

## Antibacterial Activity of *Glycine max* L. Seeds Using Different Extracts

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*Glycine max*  
*Klebsiella pneumoniae* *Bacillus cereus* *Staphylococcus aureus*  
*Pseudomonas aeruginosa*  
Tetracycline Cephalexin Chloramphenicol  
.Gentamycin  
200mg/cm<sup>3</sup>  
200mg/cm<sup>3</sup> *Ps. aeruginosa*  
*B. cereus*

### Abstract

In the present study, antibacterial properties of three different extracts from *Glycine max* L. seeds were screened against four types of Gram-positive and negative bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using agar-well diffusion method and comparing with standard antibiotics Chloramphenicol, Cephalexin, Tetracycline and Gentamycin. The ethanol extract demonstrated good inhibitory effects against most tested bacteria using the extract concentration 200mg/cm<sup>3</sup> compared with chloroform and petroleum ether extracts which gave clear inhibitory effect against *Ps. aeruginosa* using the same extract concentration the study also revealed

the complete resistance of *B. cereus* towards chloroform and petroleum ether extracts but was inhibited using all ethanol extract concentrations and the proportion between inhibitory effect and extract concentration was direct.

## Introduction

Nature has provided a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine [1]. The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide. It is recognized that in some developing countries, plants are the main medicinal source to treat infectious diseases [2]. Plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant bacteria. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources [3].

*Glycine max* L. (Soybean) is a subtropical plant (Figure 1), native to Asia. This member of the pea family (*Fabaceae*) grows from one to five feet tall and forms clusters of three to five pods, each containing two to four beans per pod. Soy has been a dietary staple in Asian countries for at least 5,000 years. The seed (Figure 2) contains up to 20% of an edible oil that has a very wide range of applications and is commonly used in the chemical industry, manufacturing of paints, linoleum, oilcloth, printing inks, soap, insecticides, and disinfectants [4].

Soybean, a staple food in many Asian countries, contains valuable constituents, including protein, isoflavones, saponins, and phytosterols. Soybean protein provides most of the essential amino acids. It's also low in fat and cholesterol-free. The isoflavones in soybean, primarily genistein and daidzein, have been well researched by scientists for their antioxidant and phytoestrogenic properties. Saponins enhance immune function and bind to cholesterol to limit its absorption in the intestine. Phytosterols and other components of soy have been reported to lower cholesterol levels. Isoflavones may reduce the risk of hormone-dependent cancers, such as breast and prostate cancer, as well as other cancers. Both animal and human studies have confirmed this [5].

Old Chinese herbals suggest that the soybean was a specific remedy for the proper functioning of the bowels, heart, kidney, liver, and

stomach. A decoction of the root is said to be astringent. Soybean oil has a high proportion of unsaturated fatty acid, and contain considerable levels of phenolic compounds. Commercial grades of natural lecithin, often derived from soybean, are reported to contain a potent vasodepressor [6]. Medicinally lecithin is indicated as a lipotropic agent.

Soybean is listed as a major starting material for stigmaterol, once known as an antistiffness factor. Sitosterol, also a soy byproduct, has been used to replace diosgenin in some antihypertensive drugs [7].



Figure 1: *Glycine max* plant

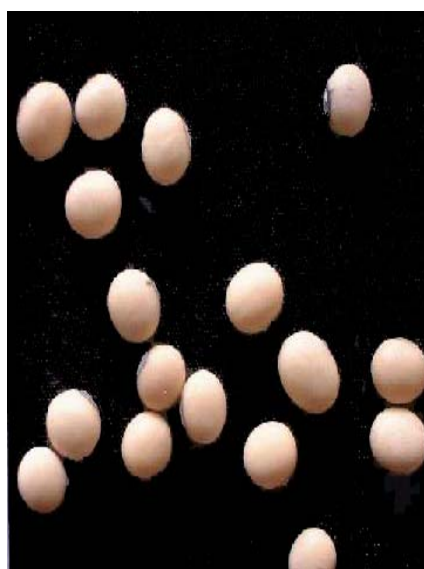


Figure 2: *Glycine max* seeds

Since no approaches have been attempted to detect antibacterial activity from this plant species, although it was reported that *Glycine wightii* demonstrated good antibacterial activity against *S. aureus* [8], therefore this study tried to discover the plant seed activity among four types of bacteria using different extracts.

