East African Scholars Journal of Medical Sciences
Abbreviated Key Title: East African Scholars J Med Sci
ISSN: 2617-4421 (Print) & ISSN: 2617-7188 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-8 | Issue-8 | Aug-2025 | DOI: https://doi.org/10.36349/easms.2025.v08i08.002

Original Research Article

Herbal Antimicrobials under the Lens: In Vitro Assessment of *Ocimum basilicum* and *Glycyrrhiza glabra* against Pathogenic Bacteria

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Article History
Received: 04.06.2025
Accepted: 09.08.2025
Published: 13.08.2025

Journal homepage:
https://www.easpublisher.com

Quick Response Code

Abstract: ***Aims*:** This study was designed to test the antibacterial activity of *Ocimum basilicum* (Sweet basil) and *Glycyrrhiza glabra* (Licorice) extracts in four different solvents i.e. petroleum ether, chloroform, methanol and water against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi*. ***Methodology*:** Each plant material was weighed and 20.0 grams of it was taken in four different bottles 500.0 mL of each solvent was added in the respective bottle. The extracts were filtered by whatmann's filter paper, dried in vacuum desiccators and the powder mass obtained was weighed and then reconstituted in respective solvent to get the final extract of known concentrations. Bacteria were inoculated separately in the Nutrient Agar medium in a concentration of 10^6 CFU/ml and the media was poured in petri dishes and was allowed to solidify. Six wells of 1.0 centimeter diameter were cut in each plate with the help of sterile cork borer. In three wells equal volume of known concentrations of plant extracts was poured with the help of micropipettes. In 4th well, penicillin G (1600µg per well) and in the 5th well Gentamicin (1000µg per well) was taken as a positive control. In the 6th well, respective solvent was taken as a negative control. The plates were incubated at 37 degree Celsius for 24 hours and the DIZ was calculated in millimeters. Each experiment was performed in five replicates. ***Results and Discussion*:** Both plant extracts showed considerable activity against gram positive and gram negative bacteria. *Ocimum basilicum* non-polar components had good activity against gram positive than gram negative bacteria while *Glycyrrhiza glabra* methanol extract showed greater activity against all tested bacteria. ***Conclusion*:** It was concluded that the extracts of both *Ocimum basilicum* (Sweet Basil) and *Glycyrrhiza glabra* (Licorice) in petroleum ether, chloroform, methanol and water have antibacterial properties except aqueous extract of *Ocimum basilicum*.

Keywords: Antimicrobial Resistance, Inhibitory Zones, *Ocimum bascilicum, Glyccyrhiza glabra*.

INTRODUCTION

Ocimum basilicum (basil) belonging to the plant family *Lamiaceae*, comprises 65 species. Basil is an annual, 20–60 cm long, white-purple flowering plant, which is originally native to India and other regions of Asia. Basil leaves containing essential oils of distinctive aroma can be used both fresh and dried to spice up various kinds of meals. Apart of culinary use, basil has been traditionally employed as a medicinal herb in the treatment of headaches, coughs, diarrhea, constipation, warts, and/or kidney malfunction (Özcan *et al.,* 2005). Leaves and flowering parts of *O. basilicum* are traditionally used as antispasmodic, aromatic, carminative, digestive, galactogogue, stomachic, and tonic agents (Chiej, 1984). Extracts from the plant are bactericidal and are also effective against internal parasites (Bown, 1995) while *Glycyrrhiza glabra* (Licorice) is used in modern medicine chiefly as a flavoring agent that masks bitter agents, such as quinine, and in cough and cold preparations for its expectorant activity. Most recently, a sample of historic licorice from 756 A.D. was analyzed and was found to still contain

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active principles even after 1200 years. Alcohol extracts of *G. glabra* also have in vitro antibacterial activity and weak in vivo antiviral activity.

Plants have many different substances like Alkaloids, Tannins, Resins, Flavonoids, Essential oils, Fixed oils, Glycosides, Steroids and etc. (Anum *et al.,* 2025). These substances have different polarities and different range of antimicrobial activity. The total antimicrobial activity of a plant extract may be either due to its non-polar components, or its polar components or both. "Simple Maceration" using a Wide range of Solvents is an effective method by which, it is possible to separate the non-polar, Intermediately polar and highly polar components by extracting plant material in different solvents and the antimicrobial activity of these extracts can be tested to know that the collective antimicrobial effect of the plant material is due to polar or non-polar components. Further, different identification tests can be performed on the extract (with maximum antimicrobial activity) to identify the group of substances (like glycosides or alkaloids or essential oils etc.) in the extract. This approach is quite useful in detecting new antimicrobial substances. So, this study is totally focused to evaluate their potential as antibacterial drug and to orient future investigations towards the finding of new, potent and safe antibacterial compounds.

MATERIALS AND METHODS

Plant Collection and Authentication

Ocimum basilicum (sweet basil) leaves and *Glycyrrhiza glabra* (Licorice) dried roots were collected from the botanical gardens of Govt. College University, Lahore and market respectively. Plants were identified by the Chairman Botany Department, Govt. College University, Lahore. Plant parts were washed with distilled water. Dried plant material was stored in room temperature in the moisture free containers. Dried leaves were ground with the help of pestle and mortar. This material was further ground with mechanical chopper rendering it fully pulverized. Dried Glycyrrhiza roots were cut into pieces. The plant was material then subjected to maceration (Afridi *et al.,*2025).

Extract Preparation

Eight reagent bottles of 500mL capacity were used for maceration. 500mL of four solvents i.e. Methanol, Distilled Water, Chloroform, and Petroleum Ether was taken in four bottles for Ocimum and in four bottles for Glycyrrhiza separately. Bottles were labeled accordingly. Twenty grams of Ocimum powder and Glycyrrhiza pieces were weighed accurately using a digital balance and added in respective bottles. The bottles were regularly shaken and placed for three days to allow the plant material to macerate. Extracts were filtered using Whatmann's filter paper. The extracts were dried in vacuum desiccators (Millipore) till dry powder was obtained. The powder was weighed and dissolved in respective solvent (1mL of solvent for each 20mg of powder) to get extract of known concentration (Bin *et al*.,2007). Extracts were subjected to Syringe Filtration, using Filters of Minisart (Sartorius) 0.2µm size in safety cabinets. The extracts were then stored in a refrigerator at 2°C to 8°C till used.

Bacterial Cultures

Isolation and Identification

Four bacteria were used for the study i.e *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* were isolated and identified by using the scheme shown in figure 1 and 2 respectively (Claus *et al*.,1992) and Biochemical testing of Escherichia coli and Salmonella typhi was performed by using api 20E Kits (bioMerieux). Manual testing was performed for the biochemical Identification of *Staphylococcus aureus* and *Bacillus subtilis* as described in Al-Joda *et al*., 2021.

