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Synergistic Activity of Phenolic Compounds of Some Plants Against Bacteria

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ABSTRACT

Medicinal plants produce great groups of secondary metabolites which are essential for medicine purpose, one of them phenolic compounds, antimicrobial activity of phenolic compounds which derivative from plants has been examined for several years. The phenolic extracts of *Sesamum indicum* and *Pimpinella anisum* seeds have antibacterial action against Gram positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*), (*Acinetobacter baumannii*), and (*Pseudomonas aeruginosa*) (*Proteus mirabilis*). The current findings show that the synergistic impact of phenolic extracts from *S. indicum* and *P. anisum* is active against a variety of pathogenic bacteria, and that the synergistic effect for two plants is more antibacterial than phenolic extracts from one plant. The results indicated Gram-negative (*P. aeruginosa*) more effected by plants, than Gram-negative (*S. aureus)* which have the lower effects. The results of HPLC indicated Sesame (*S. indicum*) have total concentration of phenolic compounds was (1313.7 µg/ml) higher than total concentration of phenolic compounds of Anise (*P. anisum*) (220.991 µg/ml), and have varied types of phenolic compounds were Pyrogallol, Gallic acid, Rutin, Kaempferol, Cinnamaldehyde, Qurctin, Eugenol, Lignan with different concentration. From this study may be conclusion Synergistic effect for two plants have more antibacterial than phenolic extracts of one plant, and Sesame (*S. indicum*) have higher antimicrobial activity than Anise (*P. anisum*).

Keywords- Medicinal plants, Synergistic antibacterial effect, Phenolic extracts, HPLC analysis.

I. INTRODUCTION

Humans are repeatedly infected by various microorganisms found in the environment, such as bacteria, viruses, molds, and yeasts. In recent years, an uncontrolled rise of pathogens and multi-drug resistant bacteria, containing numerous gram-negative and gram-positive bacteria, has become a major problem (Lambert, 2005; Sapkota *et al*., 2012). Antimicrobial resistance (AMR) is one of the most serious dangers to global health, and active, novel antimicrobials are needed to combat it (Thabit *et al*., 2015). AMR infections now claim to kill around 700,000 people each year, with the figure expected to rise to 10 million by 2050 (O'Neill, 2016). Novel anti-infectives with active modes of action are needed (Schroeder *et al*., 2017). Herbal plants are an excellent source of natural goods for discovering novel bioactive chemicals (Rossiter *et al*., 2017). Sesame (*Sesamum indicum*) is one of the world's oldest cultivated plants; it belongs to the Pedaliaceae family and the genus *Sesamum*, which has around 36 species (Saydut *et al*., 2008). Sesame seeds have antioxidant activity and contain significant amounts of flavonoids, phenols, nutrients, and minerals, suggesting that eating sesame seeds may protect against a variety of diseases (Dravie et al., 2020). Sesamin, sesamol, sesaminol, and sesamolin, among the bioactive components of *S. indicum*, play important roles in a variety of pharmacological and biological issues, and are capable of displaying therapeutic qualities against illnesses (Wu *et al*., 2019). Anise (*Pisum anisum*) is a fragrant annual plant in the Apiaceae (Umbelliferae) family. (Al-Beitawi *et al*., 2009). Plants, as associates of this family, are usually used in the food manufacturing and medicine, several plants in this family, as celery, parsley, and carrots, are common vegetable crops, in contrast, others as anise, cumin, and fennel, are identified for their therapeutic and fragrant features (Heywood *et al*., 2007). Anise seeds and essential oil have antioxidant, antibacterial, antifungal, anti-inflammatory, antidiabetic, anticonvulsant, analgesic, gastro-protective, and antiviral properties, and they are a good source of several essential B-complex vitamins like niacin, aspyridoxine, thiamin, and riboflavin, as well as minerals like calcium, potassium, copper, iron, magnesium, manganese, and zinc (Sun *et al*., 2019). The aim of this research to study synergistic antibacterial activity of phenolic extracts of *S. indicum* and *P. anisum* against bacteria, and identify new, active compounds of phenolic extracts of these plants by High Performance Liquid Chromatography (HPLC).

II. MATERIALS AND METHODS

Bacterial isolates

All isolates were collected from diverse medical isolates, then transferred to the microbiology laboratory of medicine college, Waist university. All isolates were identified in predictable diagnostic of the bacteriology basis on a cultural, gram stain, biochemical reactions and the confirmation diagnosis was done by vitek compact. Throughout the investigation, bacteria cultures were kept in their respective agar slants at 4°C and utilized as stock cultures.

Collection of plant samples

Sesame (*Sesamum indicum* L) and anise (*Pimpinella anisum* L) seeds were gathered from a local market in AL-kut, pulverized with a grinder, and kept in hygienic conditions until usage.

