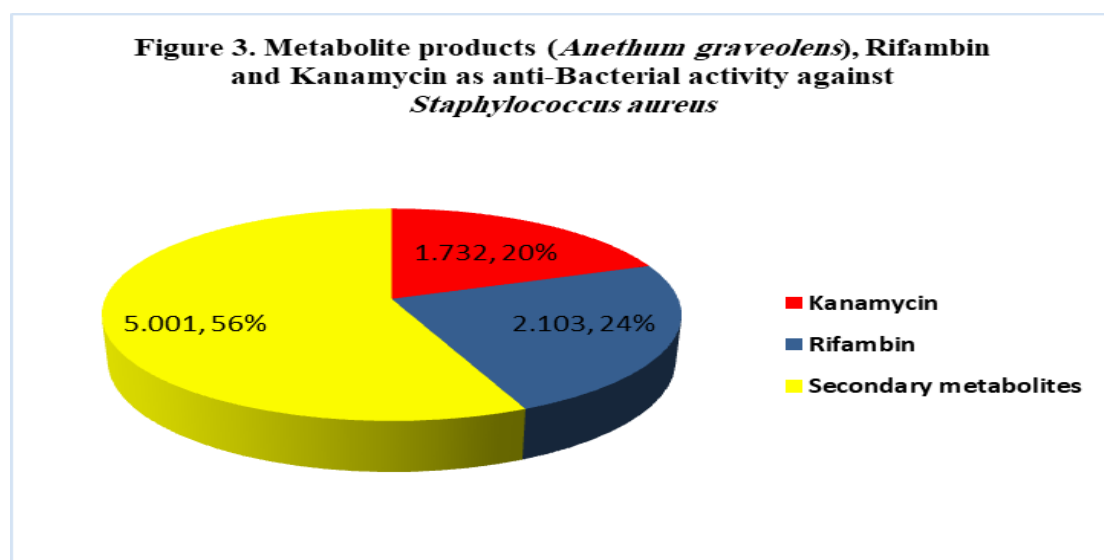


Figure 5. Metabolite products (*Anethum graveolens*), Rifambin and Kanamycin as anti-Bacterial activity against *Proteus mirabilis*

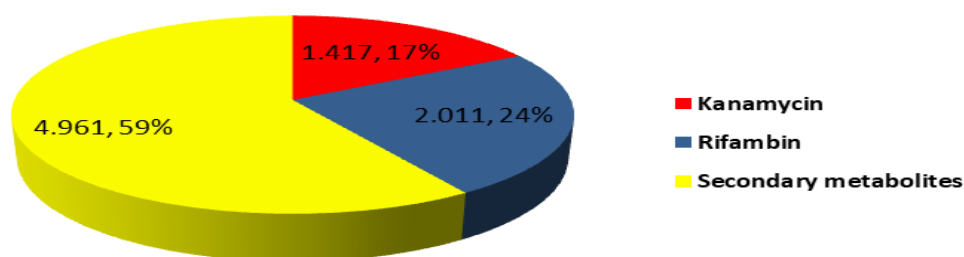


Figure 6. Metabolite products (*Anethum graveolens*), Rifambin and Kanamycin as anti-Bacterial activity against *Klebsiella pneumonia*

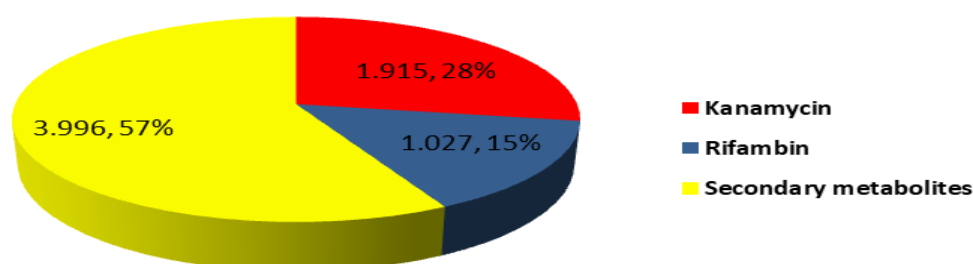


Figure 7. Metabolite products (*Plantago major*), Rifambin and Kanamycin as anti-Bacterial activity against *Staphylococcus aureus*

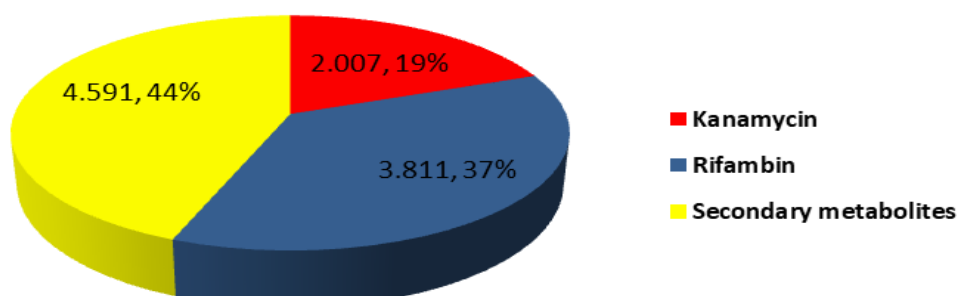


Figure 8. Metabolite products (*Plantago major*), Rifambin and Kanamycin as anti-Bacterial activity against *Escherichia Coli*