## **Materials and methods**

### **Plant material**

*Glycine max* L. subjected to *Magnoliophyta* phylum, *Magnoliopsida* class, *Fabales* order, *Fabaceae* family [9]. Soybean seeds were purchased from the local market in Mosul city Nineveh province, and identified at College of Science Department of Biology.

### **Preparation of extracts**

Soybean seeds were grinded to powder using an electrical blender, three different solvents were used (petroleum ether, ethanol and chloroform) to obtain different extracts.

### **Petroleum ether, ethanol and chloroform extracts**

Plant material (seeds) were extracted using a soxhlet extractor with solvents of increasing polarity [10] beginning with petroleum ether followed by ethanol then chloroform, each extraction was carried out for 8-10 hours continuously until the used solvents turned pure and colorless. The solvents were removed using a rotary vacuum evaporator at 40°C to give concentrated extracts which were frozen and freeze-dried until use.

### **Preparation of extract concentrations**

1gm of each extract (petroleum ether, ethanol and chloroform) was dissolved in 5ml DMSO (Dimethylsulfoxide) to give an extract with concentration 200mg/cm<sup>3</sup> which was used as a standard concentration in providing next dilutions (100, 50, 25 and 12.5mg/cm<sup>3</sup>), then were sterilized via pasturalization at 62°C for 15 minutes [11].

### **Tested bacterial strains**

The microorganisms used included two Gram positive bacteria, *Staphylococcus aureus*, *Bacillus cereus* and two Gram negative bacteria, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* which all had been obtained and identified at Department of Biology, College of Science, University of Mosul, and were further diagnosed by using characteristics features to insure.

### **Preparation of inoculum**

All microorganisms were cultured overnight at 37°C in sterile Nutrient broth (Oxoid) and used as inoculum. The turbidity of the suspensions were adjusted to the McFarland 0.5 turbidity standard.

### **Antibacterial activity**

The antibacterial tests were performed using agar-well diffusion method [12]. Agar plates were prepared using sterile Nutrient agar (Oxoid). The bacterial inoculum was evenly spread onto the surface of the agar plates using sterile swab sticks. Wells (6 mm diameter) were

punched in the plates using a sterile stainless steel borer. Ten microliters of each extract concentrations were added in each well. Ten microliters of DMSO per well was used as a negative control. Antibiotic discs (Bioanalyse) 6mm in diameter of Chloramphenicol 30µg, Cephalexin 30 µg, Tetracycline 30 µg and Gentamycin 10 µg were used as positive controls. Diffusion of the extracts and DMSO was allowed at room temperature for 30 minutes before incubating at 37°C for 24 h. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells. The size of the zones of inhibition was measured and the antibacterial activity expressed in terms of the average diameter of the zone inhibition in millimeters. The absence of a zone inhibition was interpreted as the absence of activity. Each experiment was tested in triplicate.

## Result and Discussion

Study results showed various inhibitory effects of different extracts from *G. max* seeds against tested bacteria via agar-well diffusion method, (Table 1) revealed that petroleum ether extract was active against *S. aureus* and *Ps. aeruginosa* with inhibition zones of 15 and 16mm in diameter respectively, using the extract concentration 200 mg/cm<sup>3</sup>, this extract concentration showed close or even action against *Ps. aeruginosa* compared with the antibiotic Gentamycin, meanwhile the concentrations (50, 25 and 12.5 mg/cm<sup>3</sup>) achieved weak activity against mentioned bacteria, this can be resulted by small quantity of active components in the extract, the results also revealed the complete resistance of *B. cereus* and *Kleb. pneumoniae* against the petroleum ether extract, this can be attributed to the defensive agents of these bacteria such as plasmid resistance, in addition to capsule secretion by *Kleb. pneumoniae* which is a general bacterial defensive factor against antibiotics [13,14].

**Table 1:** Antibacterial activity of petroleum ether extract from *Glycine max* seeds.

Bacteria	Petroleum ether extract concentrations (mg/ cm <sup>3</sup> )					Antibiotics			
	200	100	50	25	12.5	C	CL	TE	CN
<i>Staphylococcus aureus</i>	15	14	11	10	9	21	15	22	19
<i>Bacillus cereus</i>	-	-	-	-	-	20	12	23	18
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	17	11	20	17
<i>Pseudomonas aeruginosa</i>	16	12	10	8	8	18	19	24	16

- : No activity, C: Cloramphenicol, CL: Cephalixin, TE: Tetracycline, CN: Gentamycin.