III. PLANT EXTRACTS PREPARATION

Extraction of crude phenols

Harborne was in charge of extracting crude phenols (1984). 200 g of plant powder was divided into two equal parts, 300 ml of 1% HCL was added to one part, and 300 ml of distal water D.W. was added to the other, then the two parts were placed in an electrical blender for 5 minutes, then boiled water bath for 30-40 minutes, the two parts were cooled and filtered with muslin cloth, and then centrifuged for 10 minutes at 3000 rpm. Both supernatants were combined. NaCl and an equal amount of n-propanol were added to the mixture until the solution was divided into two layers. The bottom layer was separated using an ethyl acetate separating funnel and evaporated using a rotary evaporator at 40°C for (1-2) hours. The top layer was evaporated in a rotary evaporator at 40°C for (1-2) hours, then carried to the oven, where the extract was stored until used.

Analysis of phenolic extracts by HPLC

By injecting 20 µl of each sample's extract in HPLC (HPLC Shimadzu-C-6A) for identification, the composition of phenolic extracts and multi-vitamins of plants was fully analyzed. Mradu *et al*. (2012) utilized this method. The following equation was used to determine the concentration of each separated compound:

$$\textbf{Concentration of sample } (\mu g/ml) = \frac{\textbf{Area of the sample}}{\textbf{Area of the standard}} \times \textbf{standard conc.} \times \textbf{Dilution factor}$$

Preparation of different concentrations of phenolic extracts of plants

The following equation was used to make different concentrations of phenolic extracts (25, 50, and 75 mg/ml of plant):

$$\textbf{Concentration mg/ml} = \frac{\textbf{Weight}}{\textbf{Volume}} \times \textbf{1000}$$

Antibacterial Activity of phenolic extracts

Agar well diffusion technique was used to appearance the activity of phenolic extracts of plants in vitro and different concentration of phenols of *S. indicum* and *P. anisum* were placed into the wells with Ethylene glycols as a control.

The colony suspension of each bacterial strain was matched to 0.5 McFarland standards, yielding a concentration of 1.510^8cfu/ml. Heat sterilized 6 mm cork borers were used to fill agar wells with 25,50, and 75 mg/ml of phenols from *S. indicum*, *P. anisum*, and their synergism, respectively. The plates were incubated for 24 hours at 37°C in triplicate. After incubation, the diameter of the inhibitory zones generated was assessed.

Statistical analysis

SAS was used to do statistical analysis on the data (version 9.1). To assess significant differences between means, a three-way ANOVA and the Least Significant Differences (LSD) test were used, with P0.05 deemed statistically significant.

Results and Discussion

Table 1 shows the production of phenolic chemicals in plant extracts (1). The presence of phenols, alkaloids, glycosides, flavonoids, tannins, amino acids, phytosterols, and proteins in the methanolic extract of *S. indicum* seeds showed high antioxidant and antibacterial activity, according to Nigam *et al*. (2014).

Table 1: Yielded of phenolic compounds of plants extract expressed as %

Plants Name	Yield (%)
S. indicum	19.3
P. anisum	14.8

Pyrogallol (1132.482 µg/ml), Cinnamaldehyde (87.356 µg/ml) of *S. indicum* and *P. anisum*, respectively, had the highest concentration of phenolic compounds, whereas Gallic acid (0.251 µg/ml) and (0.030 µg/ml) had the lowest concentration of two plants, respectively. Due to changes in environmental circumstances or genetic polymorphisms, *S. indicum* lacked cinnamic chemicals. The percentage of plant needed for extraction to crude products was varied, depending on a variety of parameters such as extraction techniques, solvent employed in the extraction operation, and plant species (Henning *et al*., 2003). Table (2), Figure (1),(2),(3),(4),(5),(6),(7), and (8).

Table 2: concentration phenolic compounds in plants by HPLC

Phenolic compounds (µg/ml)	Plants species	
	S. indicum	***P. anisum***
Pyrogallol	1132.482	19.698
Gallic acid	0.251	0.030

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Rutin	40.390	0.432
Kaempferol	10.666	0.331
Cinnamic	-	4.871
Qurctin	53.652	42.687
Cinnamaldehyde	48.789	87.356
Eugenol	19.602	6.998
Lignan	7.868	58.588
Total concentration (µg/ml)	**1313.7**	**220.991**