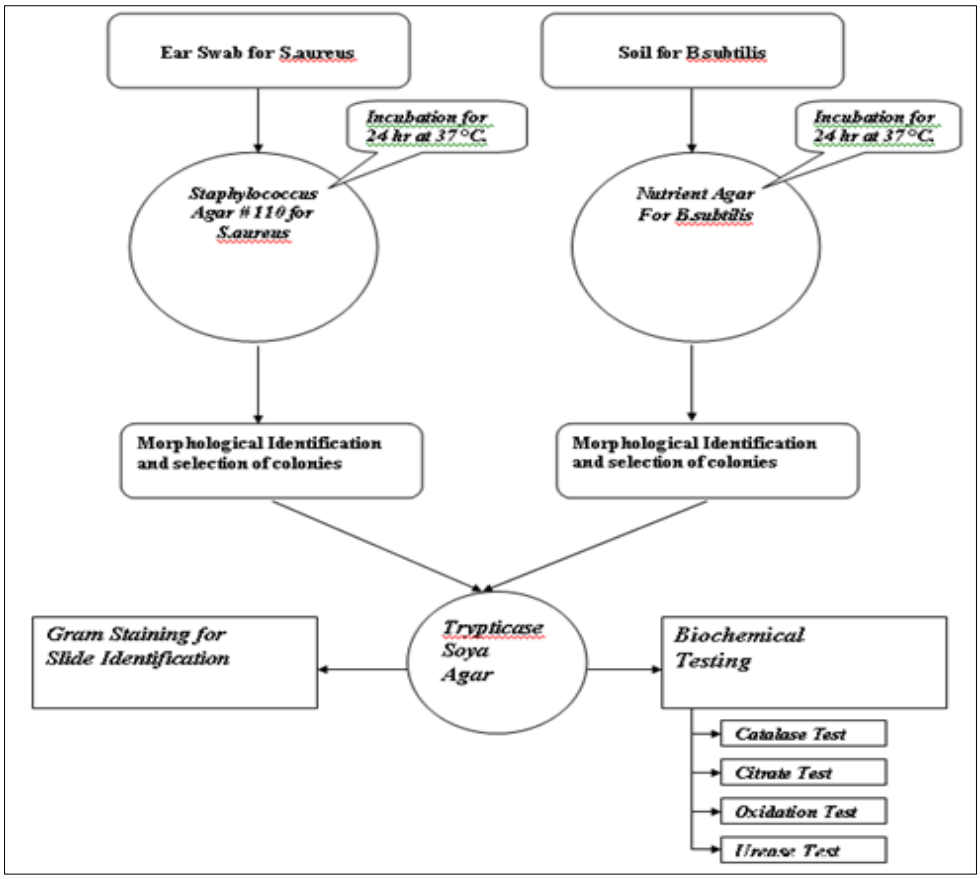


Figure 1: Isolation and Identification of Gram Positive Bacteria

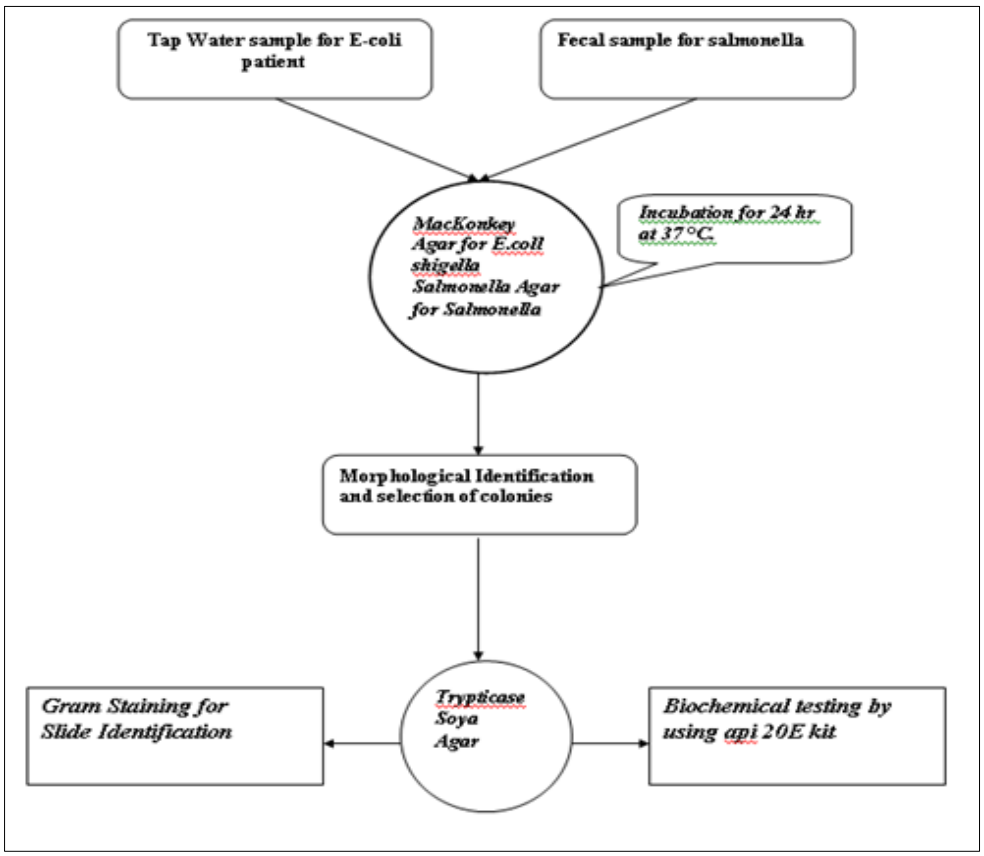


Figure 2: Isolation and Identification of Gram Negative Bacteria

Antibacterial Sensitivity Testing:

Preparation of Bacterial Suspension

10 autoclave able test tubes were washed and cleaned. 9mL of Phosphate Buffered Saline (PBS) was added in each test tube. The tubes were capped and autoclaved at 121° C and 15 lb pressure for 15-20 minutes. The tubes were labeled as 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} and 10^{-10} . Bacteria were inoculated in Nutrient Broth and incubated at 37˚C for 24 hours. 10 fold serial dilution of broth were made in Laminar flow hood. Bacterial colony forming units were determined by Viable Count Method. Briefly, 1mL from each tube was taken and spread on separate nutrient agar plates. After 2 minutes, the unabsorbed amount of suspension was sucked out in a syringe and the amount of suspension absorbed on the plate was noted. The plates were incubated for 24 hours at 37° C and after that, number of colonies were counted. From that plate which contained 30-300 colonies, number of colony forming units (CFU) per ml of the "Master Suspension" was calculated. Nutrient agar was used to culture each bacterial strain. (Colwel *et al*., 1987).

Sensitivity Testing

Antibacterial activity of plant extracts was determined by the method used by (Bin Shan *et al.,* 2007). Nutrient agar was prepared and 30 mL of media was poured in each Petri dish. Six wells of 1cm diameter were cut in each plate with the help of sterile cork borer. In one well, 50µL of extract plus 450 µL of respective solvent (total of 500 µL, Containing 1000µg of plant extract) and in the second well 250µL of extract plus 250 µL of respective solvent (total of 500 µL, Containing 5000µg of plant extract) and the 3rd well 500µL of extract (containing 10000µg of plant extract) was poured with the help of micropipettes. In 4th well, penicillin G (1600µg per well) and in the 5th well Gentamicin (1000µg per well) was taken as a positive control. In the 6th well, respective solvent was taken as a negative control. The plates were left open for 20 minutes in Laminar Flow Hood, allowing organic solvents to evaporate and then the plates were closed and incubated at 37 degree Celsius for 24 hours and the diameter of Inhibitory Zones (DIZ) was calculated in millimeters with the help of a scale. Larger the zone of inhibition, higher is the antibacterial activity (Munir *et al.,* 2025). In case of no inhibitory activity by the extract, no zone of inhibition will be developed (Westreich, 1980). Each test was performed in five replicates.