From (Table 2) it can be seen that ethanol extracts from soybean seeds demonstrated good inhibitory activity against all tested bacteria, with inhibition zones between 9-18mm in diameter, *B. cereus* (Figure 3) and *Kleb. pneumoniae* showed high susceptibility to ethanol extract in reversal of petroleum ether extract, compared with the antibiotic Cephalixin, the calculated zones reached 18 and 16mm in diameter respectively, this may refer to the ability of the ethanol solvent in dissolving active components from plants, it was also noticed that the minimum extract concentrations (25 and 12.5mg/cm<sup>3</sup>) were inactive in achieving antibacterial activities against tested bacteria except *B. cereus* which showed weak sensitivity towards these concentrations, and the proportion between activity and extract concentration was direct.

**Table 2:** Antibacterial activity of ethanol extract from *Glycine max* seeds.

Bacteria	Ethanol extract concentrations (mg/ cm <sup>3</sup> )					Antibiotics			
	200	100	50	25	12.5	C	CL	TE	CN
<i>Staphylococcus aureus</i>	13	11	10	-	-	21	15	22	19
<i>Bacillus cereus</i>	18	13	11	9	9	20	12	23	18
<i>Klebsiella pneumoniae</i>	16	13	10	-	-	17	11	20	17
<i>Pseudomonas aeruginosa</i>	12	10	9	-	-	18	19	24	16

- : No activity, C: Cloramphenicol, CL: Cephalexin, TE: Tetracycline, CN: Gentamycin.

Our results indicated that the chloroform extract concentration (200mg/cm<sup>3</sup>) from soybean seeds had moderate activity against *S. aureus*, *Kleb. pneumoniae* and *Ps. aeruginosa* (Figure 4) with inhibition zones between 13-16mm in diameter, these results were better than what the antibiotic Cephalexin achieved against *S. aureus* and *Kleb. pneumoniae* (Table 3). The investigation also showed that chloroform extract concentrations (25 and 12.5mg/cm<sup>3</sup>) showed weak inhibitory activities against *S. aureus* only, meanwhile other types resisted these concentrations, it was also noticed that *B. cereus* resisted the chloroform extract using all concentrations, this can be due to the scanty of antibacterial active components in chloroform extracts, in addition to the cooperating role between active compounds in showing inhibitory properties, if one or more of these compounds are separated it may decrease extract activity[15].

**Table 3:** Antibacterial activity of chloroform extract from *Glycine max* seeds.

Bacteria	Chloroform extract concentrations (mg/ cm <sup>3</sup> )					Antibiotics			
	200	100	50	25	12.5	C	CL	TE	CN
<i>Staphylococcus aureus</i>	16	13	10	9	9	21	15	22	19
<i>Bacillus cereus</i>	-	-	-	-	-	20	12	23	18
<i>Klebsiella pneumoniae</i>	13	9	8	-	-	17	11	20	17
<i>Pseudomonas aeruginosa</i>	15	14	11	-	-	18	19	24	16

- : No activity, C: Cloramphenicol, CL: Cephalexin, TE: Tetracycline, CN: Gentamycin.

The differences in extract activity against tested bacteria may be attributed to the cell wall complexation, muco polysaccharide extra cellular substances, in addition to the chemical complexation of bacterial cell wall and the number of its layers which also achieves resistance against antibiotics, and the role of cell wall pores in substance insertion inside bacterial cells, and ability of bacteria in changing the diameter of these pores so it can prevent large amount of substance insertion [16].

Since soybean oil has a high proportion of unsaturated fatty acid, and contain considerable levels of phenolic compounds, which are known to be synthesized by plants in response to microbial infection [17], it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [18].

Soybean seeds also contain levels of flavones which are phenolic structures containing one carbonyl group [19], these compounds exhibit



inhibitory effects against multiple bacteria and viruses, numerous studies have documented this effectiveness of flavonoids [20,21].

Finally all the extracts from *G. max* seeds showed weak activity against tested bacteria compared with the antibiotic Cloramphenicol, Tetracycline and Gentamycin, this can be attributed to small amount of active compounds in the used extract concentrations, and our conclusion could have been different if we used extract concentrations greater than 200mg/cm<sup>3</sup> due to the direct proportion between inhibitory activity and extract concentration in this study.

