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Fermentation extract of *Penicillium italicum* and *Fusarium oxysporum* and a statement of its Biological Effectiveness

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ABSTRACT

The objectives of this study were analysis of the secondary metabolite products and evaluation of Anti-bacterial activity. The FTIR analysis of *Penicillium italicum* proved the presence of functional group assignment Alkenes, Alkyl halides, Amide, and Alkane with Intensity 819.75 Bending (Strong), 1018.41 Stretch (Strong), 1238.30 Stretch (Strong), 1379.10 Stretch (Strong), 1614.42 Stretch (Bending), 2850.79 Stretch (Strong), 2920.23Stretch (Strong). The FTIR analysis of *Fusarium oxysporum* proved the presence of functional group assignment Alkenes, Alkyl halides, Amide, Acid and Alkane with Intensity 875.68 Bending (Strong =C–H), 1016.49 Stretch (Strong C-F), 1024.20 Stretch (Strong C-F), 1197.79 Stretch (Strong C-F), 1317.38 Stretch (Bending N-H), 1716.65 Stretch (Strong C=O), 2850.79 Stretch (Strong C-H), 2922.16 Stretch (Strong C-H). *Penicillium italicum* bioactive compounds and standard antibiotics were (4.66±0.21), (5.77±0.15), (6.02±0.18), (3.63±0.23), and (5.17±0.32) uses *Penicillium italicum* bioactive compounds, and (2.11±0.12), (1.25±0.13), (2.98±0.12), (2.00±0.12), and (2.04±0.12) uses Rifambin, and (1.01±0.19), (2.93±0.11), (2.40±0.12), (2.00±0.10), and (1.81±0.11) uses Cefotoxime for *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas eurogenosa* respectively. *Fusarium oxysporum* bioactive compounds and standard antibiotics were (3.07±0.24), (5.94±0.36), (4.18±0.29), (5.63±0.28), and (4.09±0.27) uses *Fusarium oxysporum* bioactive compounds, and (1.09±0.11), (2.89±0.16), (1.59±0.11), (2.25±0.12), and (0.95±0.08) uses Rifambin, and (1.59±0.19), (1.15±0.12), (1.11±0.09), (2.49±0.10), and (0.95±0.07) uses Cefotoxime for *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas eurogenosa* respectively

Keywords: *Penicillium italicum*, *Fusarium oxysporum*, Fermentation, Biological Effectiveness

INTRODUCTION

The secondary metabolite may not be directly involved in these activities, but it can have an important and good

environmental function. For example, resins, terpenes, dyes, and antibiotics. This primary metabolite can be found in many cells or organisms. Also called a central metabolite, it has a more restricted meaning (found in an independently growing organism or any cell) [1-3]. Secondary metabolites also called natural products, specialized metabolites or by-products are organic compounds that are produced by bacteria, fungi or plants that are not directly involved in the development or reproduction of an organism. Secondary metabolites play an important role in plant protection against animal and other defenses between these species. Secondary metabolites are used as drugs and dyes. Secondary host metabolites function in many important functions such as competition and species interactions, except that they are not important for survival. One quality that is necessary for identifying secondary metabolites is their specificity. Fourier transform infrared spectroscopy (FTIR) [4] is a technique that is used to obtain the infrared spectroscopy of the absorption of a solid, gas or liquid substance. The FTIR spectrometer works simultaneously with high resolution data across a large spectral range [5]. This gives a great advantage to a dispersion spectrometer, which operates at intensities over a narrow range of wavelengths simultaneously. Fourier transform spectroscopy is a less intuitive way to obtain the same information. Instead of shining a monochromatic ray of light (a beam of one wavelength) in the sample, this technique illuminates a beam that has many frequencies of light at the same time and measures how much of this beam is absorbed by the sample [6-9]. Then, the beam is modified to contain a different set of frequencies, and this creates a second data point. Also, computer processing is necessary to convert the raw data (light absorption per mirror position) to the desired result (light absorption per wavelength). It turns out that the processing required is a popular algorithm called the Fourier transform. Fourier transform of one field (in this case the displacement of the mirror in centimeters) to its inverse field (wave numbers in 1 cm). This metadata is called a "interference pattern" [10]. Multiplexer or Fellgett Advantage. This starts from that information from all wavelengths is collected. It results in a higher signal-to-noise ratio for a given scanning time limited by the participation of constant detector noise (usually in the infrared thermal spectral region where the photodetector is limited by the generation and recombination noise). The throughput or Jacquinot's advantage. This results from the fact that in a dispersive instrument, the monochromator has entrance and exit slits which restrict the amount of light that passes through it.