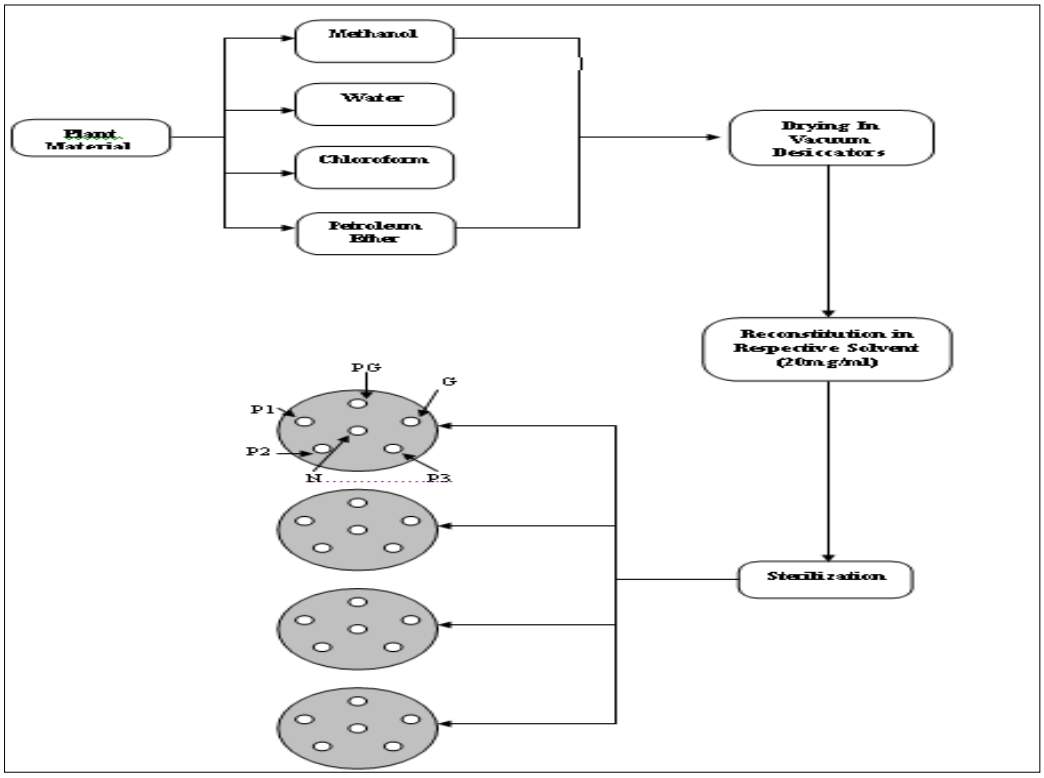


Figure 3: Experimental Design

P1 represents Plant Extract 500µL/well (Containing 10,000 µg/well)
P2 represents Plant Extract 500µL/well (Containing 5000 µg/well)
P3 represents Plant Extract 500µL/well (Containing 1000µg/well)
N represents Negative Control (Respective Solvents)
PG represents Penicillin G 1600µg/well
G represents Gentamicin 1000µg/well

RESULTS

Concentrations of Plant Extracts: The dried mass obtained after drying was weighed and the findings are given in the table 1.

Table 1: Weight of Dry Mass Obtained after Drying of Extracts in Desiccators

Extract	*Ocimum basilicum* (mg)	*Glycyrrhiza glabra* (mg)
Methanol	1050	1320
Water	600	900
Chloroform	960	1390
Petroleum Ether	520	610

Slide Identification: The results of Gram Staining and the morphological characteristics of the colonies are given in table 2.

Table 2: Morphological Characteristics of Bacterial Colonies and the Results of Gram Staining

Sr.#	Bacteria	Media Used	Bacterial Shape	Colony Morpho-Logy	Gram Staining
1	Staphylococcus aureus	Staph 110 Media	Blue clusters of balls	Smooth, with entire edges. Orange in colour	Gram Positive (Blue Stained)
2	Bacillus subtilis	Nutrient Media	Blue rods	Irregular, dull surface colonies, cream coloured	Gram Positive (Blue Stained)
3	Escherichia coli	MacConkey Agar	Red Rods	Blood Red colonies	Gram Negative (Red Stained)
4	Salmonella typhi	Shigella Salmonella Agar	Red Rods	Opaque colonies of intermediate appearance	Gram Negative (Red Stained)

Biochemical Identification

Identification of Gram Positive Bacteria: Biochemical identification of Gram positive bacteria was done and results are placed in Table 3.

Table 3: Results of Biochemical Tests Performed for the Identification of Gram Positive Bacteria

Test	*Observation*		*Inference*	
	B. subtilis	***S. aureus***	***B. subtilis***	***S. aureus***
VP	Pink Colour	No Change	+	-
Citrate	Change from Initial Green to Deep Blue Colour	No Change	+	-
Nitrate Reduction	Red Colour	Red Colour	+	+
Catalase	No Bubbles	Bubbles of Gas in capillary Tube	-	+
Glucose	Red Colour	Red Colour	+	+
Lactose	Yellow Colour	Red Colour	-	+
Xylose	Red Colour	Yellow Colour	+	-
Manitole	Yellow Colour	Red Colour	-	+
Coagulase	Plasma not Coagulated	Plasma Coagulated	-	+

+ Sign indicates that the bacteria gave positive test

- Sign indicates that the bacteria gave Negative test

Identification of Gram Negative Bacteria: Biochemical identification of gram negative bacteria was performed by using api 20E kits (Biomeriux) with results in table 4.

Table 4: Results of Biochemical Identification of Gram Negative Bacteria Using API 20E Kits

Test	*Observation*		*Inference*	
	E.coli	***S.typhi***	***E.coli***	***S. typhi***
ONPG	YELLOW	YELLOW	+	+
ADH	ORANGE	RED	+	+
LDC	RED	RED	+	+
ODC	RED	RED	+	+
CIT	GREENISH YELLOW	GREENISH BLUE	-	+
H2S	YELLOW	WHITE	-	-

URE	YELLOW	RED	-	+
TDA	WHITE	WHITE	-	-
IND	RED	WHITE	+	-
VP	WHITE	WHITE	-	-
GEL	BLACK	BLACK	+	+
GLU	YELLOW	YELLOW	+	+
MAN	YELLOW	GREENISH BLUE	+	-
INO	BLUE	BLUE	-	-
SOR	YELLOW	BLUE	+	-
RHA	YELLOW	BLUE	+	-
SAC	YELLOW	GREEN	+	-
MEL	YELLOW	BLUE	+	-
AMY	GREENISH BLUE	GREEN	-	-
ARA	YELLOW	BLUE	+	-

+ Sign indicates that the bacteria gave positive test
- Sign indicates that the bacteria gave Negative test

Antibacterial Activity Tests

Colony Forming Units Count

The results of bacterial count for *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* are given in table 5, table 6, table 7 and table 8 respectively The in vitro antibacterial activity of *Ocimum basilicum* and *Glycyrrhiza glabra* extracts was determined by measuring diameters of inhibitory zones of these extracts against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Salmonella typhi*. The results of in vitro antibacterial activity of both plants in methanol extracts, water extracts, chloroform extracts and petroleum ether extracts are given in table 9, 10, 11 and 12 respectively.