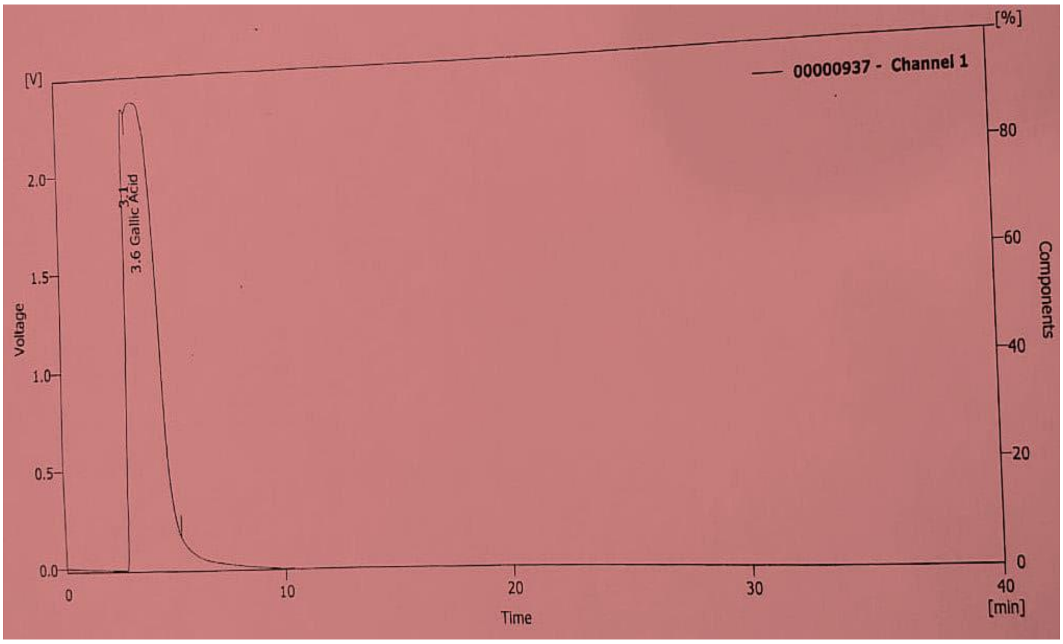

Figure 1: Gallic acid stander

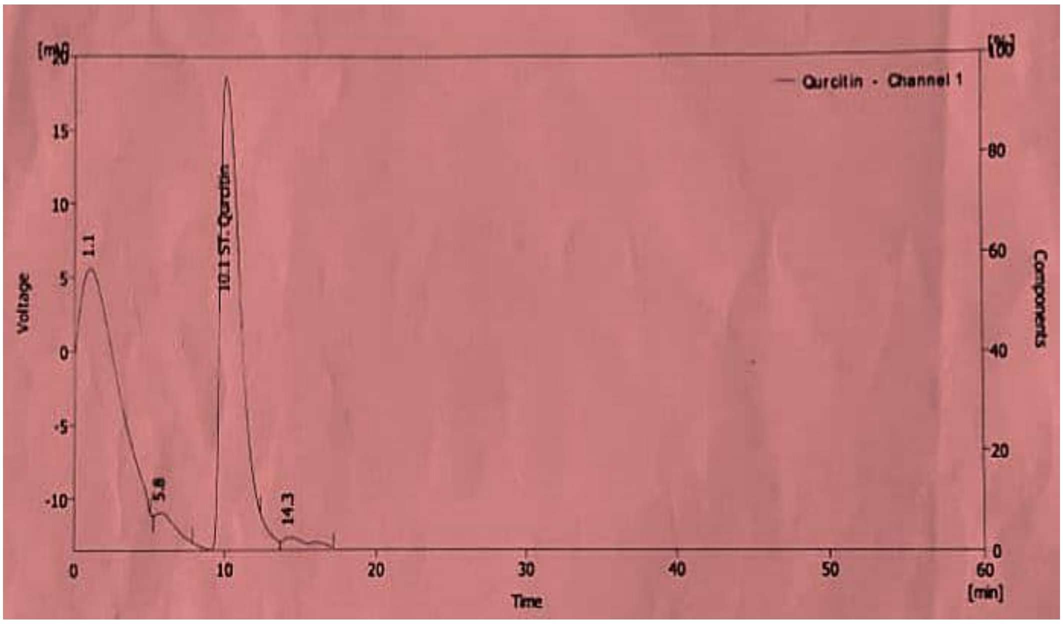