MATERIALS AND METHODS

Extraction and purification of the antibacterial agent:

Antibacterial compounds were recovered from *Penicillium italicum and Fusarium oxysporum* 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 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 *Penicillium italicum and Fusarium oxysporum* 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 *Penicillium italicum and Fusarium oxysporum* was treated for FTIR spectroscopy (Shimadzu, IR Affinity, Japan). The sample was run at infrared region between 400 nm and 4000 nm [11-13].

Determination of antimicrobial activity of crude bioactive compounds of *Penicillium italicum* and *Fusarium oxysporum*

The test pathogens were swabbed in Müller-Hinton agar plates. Sixty *µL* of *Penicillium italicum* and *Fusarium oxysporum* 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

Identification of biochemical compounds

The FTIR analysis of *Penicillium italicum* proved the presence of functional group assignment Alkenes, Alkyl halides, Amide, and Alkane with Intensity 819.75 Bending (Strong), 1018.41 Stretch (Strong), 1238.30 Stretch (Strong), 1379.10 Stretch (Strong), 1614.42 Stretch (Bending), 2850.79 Stretch (Strong), 2920.23Stretch (Strong). The FTIR analysis of *Fusarium oxysporum* proved the presence of functional group assignment Alkenes, Alkyl halides, Amide, Acid and Alkane with Intensity 875.68 Bending (Strong =C–H), 1016.49 Stretch (Strong C-F), 1024.20 Stretch (Strong C-F), 1197.79 Stretch (Strong C-F), 1317.38 Stretch (Bending N-H), 1716.65 Stretch (Strong C=O), 2850.79 Stretch (Strong C-H), 2922.16 Stretch (Strong C-H). *Fusarium oxysporum* bioactive compounds and standard antibiotics were (3.07±0.24), (5.94±0.36), (4.18±0.29), (5.63±0.28), and (4.09±0.27) uses *Fusarium oxysporum* bioactive compounds, and (1.09±0.11), (2.89±0.16), (1.59±0.11), (2.25±0.12), and (0.95±0.08) uses Rifambin, and (1.59±0.19), (1.15±0.12), (1.11±0.09), (2.49±0.10), and (0.95±0.07) uses Cefotoxime for *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas eurogenosa* respectively. Infrared spectroscopy provides a useful method for herbal analysis and elucidate the compounds structures as well as for quantitative analysis of drugs. Recently, a number of plants have been reported for antibacterial properties across the world [19-21]. It is hoped that this study would direct to the establishment of some compounds that could be used to invent new and more potent antibacterial drugs of natural origin. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

Table 1. FT-IR peak values of solid analysis of *Penicillium italicum*.

No.	Peak (Wave	Intensity	Corr.	Type of	Bond	Type of	Functional	Group

	number cm-ˡ)		Intensity	Intensity		Vibration	group assignment	frequency
1.	819.75	80.609	0.316	Strong	=C–H	Bending	Alkenes	650-1000
2.	1018.41	61.727	0.876	Strong	C-F	Stretch	alkyl halides	1000-1400
3.	1238.30	81.739	2.066	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1317.38	83.193	0.728	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1379.10	81.889	0.178	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1614.42	79.693	0.611	Bending	N-H	Stretch	Amide	1550-1640
7.	2850.79	86.184	4.720	Strong	C-H	Stretch	Alkane	2850-3000
8.	2920.23	81.949	7.339	Strong	C-H	Stretch	Alkane	2850-3000