Statistical Analysis

ANOVA was applied on the results and the ANOVA tables of Diameters of Inhibitory Zones of Plant Extracts against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Salmonella typhi* are given in Table 13, 14,15, and 16 respectively.

Graphical Representation

Figure 4 indicates *Ocimum basilicum* antibacterial activity against all four strains. Similarly, Figure 5 shows *Glycyrrhiza glabra* antibacterial activity against choosen *bacterial strains.*

Table 5: Counting of CFU/Millilitre of *Bacillus subtilis* Suspension Using Viable Method

Bacteria	Dilution of Broth	Volume of inoculum's per plate	Number Of Colonies Counted	CFU/ml of bacterial suspension	Volume of bacterial suspension containing 10^6 CFU(µL)
B. subtilis	10^{-1}	1mL	More than 300	4.5×10^9	0.22 0.22 μL of broth was added per milliliter of media.
	10^{-2}	1mL	More than 300		
	10^{-3}	1mL	More than 300		
	10^{-4}	1mL	More than 300		
	10^{-5}	1mL	More than 300		
	10^{-6}	1mL	More than 300		
	10^{-7}	1mL	More than 300		
	10^{-8}	1mL	45		
	10^{-9}	1mL	Less than 30		
	10^{-10}	1mL	Less than 30		

Colonies Were Counted only From That Plate Which Contained Colonies Within 30-300.

Table 6: Counting of CFU/Millilitre of *Staphylococcus aures* Suspension Using Viable Method

Bacteria	Dilution of Broth	Volume of inoculum's per plate	Number Of Colonies Counted	CFU/ml of bacterial suspension	Volume of bacterial suspension containing 10^6 CFU (µL)
S. aureus	10^{-1}	1mL	More than 300	3.8×10^9	0.26 0.26 μL of broth was added per milliliter of media.
	10^{-2}	1mL	More than 300		
	10^{-3}	1mL	More than 300		
	10^{-4}	1mL	More than 300		
	10^{-5}	1mL	More than 300		
	10^{-6}	1mL	More than 300		
	10^{-7}	1mL	More than 300		

	10^{-8}	1mL	38		
	10^{-9}	1mL	Less than 30		
	10^{-10}	1mL	Less than 30		

Colonies Were Counted Only from That Plate Which Contained Colonies within 30-300

Table 7: Counting of Cfu/Millilitre of *Escherichia Coli* Suspension Using Viable Method

Bacteria	Dilution of Broth	Volume of inoculum's per plate	Number Of Colonies Counted	CFU/ml of bacterial suspension	Volume of bacterial suspension containing 10^6 CFU (µL)
E. coli	10^{-1}	1mL	Mora than 300	4.5×10^9	0.22 0.22 μL of broth was added per milliliter of media.
	10^{-2}	1mL	More than 300		
	10^{-3}	1mL	More than 300		
	10^{-4}	1mL	More than 300		
	10^{-5}	1mL	More than 300		
	10^{-6}	1mL	More than 300		
	10^{-7}	1mL	More than 300		
	10^{-8}	1mL	45		
	10^{-9}	1mL	Less than 30		
	10^{-10}	1mL	Less than 30		

Colonies were counted only from that plate which contained colonies within 30-300.

Table 8: Counting of Cfu/Millilitre of *Salmonella Typhi* Suspension Using Viable Method

Bacteria	Dilution of Broth	Volume of inoculum's per plate	Number Of Colonies Counted	CFU/ml of bacterial suspension	Volume of bacterial suspension containing 10^6 CFU (µL)
S. typhi	10^{-1}	1mL	More than 300	4.2×10^9	0.23 0.23 μL of broth was added per milliliter of media.
	10^{-2}	1mL	More than 300		
	10^{-3}	1mL	More than 300		
	10^{-4}	1mL	More than 300		
	10^{-5}	1mL	More than 300		
	10^{-6}	1mL	More than 300		
	10^{-7}	1mL	More than 300		
	10^{-8}	1mL	42		
	10^{-9}	1mL	Less than 30		
	10^{-10}	1mL	Less than 30		

Colonies were counted only from that plate which contained colonies within 30-300

Table 9: Diameter of Inhibitory Zones (mm) of Methanol Extracts of *Ocimum Basilicum* and *Glycyrrhiza Glabra* against *Staphylococcus Aureus, Bacillus Subtilus*, *Salmonella Typhi* and *Escherichia Coli* By Agar Well Diffusion Method

Bacteria	Diameter of Inhibition Zone (mm)							
	Ocimum basilicum			*Glycyrrhiza glabra*			Gentamicin (1000µg/well) Mean ± S.Err	Penicillin G (1600 µg/well) Mean ± S.Err
	(10,000 µg/well) Mean±S. Err	(5,000 µg/well) Mean ± S.Err	(1000 µg/well) Mean ± S.Err	(10,000 µg/well) Mean ± S.Err	(5,000 µg/well) Mean ± S.Err	(1000 µg/well) Mean ± S.Err		
Staphylococcus aureus	11.77±0.4523	7.56±0.9125	4.22±1.0692	28.56±0.2356	20.78±0.8569	14.91±0.6598	30.25±1.0369	16.53±1.1562
Bacillus subtilis	10.16±0.1965	6.55±0.8254	4.11±1.0015	24.59±0.9562	17.26±0.9658	12.87±0.8654	21.49±0.9653	27.58±0.9632
Salmonella typhi	0±0.0000	0±0.0000	0±0.0000	22.47±0.7856	16.09±1.0095	10.11±1.0244	26.16±1.0235	0±0.0000
Escherichia coli	9.11±1.0263	5.55±0.9156	3.44±1.0254	20.13±1.0693	13.41±0.6897	9.26±1.0326	17.25±0.8226	0±0.0000

Number of replicates for each test = n= 5

Table 10: Diameter of Inhibitory Zones (mm) of Water Extracts of *Ocimum Basilicum* and *Glycyrrhiza Glabra* against *Staphylococcus Aureus, Bacillus Subtilus*, *Salmonella Typhi* and *Escherichia Coli* by Agar Well Diffusion Method