Figure 2: Qurctin stander

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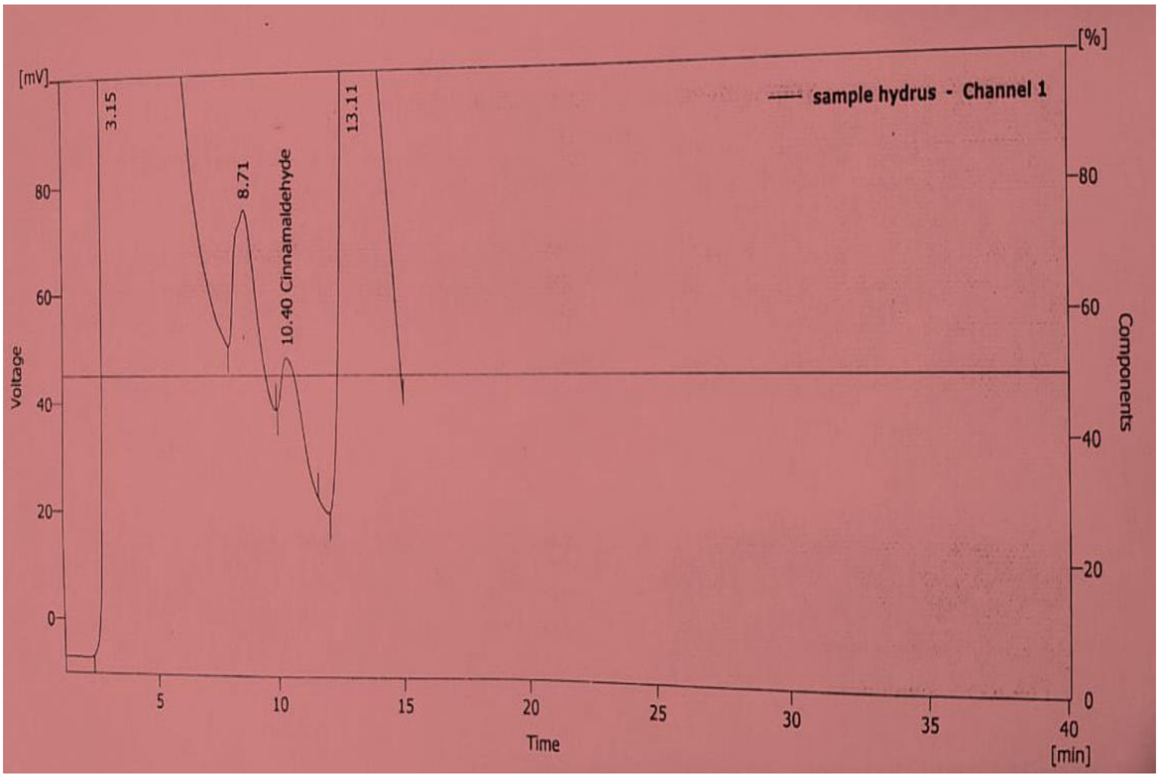