Table 2. FT-IR peak values of solid analysis of *Fusarium oxysporum*

No.	Peak (Wave number cm-ˡ)	Intensity	Corr. Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	875.68	81.563	1.123	Strong	=C–H	Bending	Alkenes	650-1000
2.	1016.49	63.828	0.776	Strong	C-F	Stretch	alkyl halides	1000-1400
3.	1024.20	63.550	0.936	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1197.79	80.489	0.124	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1317.38	81.051	2.838	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1614.42	80.833	0.751	Bending	N-H	Stretch	Amide	1550-1640
7.	1716.65	89.259	0.978	Strong	C=O	Stretch	Acid	1700-1725
8.	2850.79	89.239	2.861	Strong	C-H	Stretch	Alkane	2850-3000
9.	2922.16	85.898	4.577	Strong	C-H	Stretch	Alkane	2850-3000

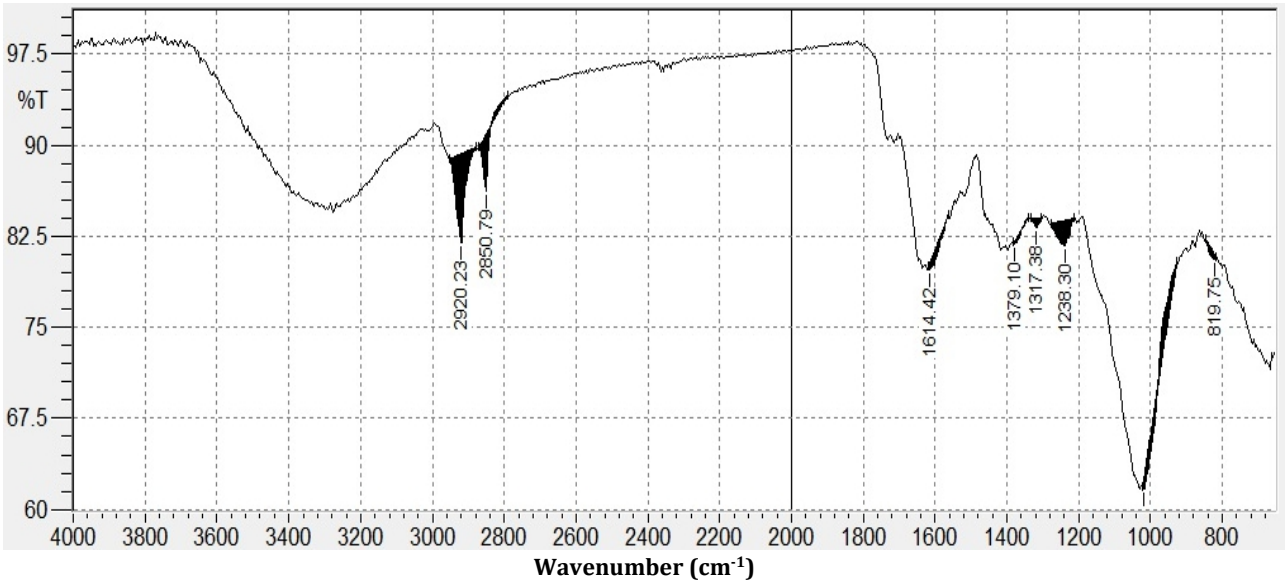

Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Penicillium italicum*

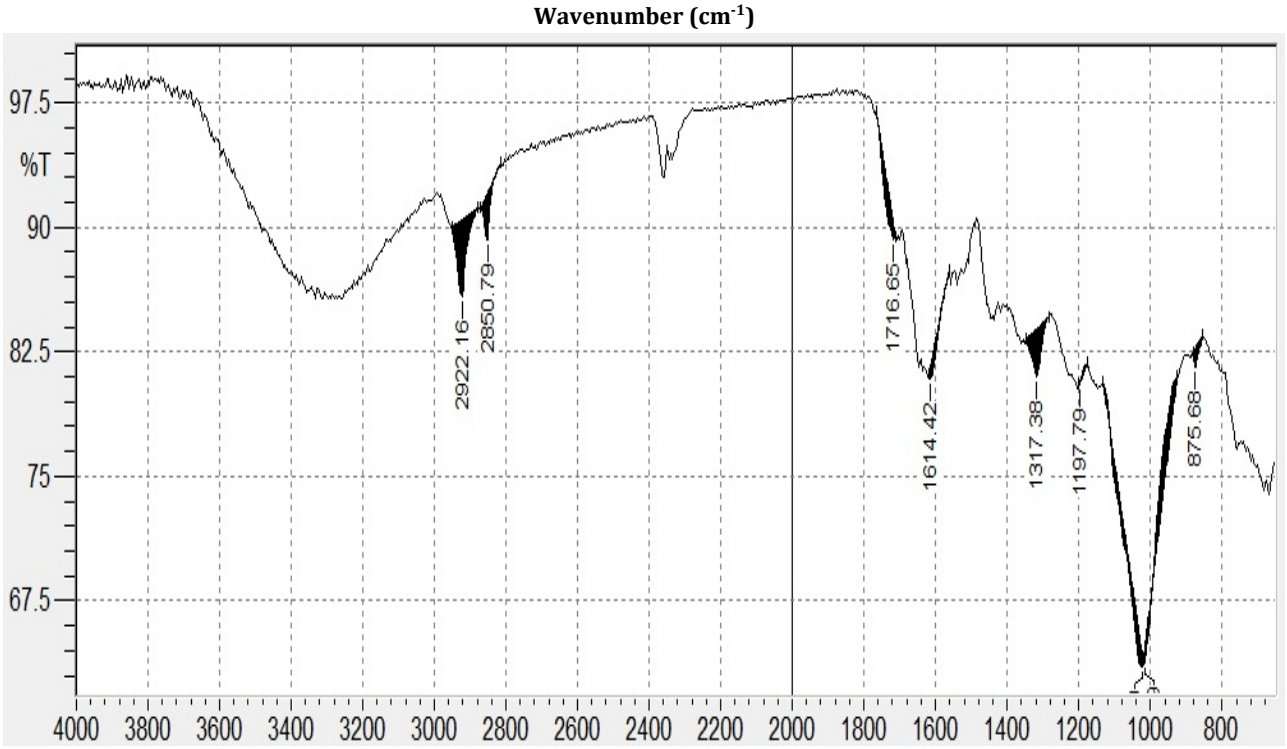

Figure 2. Fourier-transform infrared spectroscopic profile solid analysis of *Fusarium oxysporum*

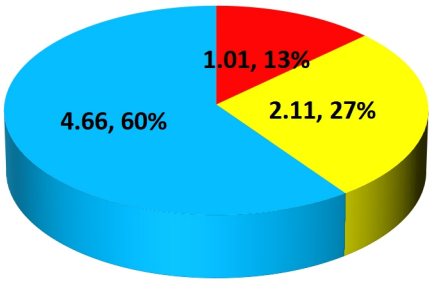

Figure 3. Metabolite products, rifambin and kanamycin as anti-Bacterial activity against *Staphylococcus aureus*

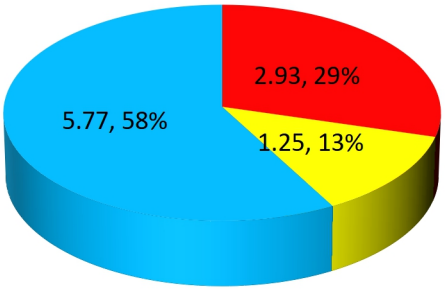

Figure 4. Metabolite products, rifambin and kanamycin as anti-Bacterial activity against *Escherichia Coli*

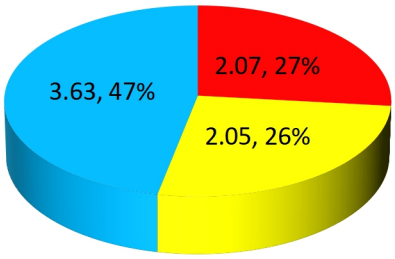

Figure 6. Metabolite products, rifambin and kanamycin as anti-Bacterial activity against *Klebsiella pneumonia*