Bacteria	Diameter of Inhibition Zone (mm)							
	Ocimum basilicum			*Glycyrrhiza glabra*			Gentamicin (1000µg/well) Mean ± S.Err	Penicillin G (1600 µg/well) Mean ± S.Err
	(10,000 µg/well) Mean±S. Err	(5,000 µg/well) Mean ± S.Err	(1000 µg/well) Mean ± S.Err	(10,000 µg/well) Mean ± S.Err	(5,000 µg/well) Mean ± S.Err	(1000 µg/well) Mean ± S.Err		
Staphylococcus aureus	0±0.0000	0±0.0000	0±0.0000	24.45±0.9632	17.46±0.8659	12.96±0.4526	30.25±1.0369	16.53±1.1562
Bacillus subtilis	0±0.0000	0±0.0000	0±0.0000	21.16±1.2301	16.48±0.4539	10.46±0.8626	21.49±0.9653	27.58±0.9632
Salmonella typhi	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	26.16±1.0235	0±0.0000
Escherichia coli	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	17.25±0.8226	0±0.0000

Number of replicates for each test = n= 5

Table 11: Diameter of Inhibitory Zones (mm) of Chloroform Extracts of *Ocimum Basilicum* and *Glycyrrhiza Glabra* against *Staphylococcus Aureus, Bacillus Subtilus*, *Salmonella Typhi* and *Escherichia Coli* by Agar Well Diffusion Method

Bacteria	Diameter of Inhibition Zone (mm)							
	Ocimum basilicum			*Glycyrrhiza glabra*			Gentamicin (1000µg/well) Mean ± S.Err	Penicillin G (1600 µg/well) Mean ± S.Err
	(10,000 µg/well) Mean±S. Err	(5,000 µg/well) Mean ± S.Err	(1000 µg/well) Mean ± S.Err	(10,000 µg/well) Mean ± S.Err	(5,000 µg/well) Mean ± S.Err	(1000 µg/well) Mean ± S.Err		
Staphylococcus aureus	13.55±1.0235	9.13±0.5845	5.55±0.4585	13.23±0.8795	9.23±0.7598	6.55±0.9548	30.25±1.0369	16.53±1.1562
Bacillus subtilus	11.30±0.81254	8.42±0.1965	4.33±0.9123	12.51±1.0256	8.85±0.1659	6.33±1.0056	21.49±0.9653	27.58±0.9632
Salmonella typhi	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	26.16±1.0235	0±0.0000
Escherichia coli	10.13±0.5689	7.29±1.0233	4.37±0.8659	0±0.0000	0±0.0000	0±0.0000	17.25±0.8226	0±0.0000

Number of replicates for each test = n= 5

Table 11: Diameter of Inhibitory Zones (mm) of Chloroform Extracts of *Ocimum Basilicum* and *Glycyrrhiza Glabra* against *Staphylococcus Aureus, Bacillus Subtilus*, *Salmonella Typhi* and *Escherichia Coli* by Agar Well Diffusion Method

Bacteria	Diameter of Inhibition Zone (mm)							
	Ocimum basilicum			*Glycyrrhiza glabra*			Gentamicin (1000µg/well) Mean ± S.Err	Penicillin G (1600 µg/well) Mean ± S.Err
	(10,000 µg/well) Mean±S. Err	(5,000 µg/well) Mean ± S.Err	(1000 µg/well) Mean ± S.Err	(10,000 µg/well) Mean ± S.Err	(5,000 µg/well) Mean ± S.Err	(1000 µg/well) Mean ± S.Err		
Staphylococcus aureus	13.55±1.0235	9.13±0.5845	5.55±0.4585	13.23±0.8795	9.23±0.7598	6.55±0.9548	30.25±1.0369	16.53±1.1562
Bacillus subtilis	11.30±0.81254	8.42±0.1965	4.33±0.9123	12.51±1.0256	8.85±0.1659	6.33±1.0056	21.49±0.9653	27.58±0.9632
Salmonella typhi	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	26.16±1.0235	0±0.0000
Escherichia coli	10.13±0.5689	7.29±1.0233	4.37±0.8659	0±0.0000	0±0.0000	0±0.0000	17.25±0.8226	0±0.0000

Number of replicates for each test = n= 5

Table 12: Diameter of Inhibitory Zones (mm) of Petroleum Ether Extracts of *Ocimum Basilicum* and *Glycyrrhiza Glabra* against *Staphylococcus Aureus, Bacillus Subtilus*, *Salmonella Typhi* and *Escherichia Coli* by Agar Well Diffusion Method

Bacteria	Diameter of Inhibition Zone (mm)							
	Ocimum basilicum			*Glycyrrhiza glabra*			Gentamicin (1000µg/well) Mean ± S.Err	Penicillin G (1600 µg/well) Mean ± S.Err
	(10,000 µg/well) Mean±S. Err	(5,000 µg/well) Mean ± S.Err	(1000 µg/well) Mean ± S.Err	(10,000 µg/well) Mean ± S.Err	(5,000 µg/well) Mean ± S.Err	(1000 µg/well) Mean ± S.Err		
Staphylococcus aureus	15.42±0.1256	12.56±1.2032	8.50±0.9654	10.12±0.6598	7.17±0.8569	5.66±0.1235	30.25±1.0369	16.53±1.1562
Bacillus subtilis	14.11±0.2356	9.23±1.0325	6.11±0.5689	9.08±0.1562	6.33±0.4587	4.13±0.6235	21.49±0.9653	27.58±0.9632
Salmonella typhi	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	26.16±1.0235	0±0.0000
Escherichia coli	13.71±0.8659	9.31±1.0965	5.79±0.4598	0±0.0000	0±0.0000	0±0.0000	17.25±0.8226	0±0.0000

Number of replicates for each test = n= 5

Table 13: Results of F-Test (Anova) Applied on the Diameters of Inhibitory Zones of Plant Extracts and Positive Reference (Penicillin G and Gentamicin) Against *Staphylococcus Aureus* Dependent Variable: DIZ

Source		Type III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Hypothesis	2312.96	1	2312.96	1623.436	.000	.535	1623.436	1.000
	Error	7.563	4	1.891(b)					
Extract	Hypothesis	1995.623	23	86.766	111.326	.000	..456	111.326	1.000
	Error	24.536	92	.266(c)					
Replicate	Hypothesis	4.236	4	1.059	2.265	0.01	.236	13.212	.536
	Error	56.236	92	.611(c)					
Extract * Replicate	Hypothesis	71.265	92	.774	.	.	.996	.	.
	Error	..236	0	.(d)					

Computed Using alpha=0.05

Table 14: Results of F-Test (Anova) Applied on the Diameters of Inhibitory Zones of Plant Extracts and Positive Reference (Penicillin G and Gentamicin) Against *Bacillus Subtilis* Dependent Variable: DIZ

Source		Type III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Hypothesis	3165.235	1	3165.235	6446.507	.000	.489	3125.231	.992
	Error	1.965	4	.491(b)					
Extract	Hypothesis	1895.611	23	82.417	81.235	.000	.896	3425.236	1.000
	Error	56.235	92	.611(c)					
Replicate	Hypothesis	2.163	4	.541	1.236	.939	.032	3.025	.299
	Error	61.546	92	.669(c)					
Extract * Replicate	Hypothesis	61.253	92	..666		.	1.000	.	.
	Error	.000	0	.(d)					

Computed using alpha = .05

Table 15: Results of F-Test (Anova) Applied on the Diameters of Inhibitory Zones of Plant Extracts and Positive Reference (Penicillin G and Gentamicin) Against *Escherichia Coli* Dependent Variable: DIZ