Figure 3: Cinnamaldehyde stander

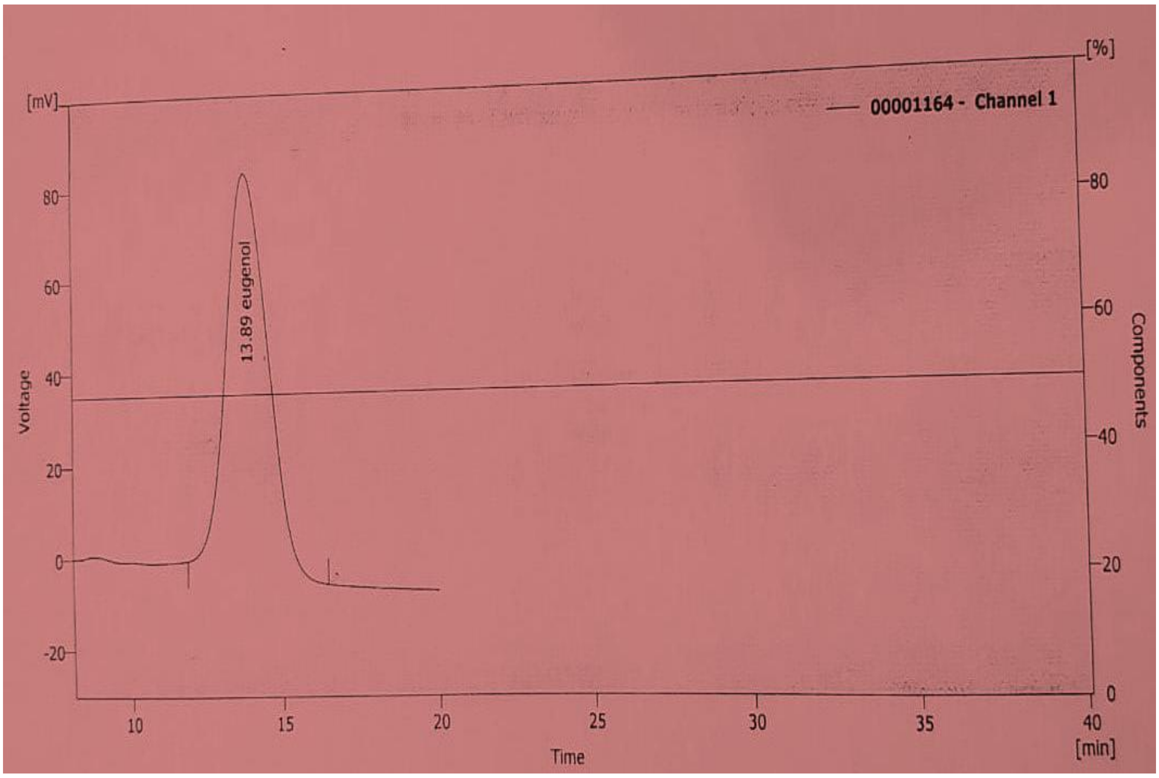

Figure 4 Eugenol stander

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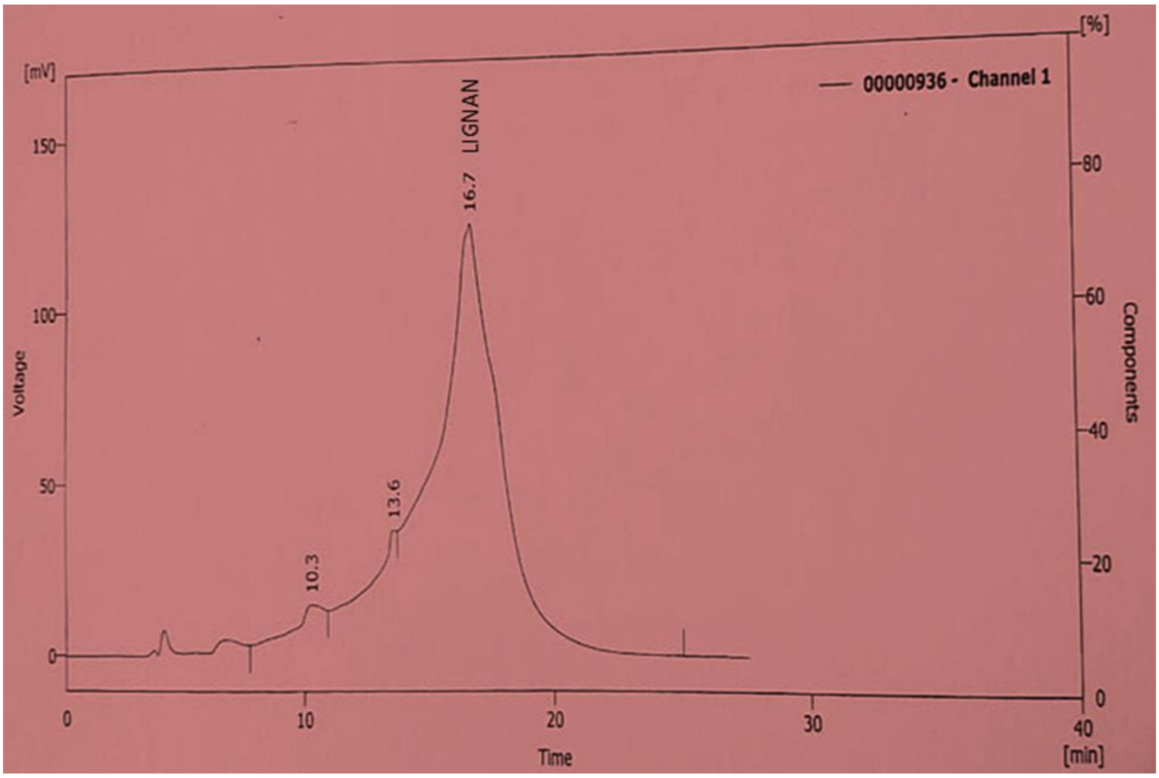