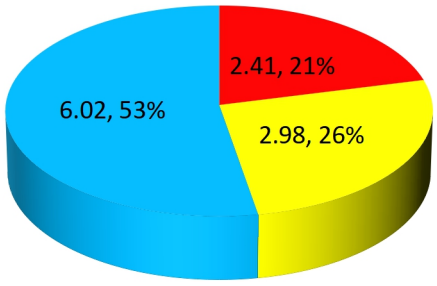

Figure 5. Metabolite products, rifambin and kanamycin as anti-Bacterial activity against *Proteus mirabilis*

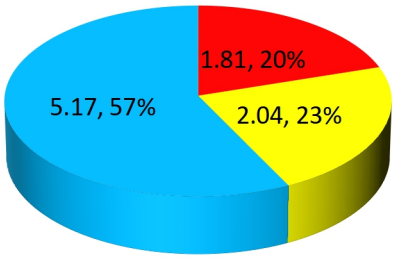

Figure 7. Metabolite products, rifambin and kanamycin as anti-Bacterial activity against *Pseudomonas eurogenosa*

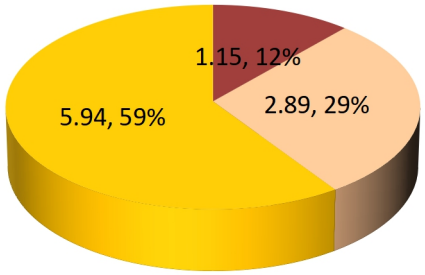

Figure 9. *Fusarium oxysporum* metabolite products, rifambin and kanamycin as anti-

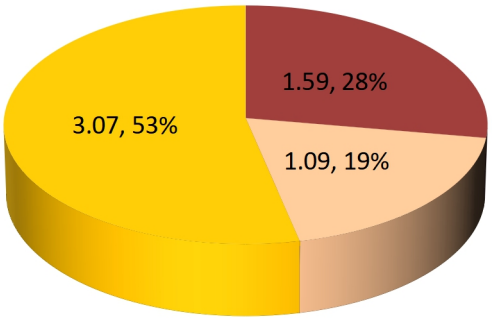

Figure 8. *Fusarium oxysporum* metabolite products, rifambin and kanamycin as anti-Bacterial activity against *Staphylococcus aureus*

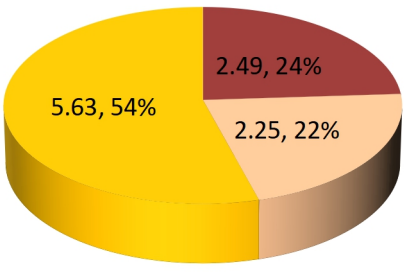

Figure 11. *Fusarium oxysporum* metabolite products, rifambin and kanamycin as anti-Bacterial activity against *Klebsiella pneumonia*

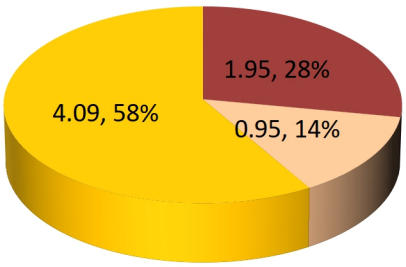

Figure 12. *Fusarium oxysporum* metabolite products, rifambin and kanamycin as anti-Bacterial activity against *Pseudomonas eurogenosa*

CONCLUSION

The FTIR analysis of *Penicillium italicum* proved the presence of functional group assignment Alkenes, Alkyl halides, Amide, and Alkane. *Penicillium italicum* was very highly active against *Escherichia coli* (6.02±0.18). The FTIR analysis of *Fusarium oxysporum* proved the presence of functional group assignment Alkenes, Alkyl halides, Amide, Acid and Alkane. *Fusarium oxysporum* was very highly active against *Escherichia coli* (5.94±0.36). FTIR analysis of *Penicillium italicum* and *Fusarium oxysporum* revealed the existence of thirty nine bioactive chemical compounds.

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