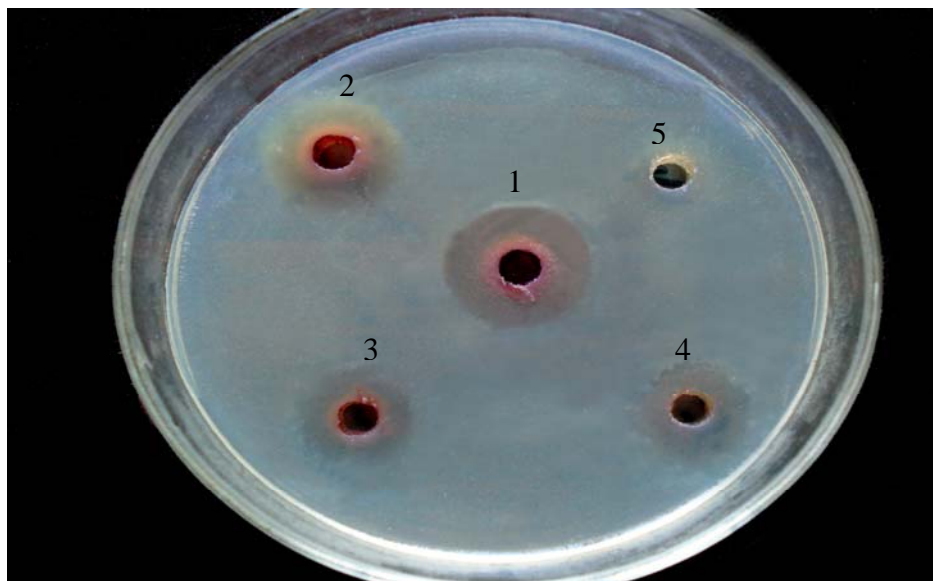


Figure 3: Effect of ethanol extract concentrations from *G. max* seeds against *B. cereus* 1(200mg/cm<sup>3</sup>), 2(100mg/cm<sup>3</sup>), 3(50mg/cm<sup>3</sup>), 4(25mg/cm<sup>3</sup>), 5(12.5mg/cm<sup>3</sup>).

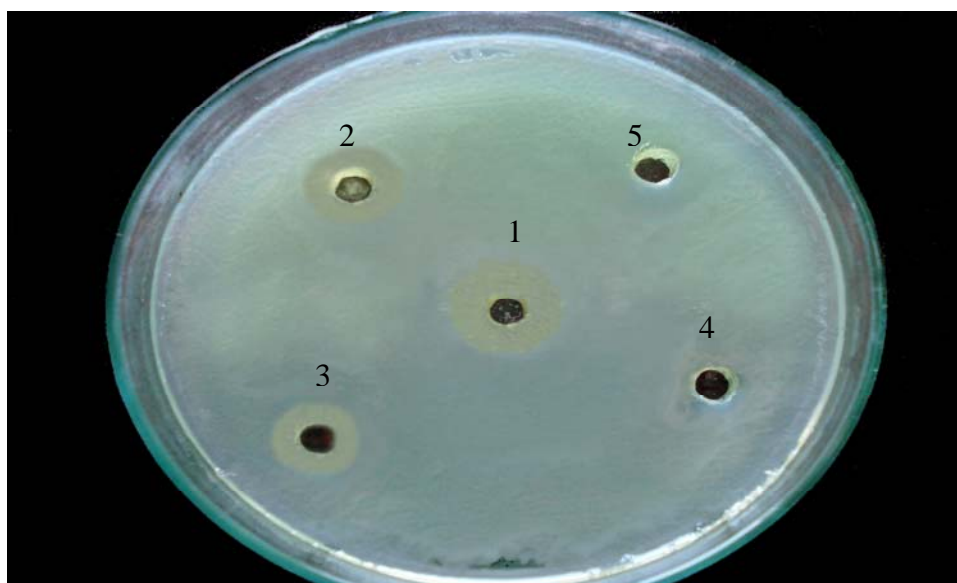


Figure 4: Effect of chloroform extract concentrations from *G. max* seeds against *Ps. aeruginosa* 1(200mg/cm<sup>3</sup>), 2(100mg/cm<sup>3</sup>), 3(50mg/cm<sup>3</sup>), 4(25mg/cm<sup>3</sup>), 5(12.5mg/cm<sup>3</sup>).

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