Figure 5: Lignan stander

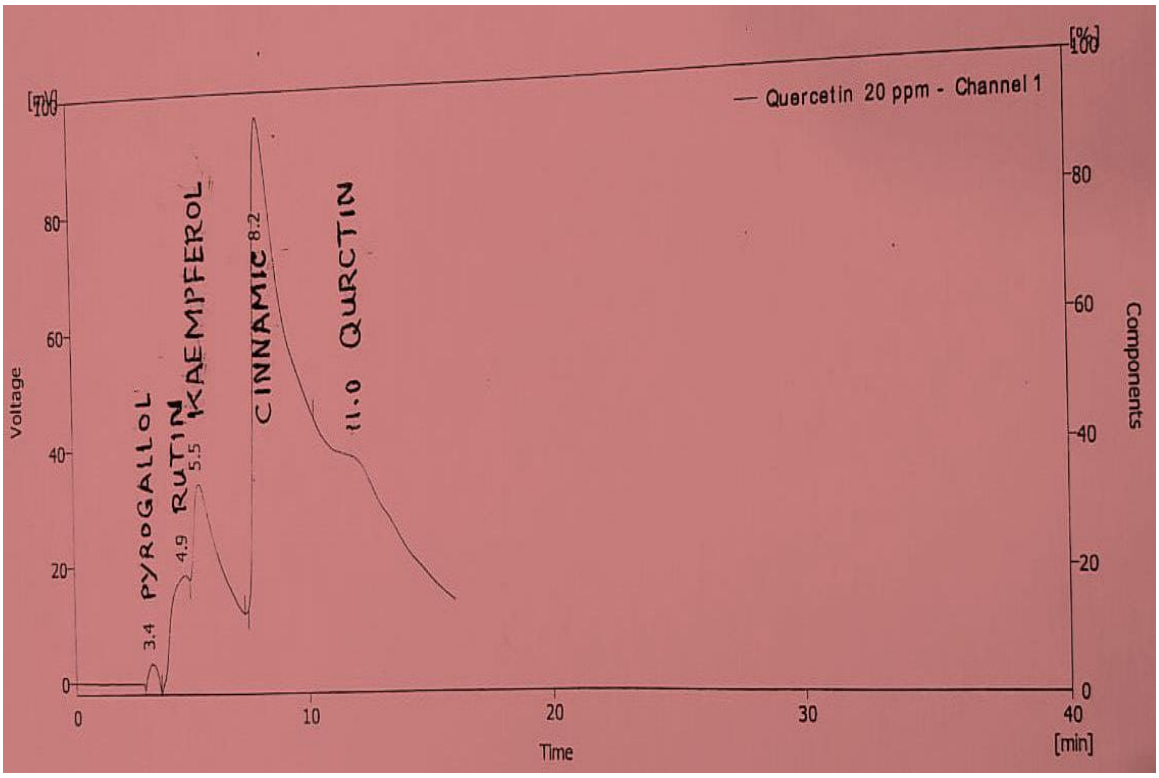

Figure 6: Mix stander (Pyrogallol, Rutin, Kaempferol, Cinnamic, Qurctin)

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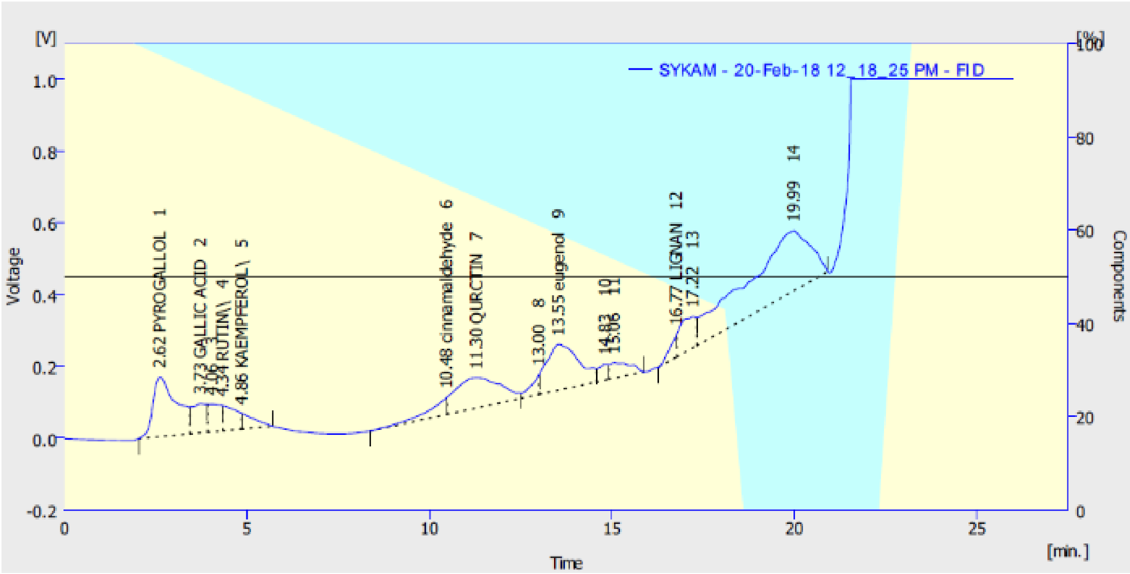

Figure 7: Phenolic compounds of *S. indicum* plants
(Pyrogallol, Gallic acid, Rutin, Kaempferol, Cinnamaldehyde, Qurctin, Eugenol, Lignan)