Source		Type III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Hypothesis	2235.123	1	2235.123	3788.344	.000	.596	5263.023	1.000
	Error	2.362	4	.590(b)					
Extract	Hypothesis	2536.133	23	110.266	312.235	.000	1.000	4256.032	1.000

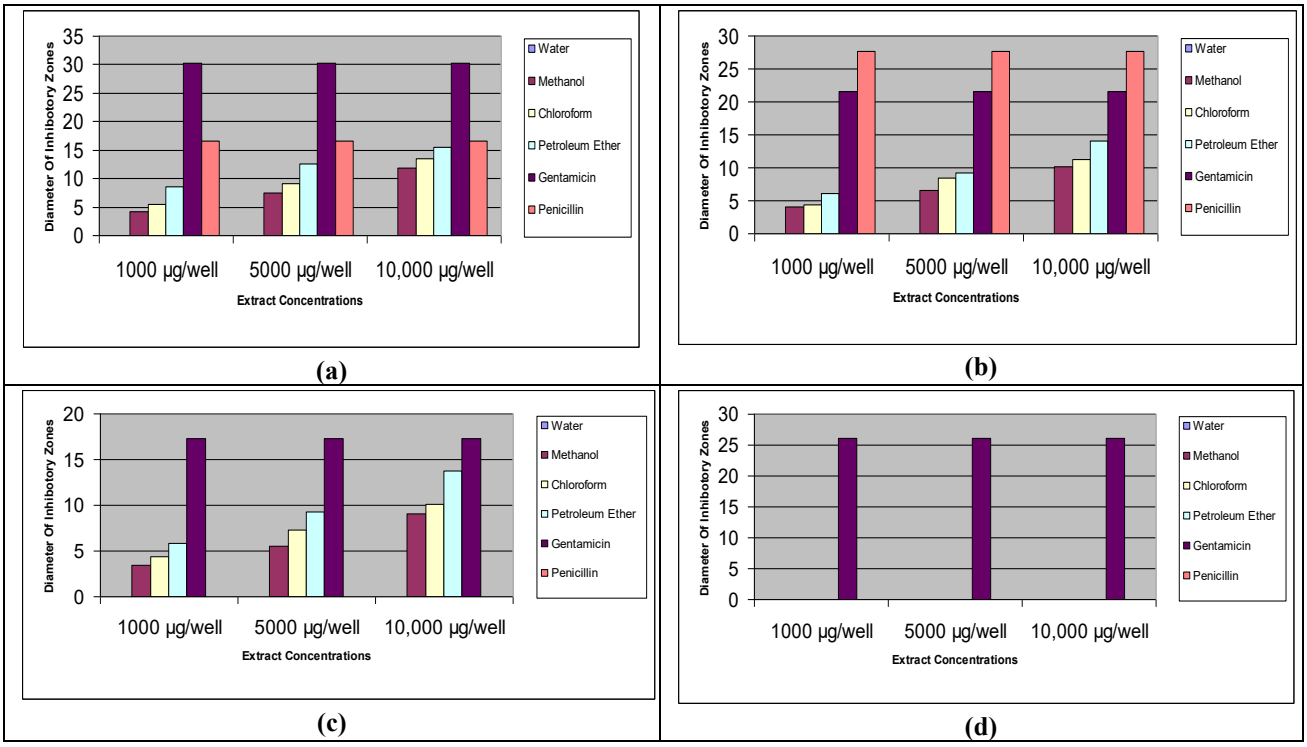
	Error	2.366	92	.025(c)					
Replicate	Hypothesis	236.636	4	59.159	.956	.296	.069	1.039	.562
	Error	8.236	92	.089(c)					
Extract *	Hypothesis	36.25	92	.394	.	.	.923	.	.
Replicate	Error	.000	0	.(d)					

Computed using alpha = .05

Table 16: Results of F-Test (Anova) Applied on the Diameters of Inhibitory Zones of Plant Extracts and Positive Reference (Penicillin G and Gentamicin) Against *Salmonella Typhi* Dependent Variable: DIZ

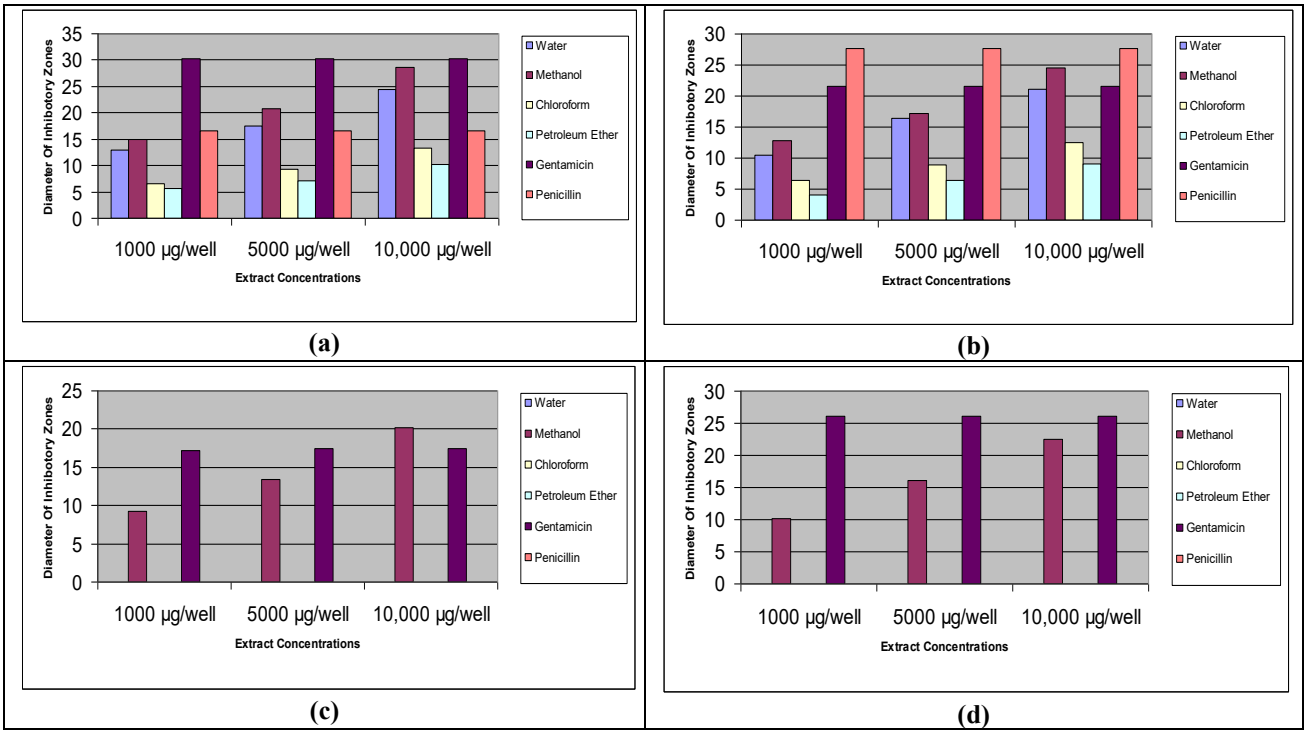
Source		Type III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Hypothesis	235.235	1	235.235	5213.236	.000	1.000	3623.032	1.000
	Error	.125	4	.031(b)					
Extract	Hypothesis	1253.235	23	54.488	1253.235	.000	.925	1325.123	1.000
	Error	5.235	92	.056(c)					
Replicate	Hypothesis	1.236	4	.309	.235	.923	1.000	1.369	.933
	Error	3.635	92	.039(c)					
Extract *	Hypothesis	1.236	92	.013	.	.	1.000	.	.
Replicate	Error	.000	0	.(d)					