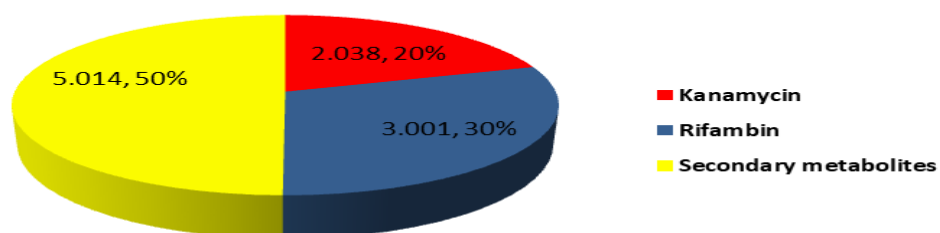


Figure 9. Metabolite products (*Plantago major*), Rifambin and Kanamycin as anti-Bacterial activity against *Proteus mirabilis*

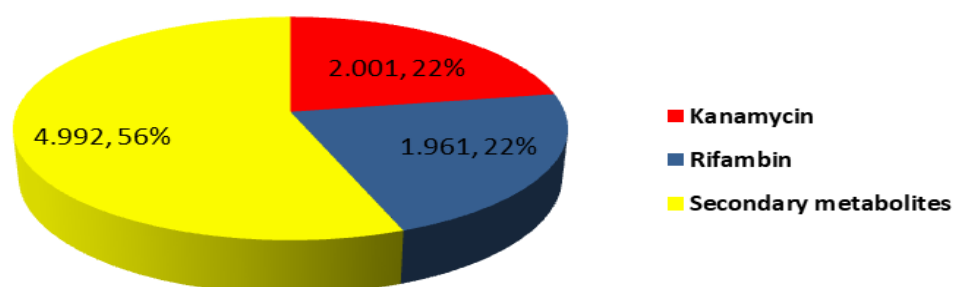
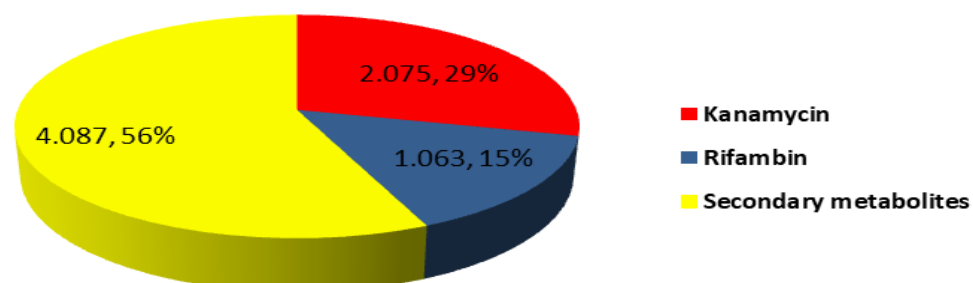


Figure 10. Metabolite products (*Plantago major*), Rifambin and Kanamycin as anti-Bacterial activity against *Klebsiella pneumonia*



CONCLUSION

The FTIR analysis of *Anethum graveolens* proved the presence of functional assignment Alkenes, Alkyl halides, Amide, and Alkane with Peak (Wave number cm^{-1}) 667.3, 758.0, 1026.1, 1238.6, 1317.2, 1375.2, 1608.5, 1732.1, 2850.4 and 2920.9, The FTIR analysis of *Plantago major* proved the presence of functional assignment Alkenes, Alkyl halides, Amide, and Alkane with Peak (Wave number cm^{-1}) 1001.06, 1049.28, 1238.30, 1386.82, 1394.53, 1517.98, 1635.64, 2854.65, 2926.01. Zone of inhibition (mm) of test bacterial strains to *Anethum graveolens* bioactive compounds and standard antibiotics were (5.001 ± 0.31) , (4.006 ± 0.23) , (4.961 ± 0.21) , and (3.996 ± 0.20) uses *Anethum graveolens* bioactive compounds, and (2.103 ± 0.11) , (2.431 ± 0.11) , (2.011 ± 0.11) , and (1.027 ± 0.01) uses Rifambin, and (1.732 ± 0.10) , (2.009 ± 0.11) , (1.417 ± 0.10) , and (1.915 ± 0.11) uses Kanamycin for *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia* respectively. Zone of inhibition (mm) of test bacterial strains to *Plantago major* bioactive compounds and standard antibiotics were (4.591 ± 0.21) , (5.014 ± 0.30) , (4.992 ± 0.30) , and (4.087 ± 0.21) uses *Plantago major* bioactive compounds, and (3.811 ± 0.21) , (3.001 ± 0.19) , (1.961 ± 0.12) , and (1.063 ± 0.10) uses Rifambin, and (2.007 ± 0.10) , (2.038 ± 0.11) , (2.001 ± 0.10) , and (2.075 ± 0.11) uses Kanamycin for *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia* respectively.

Financial disclosure

There is no financial disclosure.

Conflict of interest

None to declare.

Ethical Clearance

In our research, all protocols were approved and all methods were carried out in accordance with approved guidelines.

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