



Phytochemical screening, bioautography and antibacterial evaluation of the methanolic extract of *Glycine Max* (Soybean)

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ABSTRACT

Glycine max belongs to the family Fabaceae. It is called Soybean. Phytochemical analysis of *Glycine max* has revealed that numerous compounds in plants traditionally used for medicinal purposes have many therapeutical properties. The result of the phytochemical studies revealed the presence of Saponins, Tannins, Alkaloids, Steroids and numerous other chemicals. Saponins, Tannins and alkaloids are chemicals that are known to have anti-bacterial properties. The concentrations of the plant used were 25 mg/ml, 50 mg/ml and 100mg/ml respectively. At these concentrations, the extract inhibited the growth of *Escherichia Coli*, *Pseudomonas Aeruginosa* and *V. Harveyi* and produced percentage inhibition ranging between 67.6% to 82.8%. The anti-bacterial activity demonstrated by the plant extract may due to the presence of the phytochemicals present in the plant.

Keywords: *Glycine Max*, Phytochemical Screening, Saponins, Antibacterial, *V. Harveyi*

INTRODUCTION

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity.¹ There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious disease. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections.

Green plants are the indispensable storehouse of many chemical metabolites which are grouped into two categories namely: primary and secondary metabolites. Secondary metabolites are the

substances produced by plants as defense chemicals. They include alkaloids, flavonoids, essential oils, phenols, saponins etc. In India, different regions have specific features according to the climatic conditions. These plants including medicinal plants are also used as a feeding for animals. They are indirectly shown by their effects by which animals do not suffer by any types of diseases. Growing plants are one of the cheapest sources of feeding for animals having crude proteins of 14-25%.²

In addition to the high content of protein and fibres, legumes contain saponins, which are bioactive compounds. Saponins are a group of surface-active glycosides, which distinguishes them from other glycosides.³ The highest concentration of saponins occurs in soybeans (6500 mg kg⁻¹).⁴ The physiological role of saponins in plants is not yet completely cleared up. Saponins are secondary metabolites and play a role in the protection of plants against

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microorganism. Many saponins show strong antibacterial activities. As saponins are probably a part of plants' defence systems, they have been included in a group of protective molecules in plants called phytoprotectant.⁵

The effects of saponins at the intestinal level may also need attention, given its presence in some common dietary ingredients. Also several important systemic infections gain access to the body via the intestinal route. Infections of the intestine, along with the respiratory tract, and urogenital tract are the most common cause of mortality and morbidity in man⁶ has long been considered that a single dose of orally administered sub-unit vaccine would be the most useful means of protecting against these disorders.⁷ Saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss etc. according to medical field. It is a bioactive antibacterial agent of plants.⁸ The present study was designed to evaluate the fundamental phytochemical constituents and antibacterial activities of the *Glycine max*.

MATERIALS AND METHODS

Collection and Extraction

Soybean seeds were purchased from the commercial market at Nagercoil, kanayakumari district, Tamilnadu, India. The samples were collected in sterile polyethylene bags. The fresh seeds were dried under shade. Weighed around 10 gram of soybeans seed and washed air, dried at room temperature and coarsely powdered in a mixer sieved, and then mixed 5 gram fine powder of soybean with 80% of ethanol and stirred it, allow it freely for 3 days. It was then filtered and the residues were discarded. The filtrate was poured in plates, allow it to dry, and sample is successively extracted with methanol. The resulting extract was evaporated to dryness using hot air oven and stored at 4°C for further use.

Phytochemical screening^{9,10}

Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for saponins

0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for steroids

One ml of the extracts was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for terpenoids

2 ml of chloroform was added to 0.5 g each of the extract. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for triterpenoids:

Ten mg of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc. H₂SO₄. Formation of reddish violet colour indicates the presence of triterpenoids.

Test for anthraquinones

0.5g of the extract was boiled with 10 ml of sulphuric acid (H₂SO₄) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for Flavonoids

A few chop of 1% NH₃ solution is added to the aqueous extract of plant sample in a test tube. A yellow coloration is observed if flavonoids compound are present.

Test for alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloid base. The



chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides

To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Saponin Estimation Procedure

Weigh accurately 1.5 to 2 gm of the material in a beaker add 50 ml of petroleum ether and gently heat to 40°C on a water bath for 5 minutes with regular shaking. Filter the petroleum ether repeat the operation with further 2 X 50 ml of petroleum ether. Discard petroleum ether and preserve the marc. Extract the marc obtained in the previous test with 4 X 60 ml of methanol with mild heating. Filter the methanol layer to another beaker. Concentrate the combined methanol layer to about 25ml. Add 150 ml of dry acetone to precipitate the saponins. Filter the saponins through a filter paper and dry at 100°C for constant weight.