Computed using alpha = .05



1000 *Ocimum bascilicum L.*. Extract 1000 µg/well Concentration
5,000 *Ocimum bascilicum L.*. Extract 5,000 µg/well Concentration
10,000 *Ocimum bascilicum L.*. Extract 10,000 µg/well Concentration
PENICILLIN G 1600 µg/well Concentration
GENTAMICIN 1000µg/well Concentration

Figure 4: In Vitro Antibacterial Activity of *Ocimum Basilicum L.* Extracts Against *Staphylococcus Aureus (a), Bacillus Subtillis (b), Escherichia Coli (c) And Salmonella Typhi (d)*



1000 *Glycyrrhiza Glabra* Extract 1000 µg/well Concentration
5,000 *Glycyrrhiza Glabra* Extract 5,000 µg/well Concentration
10,000 *Glycyrrhiza Glabra* Extract 10,000 µg/well Concentration
PENICILLIN G 1600 µg/well Concentration
GENTAMICIN 1000µg/well Concentration

Figure 5: In Vitro Antibacterial Activity of *Glycyrrhiza Glabra* Extracts Against *Staphylococcus Aureus (a)*, *Bacillus Subtillis (b), Escherichia Coli (c) And Salmonella Typhi(d)*

DISCUSSION

The spread of multidrug-resistant (MDR) strains of bacteria necessitates the discovery of new classes of antibacterial and compounds that inhibit these resistance mechanisms (Khan *et al.,* (2025). So, this study was designed with the same idea that herbs have antimicrobial activity. Two herbs *Ocimum basilicum* and *Glycyrrhiza glabra* were extracted in four different solvents i.e. petroleum ether, chloroform, methanol and water and each extract was tested for its antibacterial activity against four different bacteria i.e. *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi.*

All extracts of *Ocimum basilicum* except aqueous extract has an antimicrobial effect against the genera *Staphylococcus, Bacillus and Escherichia except Salmonella*. The maximal inhibition zones for *Staphylococcus, Bacillus, and Escherichia* to *Ocimum basilicum* petroleum ether extracts were in the range of 8.50-15.42 mm, 6.11-14.11mm and 5.79-13.71 mm respectively at concentrations in the range of 1000-10,000µg/well. In case of the chloroform extract, the maximal inhibition zones of the above mentioned bacteria sensitive to the extract were 5.55-13.55mm, 4.33-11.30mm and 4.37-10.13mm at same concentration range. Methanolic extract showed the maximal inhibition zones in the range of 4.22-11.77 mm, 4.11-10.16 mm and 3.44-9.11 mm for *Staphylococcus, Bacillus*, and *Escherichia* respectively. Aqueous extract showed no activity against any bacteria tested. The petroleum ether extract has a stronger and broader spectrum of antimicrobial activities compared with the chloroform, and methanol and water extracts. Based on mean values of inhibitory activity gram positive bacteria demonstrated more sensitivity to the extracts than gram negative bacteria (figure 4, Table 9, 10, 11, and 12). *S. aureus* was the most sensitive to all extracts, while *S.typhi* was most resistant. The highest sensitivity of *S. aureus* may be due to its cell wall structure and outer membrane. Antibacterial activity of *Ocimum basilicum* was greater against Gram-positive bacteria. These findings supports the findings of Bozin *et al*., (2006) that the Gram-positive strains of bacteria shows higher sensitivity to *Ocimum basilicum* essential oils. The antimicrobial activities of essential oils from *Ocimum basilicum* may be in due part to the presence of high content of linalool (Koutsoudaki, *et al*., 2005). Since essential oils are polar in in nature. So they are better soluble in non polar solvents like petroleum ether and less soluble or insoluble in polar solvent like water. These findings are in accordance with the findings of Ahmet *et al.*, (2005) who demonstrated the antimicrobial Effects of *Ocimum basilicum* extracts which revealed that hexane (non-polar solvent) extract showed a

stronger and broader spectrum of antibacterial activity followed by the methanol and ethanol (polar solvents) extracts. The change in values for zone of inhibition of methanolic oscimum extract against S.aureus and E.coli may be due to seasonal change, different extraction process or plant species since it effects the distribution of essential oils in plant parts. (Figure 4, Table 9, 10, 11 and 12)

Antimicrobial activity of Glycyrrhiza is evident from a number of studies. Fukai**,** *et al.,* (2002) investigated the antimicrobial activity of licorice flavonoids against methicillin-resistant Staphylococcus aureus. Glabridin and glabrene (components of Glycyrrhiza glabra) exhibited inhibitory activity against the growth. Anticariogenic activity of some tropical medicinal plants including G.glabra methanol extract was investigated by Hwang, *et al.,* (2004) against *Streptococcus mutans*.

Antibacterial activity of Glycyrrhiza was stronger in polar solvents (methanol and water) against gram positive bacteria, however, activity of non- polar solvents (petroleum ether and chloroform) was zero against gram negative bacteria. The range of Diameter of inhibitory zone (DIZ) was 14.91-28.56mm, 12.87-24.59mm, 10.11-22.47mm and 9.26-20.13mm in methanol extract (with concentration range of 1000-10,000µg/well) against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Escherichia coli* respectively. Similarly, it was 12.96-24.45mm, 10.46-21.16mm, 0.0mm and 0.0mm in water extracts (with the same concentration range) against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* respectively. However, the DIZ range was 6.55-13.23mm, 6.33-12.51mm, 0.0mm and 0.0mm in chloroform extracts (with similar concentrations) against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* respectively and similarly, it was 5.66-10.12mm, 4.13-9.08mm, 0.0mm and 0.0 mm in petroleum ether extracts (in same range of concentration) against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* respectively. It is indicated that the activity of the plant against gram positive bacteria was greater than gram negative bacteria i.e. presented in Figure 5, table 9, 10, 11 and 12.

Methanol and Aqueous extracts of Glycyrrhiza were considerably more active against *Staphylococcus aureus* when compared to *Bacillus subtilis*. In case of gram negative bacteria, activity of polar extract of Glycyrrhiza was better against *Salmonella typhi* than against *Escherichia coli* .The results showed that non-polar extracts (petroleum ether and chloroform) of Glycyrrhiza had considerable activity against tested gram positive bacteria but no activity against tested gram negative bacteria.