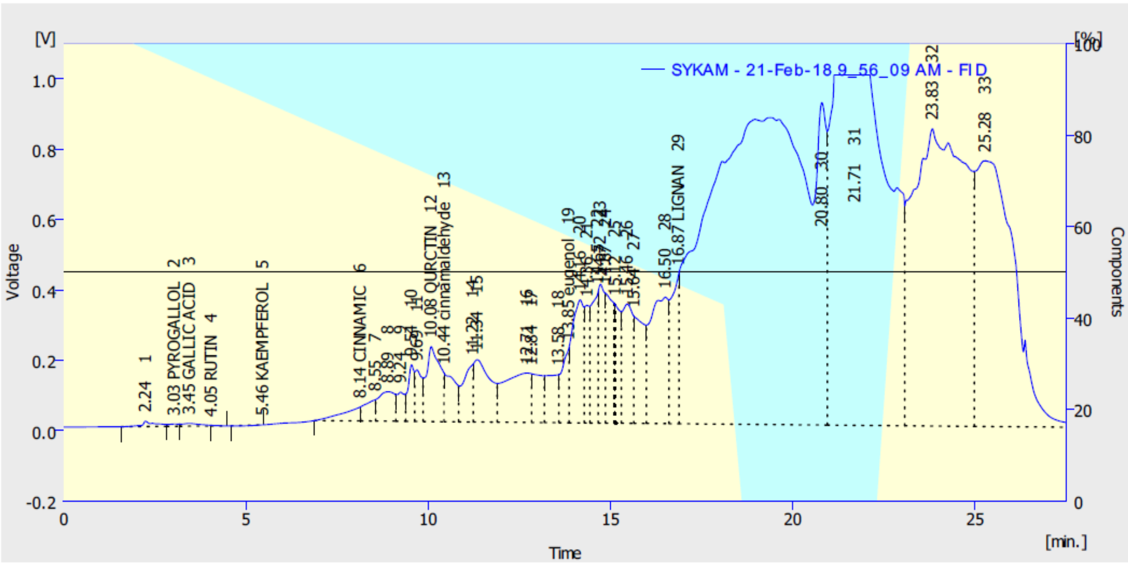

Figure 8: Phenolic compounds of *P. anisum* plant
(Pyrogallol, Gallic acid, Rutin, Kaempferol, Cinnamic, Qurctin, Cinnamaldehyde, Eugenol, Lignan)

Antibacterial Activity

An antibacterial experiment utilizing the agar dilution method was achieved with five microbes *S. aureus*, *E. Coli*, *A. baumannii*, *P. aeruginosa*, and *P. mirabilis*, and inhibition zone were calculated to evaluate the antibacterial activity of phenolic extracts of *S. indicum* and *P. anisum*, the results shown that the differences among type of bacteria within each concentration of phenolic extracts of plants are significant at ($P \leq 0.05$) as shown table (3), figure (9).

Results in table (3) indicated phenolic extracts of *S. indicum* and *P. anisum* plants have antibacterial activity against selected bacteria species. The results indicated *S. indicum* have antibacterial activity against selected bacteria more than *P. anisum*. Synergistic effect of phenolic extracts for two plants has more antibacterial activity than phenolic extracts of one plant (Table 3). Infectious diseases are a leading cause of death and morbidity in humans, especially in developing countries. In recent years, several natural antibacterial products have been developed from a wide range of plants, such as *S. indicum* and *P. anisum*, each of which has a different class of compounds that act as therapeutic potential in the treatment of human infections and are now considered to be safe (Akinsulire *et al*. 2007).

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All bacteria including Gram-positive (*S. aureus*) and Gram-negative (*E. Coli, A. baumannii, P. aeruginosa,* and *P. mirabilis*), were affected by phenolic extracts from two plants, with all bacteria being more affected by the synergistic actions of these plants. The results indicated Gram- negative (*P. aeruginosa*) more effected by plants extracts, than Gram-positive (*S. aureus)* lower effects (Table 3). Al-Bayati (2008) found that the methanol extract and essential oil of *Thymus vulgaris* and *Pimpinella anisum* had synergic antibacterial activity against nine pathogenic bacteria, and that the methanol extract and essential oil of these plants had antibacterial activity against the most experienced pathogens. According to Anilakumar *et al*. (2010), sesame seeds exhibit antibacterial properties against common skin infections such as *Staphylococcus* and *Streptococcus* spp., as well as antifungal, anti-inflammatory, and antiviral properties.

Anise has been tested for its antibacterial (Tabanca *et al.*, 2003), antioxidant (Gulcin *et al.*, 2003), antimicrobial (Al-Kassie, 2008). The antibacterial activity of aniseed, bay leaf, black pepper, and coriander aqueous extracts against 176 bacterial isolates from 12 distinct genera was determined using the disc diffusion technique (Chaudhry and Tariq, 2006). The results indicated Sesame (*S. indicum*) have antibacterial activity against selected bacteria more than Anise (*P. anisum*) due to total concentration of phenolic compounds of Sesame was (1313.7 µg/ml) higher than total concentration of phenolic compounds of Anise (220.991 µg/ml), and have varied types of phenolic compounds were Pyrogallol, Gallic acid, Rutin, Kaempferol, Cinnamaldehyde, Qurctin, Eugenol, Lignan with different concentration.

Table 3: Antibacterial activity and synergistic effect of *S. indicum* and *P. anisum* phenolic extracts against pathogenic bacteria statistically at (P≤0.05), inhibition zone diameters in mm.

Bacteria species	**Plant species**	**Concentration of phenolic extracts (mg/ml)**		
		25	**50**	**75**
E. coli	*S. indicum*	A17.00±0.57a	A18.00±1.15a	A21.00±0.57b
	P. anisum	B14.00±0.57a	B15.00±1.15a	B15.00±0.57a
	Synergistic effect	C20.00±0.57a	C21.00±0.57a	C22.00±1.15a
A. baumannii	*S. indicum*	A20.00±1.15a	A25.00±0.57b	A30.00±1.15c
	P. anisum	BD17.00±1.15a	B19.00±0.57ab	B21.00±0.57b
	Synergistic effect	C24.00±1.15a	C28.00±1.15b	AC32.00±0.57c
S. aureus	*S. indicum*	AB16.00±0.57a	A17.00±0.57a	A18.00±1.15ab
	P. anisum	B12.00±0.57a	B13.00±1.15ab	A15.00±0.57b
	Synergistic effect	AC17.00±1.15a	AC19.00±0.57a	AC19.00±0.57a
P. aeruginosa	*S. indicum*	A22.00±0.57a	A25.00±1.15b	A28.00±1.15c
	P. anisum	B19.00±1.15a	B22.00±0.57b	B25.00±0.57c
	Synergistic effect	C25.00±0.57a	C28.00±1.15b	AC30.00±0.57c
P. mirabilis	*S. indicum*	AC18.00±1.15a	A20.00±1.15a	A28.00±1.15b
	P. anisum	AC16.00±0.57a	A18.00±1.15ab	B20.00±0.57b
	Synergistic effect	B21.00±0.57a	B23.00±0.57a	AC29.00±1.15b
LSD	2.61			