Calculation

Percentage of total saponins =

$$\frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

Bioautography

A TLC Bioautographic method was used to detect active components. After application of the extract on a silica gel plate, thin layer chromatography (TLC) was developed using ethylacetate:methanol (9:1) as the eluent system for *Glycine max*. Observe the

bands, the TLC plates were dried for complete removal of solvents. Then the fractions of TLC were spotted on already swabbed agar plates by bioautography method to evaluate the activity of the different essential compounds, and the plates were incubated at 35°C for 24 hours. The activity of compound can detect by its zone formation.

Antibacterial Screening

Test Organisms

The test organisms were standard laboratory strains of *Escherichia Coli*, *Pseudomonas Aeruginosa* and *V.Harveyi*. The organisms were obtained from the Department of marine Science (CMST), Manonmaniam Sundaranar University, Rajakkamangalam, Kanyakumari district, Tamilnadu, India.

Antibacterial activity

Muller-Hinton agar were poured on to sterile Petri plates. When the media solidified, 0.1ml of inoculums with 0.5 OD was poured over feeder layer and spread evenly with a sterile spreader. A well of 6mm diameter was made by using a sterile cork borer. Each well-received the extract was tested in a different concentration (25 mg/ml, 50 mg/ml and 100 mg/ml). Distilled water was used as negative control while ampicillin was used as positive control. And the commercial antibiotics like as Ampicillin and Tetracycline tested against pathogens. They were incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zone was measured.

RESULTS

Phytochemical Screening

The phytochemical screening of methanolic extracts showed the presence of different types of active constituents, namely alkaloids, anthraquinones, cardiac glycosides, flavonoids, terpenoids, tannins, Saponins, Sterols and triterpenes. These compounds were present in almost all the plants extracts. The details were given in the (Table 1). The total percentage of saponin was estimated from the *Glycine max*, and it was found that 2 g of *Glycine max* contains 40% of saponin molecule.



Table 1 Summary of the Results of Phytochemical Analysis of *Glycine Max*

S.No.	Phytochemical group	Result
1.	Alkaloids	+
2.	Saponins	+
3.	Flavonoids	+
4.	Tannins	+
5.	Steroids	+
6.	Terpenoides	+
7.	Titerpenoides	+
8.	Anthraquinones	+
9.	Cardiac glycosides	+

Note: + = Present

TLC Studies on *Glycine max*

On TLC analysis for the hot water extract *Glycine max* was revealed that, the single spot were obtained, and

it observed under UV-illuminator. The fraction obtained having the R_f values of 0.94. And it shows on Fig (1).



Fig 1: Thin Layer Chromatography (TLC) for Steroidal Saponin from *Glycine Max*

Bioautography

Bioautography method was used to detect active components by its zone formation. The maximum zone of inhibition is measured in 6.1 mm in dm. The minimum zone of inhibition for the fraction of *Glycine max* is 1.8 mm against *V.harveyi* (Table 2).



Table 2 Bioautography of the saponin activities of *Glycine max* against some pathogenic bacteria

S.No	Concentration (g/ml)	Pathogenic bacteria		
		Zone of Inhibition(mm)		
		<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>V.harveyi</i>
1	1.0 gram	4.2 mm	3.8 mm	4.5 mm
2	2.0 gram	5.9 mm	5.4 mm	6.1 mm
3	3.0 gram	2.7 mm	1.4 mm	1.8 mm

Antimicrobial activity.

The antimicrobial activities of the plant extracts against the three bacteria strains examined were assessed by the presence or absence of inhibition zones. The aqueous extract of *Glycine max* exhibited moderate level antimicrobial activity against

Escherichia coli, *Pseudomonas aeruginosa* and *V.harveyi* the test organisms. Methanol extract of *Glycine max* was active against all the test organisms except *Pseudomonas aeruginosa*. On the other hand, it was found that the methanol extract of *Glycine max* exhibited high activity against *Escherichia coli* and *V.harveyi* (Fig. 2)

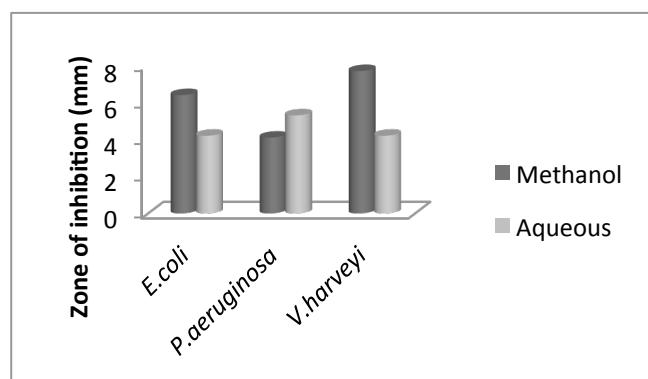


Fig 2: Antibacterial activity *Glycine max* against pathogenic microorganism

To screen the antibacterial activity against tested organisms, ampicillin and tetracycline were used as a standard. It was found that tetracycline (5µg/ml)

standard showed higher activity than ampicillin (30µg/ml) standard against tested microorganisms (Fig. 3).