The antimicrobial activity of *G. glabra* is well known and glabridin has been reported to possess antibacterial activities against some strains (Fukai *et al*., 2002). The antitubercular phenolic compounds from *G. glabra* and *G. inflate* were previously identified by Moller *et al*., (2002) as licoisoflavone and licochalcone A. Additionally, glabridin was more active against gram-positive strains than gram-negative. The results obtained in the present study also revealed *G. glabra* extracts are more active against gram-positive bacteria. No antibacterial activity could be seen with water, chloroform and petroleum ether extracts against selected gram-negative bacteria. A possible explanation for these observations may lie in the significant differences in the outer layers of gram-negative and gram-positive bacteria. Gram-negative bacteria possess an outer membrane and a unique periplasmic space not found in gram-positive bacteria as described by Duffy and Power, (2001). Gram-positive bacteria do not have such outer membrane and cell wall structure. Antibacterial substances can easily destroy the bacterial cell wall and cytoplasmic membrane and result in the leakage of cytoplasm and its coagulation. This study revealed that methanolic extract of *Glycyrrhiza glabra* possess remarkable growth inhibitory activities against selected gram positive and negative bacteria. It indicates that polar or highly polar components of *Glycyrrhiza glabra* extract have stronger antibacterial activity than non-polar components. It suggests that in some future effort, methanol may be used to determine the antibacterial potential of both plants. However it is an established fact through these studies that extracts of both plants can be exploited as an antimicrobial agent against various food born diseases.

Two antibiotics were used as positive control. Penicillin-G showed activity against gram positive bacteria (diameter of inhibitory zone was 27.58mm and 16.53mm against *Bacillus subtilis* and *Staphylococcus aureus* respectively) but the gram negative bacteria were found to be resistant to penicillin-G (DIZ was 0.0mm for both *Escherichia coli* and *Salmonella typhi* I.e. presented in Figure 4, 5 and Table 9, 10, 11, 12, 13, 14, 15 and 16. Gentamicin showed activity against both gram positive and gram negative bacteria (diameter of inhibitory zone was 21.49, 30.25, 17.25 and 26.16 against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* respectively). Penicillin-G was much effective against gram positive bacteria. Beacause Penicillin-G showed zero activity against gram negative bacteria. Gentamicin is a broad spectrum antibiotic and it showed appreciable activity against both gram positive and gram negative bacteria (Figure 4,5 and Table 9, 10, 11, 12, 13, 14, 15 and 16).

REFERENCES

- Adigüzel, A., Güllüce, M., Şengül, M., Öğütcü, H., Şahin, F., & Karaman, I. (2005). Antimicrobial effects of Ocimum basilicum (Labiatae) extract. *Turkish Journal of Biology*, *29*(3), 155-160.
- Afridi, M. S. K., Muneeb, M., Tahir, H., Asghar, M. H., Azmat, M., Habib, N., Raza, T., & Zahra, A. (2025). Evaluation of Anti-Ulcer Potential of Methanolic Extract of *Amaranthus viridis* L. In Experimental Rats. *East African Scholars Journal of Medical Sciences, 8(6),* 243-254.
- Ahmad, I., & Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of ethnopharmacology*, *74*(2), 113-123.
- AL-Joda, B. M. S., & Jasim, A. H. (2021). Biochemical testing revision for identification several kinds of bacteria. *Journal of University of Babylon for Pure and Applied Sciences*, *29*(2), 168-176.
- Bown, D. (1995). *The Royal Horticultural Society encyclopedia of herbs & their uses* (pp. 424-pp).
- Bozin, B., Mimica-Dukic, N., Simin, N., & Anackov, G. (2006). Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *Journal of agricultural and food chemistry*, *54*(5), 1822-1828.
- Chiej, R. (1984). The Macdonald encyclopedia of medicinal plants.
- Claus, D. (1992). A standardized Gram staining procedure. *World journal of Microbiology and Biotechnology*, *8*(4), 451-452.
- Duffy, C. F., & Power, R. F. (2001). Antioxidant and antimicrobial properties of some Chinese plant extracts. *International journal of antimicrobial agents*, *17*(6), 527-530.
- Friis-Møller, A., Chen, M., Fuursted, K., Christensen, S. B., & Kharazmi, A. (2002). In vitro antimycobacterial and antilegionella activity of licochalcone A from Chinese licorice roots. *Planta medica*, *68*(05), 416-419.
- Fukai,T. ; A. Marumo; K. Kaitou; T. Kanda; S. Terada and T. Nomura(2002). Anti-Helicobacter pylori flavonoids from licorice extract. *Life Sciences 71(12):1449-1463.*
- Fukai,T.; A.Marumo; K.Kaitou; T.Kanda; S.Terada and T.Nomura (2002). Antimicrobial activity of licorice flavonoids against methicillin-resistant Staphylococcus aureus. *Fitoterapia*, *73(6):536-539.*
- Hwang,J.K.; Jae-Seok Shim and Jae-Youn Chung(2004). Anticariogenic activity of some tropical medicinal plants against Streptococcus mutans. *Fitoterapia*, *75(6): 596-598.*
- Khan, M. S., Iqbal, M. S., Sonehri, H., Ishfaq, N., & Basit, A. (2025). Antibiotic Resistance: A Looming Threat to Public Health. *Research Journal of Psychology*, 3(2), 171-181.
- Koutsoudaki, C., Krsek, M., & Rodger, A. (2005). Chemical composition and antibacterial activity of the essential oil and the gum of Pistacia lentiscus Var. chia. *Journal of agricultural and food chemistry*, *53*(20), 7681-7685.
- Munir, H., Sheraz, H. M., Mahreen, B., Aslam, M., Mushtaq, S., Fatima, N., ... & Afridi, M. S. K. (2025). Evaluation of antibacterial, antifungal, and antioxidant potential of cr-doped manganese oxide nanoparticles. *Frontiers in Health and Medical Research*, *3*(5) 1358-1374.
Özcan, M., Arslan, D., & Ünver, A. (2005). Effect of drying methods on the mineral content of basil (Ocimum basilicum L.). *Journal of Food Engineering*, *69*(3), 375-379.
- Roszak, D. B., & Colwell, R. R. (1987). Metabolic activity of bacterial cells enumerated by direct viable count. *Applied and environmental microbiology*, *53*(12), 2889-2893.
- Shan, B., Cai, Y. Z., Brooks, J. D., & Corke, H. (2007). The in vitro antibacterial activity of dietary spice and medicinal herb extracts. *International Journal of food microbiology*, *117*(1), 112-119.
- Shoukat, A., Khursheed, N., Khalid, M. U., Zarrish, M., Ahmad, A., Afridi, M. S. K., & Sadiq, S. (2025). Phyllanthus emblica: Its Health Benefits and Phytochemistry; A Detailed Review. *Scholars Academic Journal of Biosciences*, 13(*6)*, 819-830.
- Wistreich, G.A. and Lechtman, M.D.1980. Antimicrobial chemotherapy. Microbiology 3rd edition. McMillan Publishing Company, New York, U.S.A.

Cite This Article: Humaira Tahir, Muhammad Shahbaz Khan Afridi, Muhammad Sohail, Faeza Hasnain, Shumaila Miraj, Hafiz Abu Bakar (2025). Herbal Antimicrobials under the Lens: In Vitro Assessment of *Ocimum basilicum* and *Glycyrrhiza glabra* against Pathogenic Bacteria. *East African Scholars J Med Sci, 8*(8), 311-323.