Means in the same column with a different capital letter are significantly different at (P≤0.05).
Means in the same row with a different small letter are significantly different at (P≤0.05).

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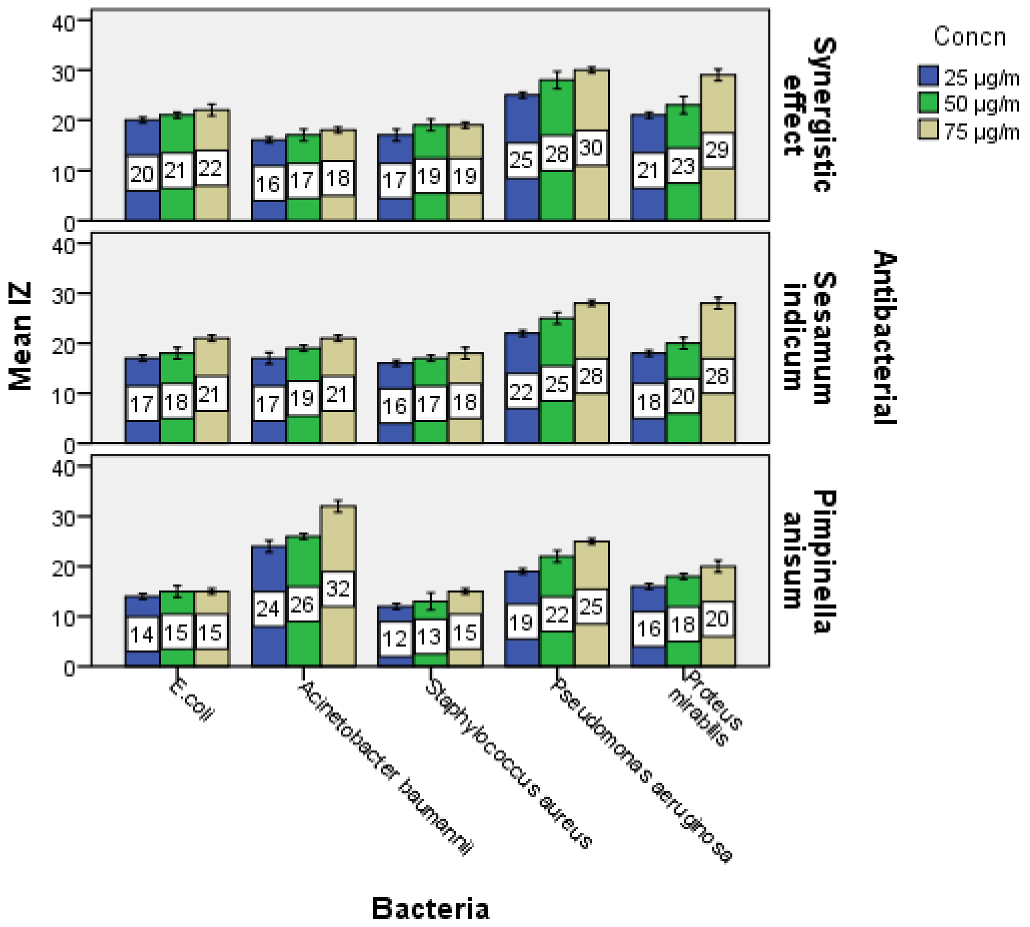

Figure 9: Antibacterial activity and synergistic effect of *S. indicum* and *P. anisum* phenolic extracts against pathogenic selected bacteria.

IV. CONCLUSION

The current results revealed that synergistic effect of *S. indicum* and *P. anisum* phenolic extracts are active against different pathogenic bacteria, and Synergistic effect for two plants have more antibacterial than phenolic extracts of one plant. The results indicated Gram- negative (*P. aeruginosa*) more effected by plant extracts, than Gram-positive (*S. aureus)* which have the lower effects. The results of HPLC indicated Sesame (*S. indicum*) have total concentration of phenolic compounds was (1313.7 µg/ml) higher than total concentration of phenolic compounds of Anise (*P. anisum*) (220.991 µg/ml), and have varied types of phenolic compounds were Pyrogallol, Gallic acid, Rutin, Kaempferol, Cinnamaldehyde, Qurctin, Eugenol, Lignan with different concentration.

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