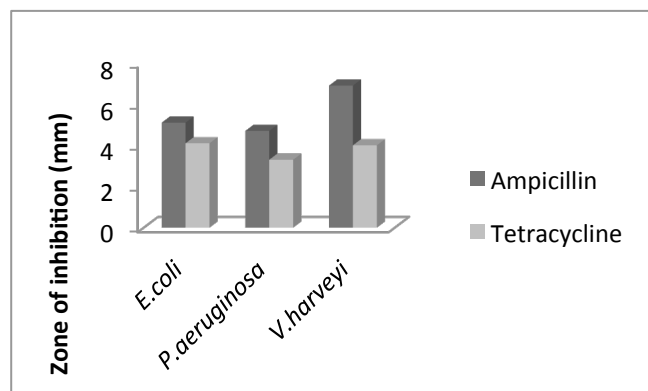


Fig 3: Antibacterial activity Ampicillin and Tetracycline against pathogenic microorganism



DISCUSSION

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials.¹¹ Continued further exploration of plant-derived antimicrobials is needed today. The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism.¹² These compounds have significant therapeutic application against human and aquaculture pathogens including bacteria, fungi or virus.

Vegetable soybean is rich in phytochemicals beneficial to the human being and is therefore considered a nutraceutical or a functional food crop.¹³ Saponins may be considered a part of plants' defence systems, and as such have been included in a large group of protective molecules found in plants.¹⁴ The present study focuses on both the phytochemical analysis and antimicrobial potential of *Glycine max*. In the present investigation, different extracts of *Glycine max* was evaluated for exploration of their

antimicrobial activity against certain bacteria, which was regarded a pathogenic microorganism. Susceptibility of plant extract was tested by agar well diffusion method was determined.

The results of our studies have shown that *Glycine max* contains Saponins, Tannins, Flavonoids, Steroids, Alkaloids and Cardiac glycosides. The plant extract also showed antibacterial activity at concentrations of 25 mg/ml, 50 mg/ml and 100mg/ml respectively. At these concentrations, the extract inhibited the growth of *Escherichia coli*, *Pseudomonas aeruginosa* and *V.harveyi* and produced percentage inhibition ranging between 67.6% to 82.8%. Therefore, the ethnomedical application of the plant in the treatment of bacterial infections is justified.

The antibacterial activity of aqueous extract (25 mg/ml, 50 mg/ml, and 100 mg/ml) showed that the extract has activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *V.harveyi* (Table. 3). Tannins are polyhydroxy compounds and have been reported show both antioxidant and antimicrobial activity (Min *et. al*, 2008). Flavonoids are also a group of polyhydroxy compounds that is known to have both antimicrobial and antioxidant activity. At 100 mg/ml, the extract produced the highest percentage inhibition of 80.8% on *V.harveyi*. At 25 mg/ml, the extract produced least percentage inhibition of 67.6% on *V.harveyi* (Table 3).

Table 2 Bioautography of the saponin activities of *Glycine max* against some pathogenic bacteria

Test organisms	Percentage inhibition of growth (%)				
	<i>Glycine max</i> (25mg/ml)	<i>Glycine max</i> (50mg/ml)	<i>Glycine max</i> (100mg/ml)	Ampicillin (100 mg/ml)	Distilled water
<i>Escherichia coli</i>	65.0	70.6	73.0	98.8	0.0
<i>Pseudomonas aeruginosa</i>	65.0	65.2	75.0	98.8	0.0
<i>V.harveyi</i>	67.6	68.0	82.8	98.8	0.0

Also, the percentage inhibition of growth produced by the extract on the other bacterial strains tested ranges between 65% to 75% (see Table 3). By

observing Table 2, it can be deduced that *Glycine max* showed a remarkable antibacterial activity when compared with Ampicillin (positive control) which produced a percentage inhibition of 98.8% (Table 3).



Therefore, the antibacterial activity exhibited by this plant extract may be due to the presence tannins, saponins and flavonoids in plant which have been reported to have antibacterial properties.

In conclusion, of the present investigation *Glycine max* contain potential antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against

various diseases. The aqueous extracts of *Glycine max* possess significant inhibitory effect against tested pathogens. The results of the study support the folklore claim along with the development of new antimicrobial drugs from the plant.

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