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Research Paper

Antibacterial Evaluation of the Methanolic and Chloroform Extract of *Glycine max* (Soybean)

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ABSTRACT

Glycine max is one of the most popular and nutritious foods with high antimicrobial effects. The present work was done to study the antibacterial activity of Methanolic and chloroform extracts of *Glycine max* against gram negative bacteria such as *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa,* the gram-positive bacteria such as *Basillus subtilis* and *Staphylococcus aureus.* The concentrations of the seed used were 500 µg/ml, 750 µg/ml and 1000 µg/ml respectively. At these concentrations the extract inhibited the growth of *Escherichia coli, Pseudomonas aeruginosa, Salmonella thphi*, *Bacillus subtilis* and *Staphylococcus aureus* and produced zone of inhibition ranging between 7 mm to 10 mm in Methanolic extract and 6 mm to 14 mm in chloroform extract. The antibacterial activity reported by the Soybean extract may be due to the presence of the phytochemical compounds present in the seeds. Highest zone of inhibition 10 mm was noted in *Staphylococcus aureus* at 1000 µg/ml and 750 µg/ml concentration and lowest zone of inhibition 7 mm was found in *Escherchia coli* at 750 µg/ml and 500 µg/ml, *Bacillus sabtilis* at 500 µg/ml, and *Pseudomonas aeruginosa* at 750 µg/ml and 500 µg/ml respectively.

***Key words*:** *Glycine max,* Methanolic, Chloroform, Antibacterial, Zone of inhibition

Medicinal plants have continued to attract attention in the global search for effective antibacterial agents that can combat resistant pathogens that have been rendering many conventional drugs absolute in the treatment of infections. The antibacterial compounds produced by plants are active against plant and human pathogenic microorganisms [1]. The active principles isolated from plants appear to be one of the important alternatives when compared with many substandard orthodox synthetic medicines because of their less or no side effect and better bioavailability. Plant extracts have been studied against pathogens for years for assays to detect new and previously undiscovered antibacterial from plant sources [2].

In spite of the considerable progress of medicine, treatment of infectious diseases faced with different unfavorable problems. Occurrence of severe levels of the antibiotic resistance is the main issue facing medical practitioners [3]. Antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria [4]. It is an increasingly serious threat with global public health impact that requires action across all government sectors and society [5]. Resistant bacteria caused more severe clinical diseases for longer period of time which causes abundant economic losses [6]. Documented data revealed that pathogenic bacteria and especially Staphylococcus aureus (*S. aureus*), *Escherichia coli* (*E. coli*), *Bacillus cereus* (*B. cereus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Listeria monocytogenes* (*L. monocytogenes*), *Klebsiella pneumoniae* (*K. pneumoniae*) *and Salmonella typhi (S. typhi*) exhibited the high levels of resistance against aminoglycosides, tetracyclines, lincosamides, macrolides, beta-lactams, quinolones, fluoroquinolone and cephems groups of antibiotics [7]. Therefore, therapeutic and pharmacological factories tried to use from novel sources for antimicrobial agents to produce strong antibiotic drugs. Application of medicinal plants for producing of antimicrobial agents had an ancient history [8]. Soybean (*Glycine max*) belongs to a large botanical family Leguminosae which classically grows in tropical, subtropical and temperate climatic regions like Iran [9]. Soybean is an oilseed and consists of 20% oil content. It is considered as the most significant crop for the production of edible oil. Soybean plant holds great importance in today's world mainly because of its high protein and lipid content and other major constituents including vitamins, minerals, fatty acids and other essential nutritional factors [10].

Besides. Soybean holds much importance from medicinal perspective. Recorded data revealed the high antimicrobial and antioxidant content of the *G. max*. Soybean seeds are rich in proteins, isoflavones and phytoestrogens, while Genistein, a soy isoflavone, has also been reported to possess anti-cancerous, antioxidant, anti-inflammatory and anti-osteoporosis effects and is considered as potential compound for metabolic disorders' treatment. 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 [11]. There is a

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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.

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 [12]. The highest concentration of saponins occurs in soybeans (6500 mg kg^{-1}) [13]. 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 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 [14]. Soybean is an oilseed and consists of 20% oil content. Because of this characteristic, it is considered as the most important crop for the production of edible oil. The top five countries with maximum soybean production at present are USA, Brazil, Argentina, China and India, and these contribute to 92% of the world's soybean production. Soy contains significant amounts of all the essential amino acids for humans, and so is a good source of protein, which is generally used to prepare extracts or powders for medicinal use. The current investigation aims to explore scientifically the antibacterial potential of crude and pure saponin seed extract of *Glycine max* plant.

MATERIALS AND METHODS

Plant materials

Glycine max L., subjected to Magnoliophyta phylum, Magnolipsida class, Fabales order, Fabaceae family [15]. Soybean seeds were purchased from the local market in Kanchipuram district of Tamil Nadu and identified at P.G. and Research Department of Botany, Arignar Anna Government Arts College, Cheyyar, Thiruvannamalai district of Tamil Nadu, India.

Preparation of extracts

Soybean seeds were grinded to powder using an electrical blender two different solvent were used chloroform and methanol to obtain different extracts.

Methanol and chloroform extracts

Plant materials (seeds) were extracted using a Soxhlet extractor with solvents beginning with methanol than chloroform, each extraction was carried out by 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.

Tested bacterial strains

The microorganism used induced two-gram positive bacteria stphylococus aureus, *Bacillos sabtilis* and three-gram negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* along with standard Amphieillin (20 µl/disc). Stock cultures were maintained at 4°C on nutrient agar slant. Active cultures or experiments were prepared by transferring a loop fell of culture from the stock cultures into the test tubes containing nutrient broth, that were incubated at 24 hrs at 37°C. The assay was performed by agar disc diffusion method.

Agar disc diffusion method (NCCLS, 1993)

Antibacterial of sample was determined by disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton agar (MHA) medium is poured into the Petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc was placed in MHA plates and add 20 µl of sample (concentration 1000 µg, 750 µg, and 500 µg) were placed in the disc. The plates were incubated at 37°C for 24 hrs. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition.

RESULTS AND DISCUSSION

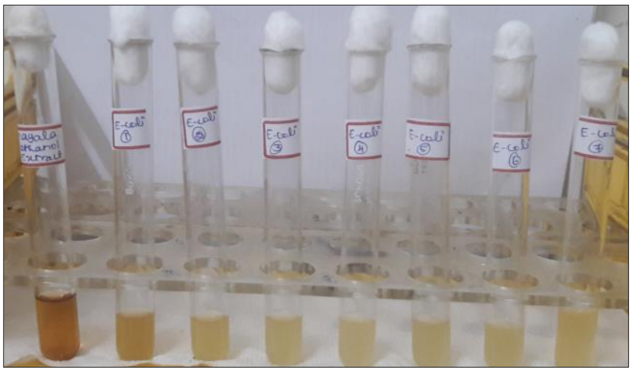
Study results showed various inhibitory effects of different extracts from *Glycine max* seeds against tested bacteria via agar-well diffusion method (Table 1-2, Fig 1-2) revealed the methanol. Extract was active against *Staphylococcus aureus* with zone of inhibition 10 mm in diameter at 1000 µg/ml and 750 µg/ml respectively. *E. coli* showed 9 mm, 7 mm and 7 mm at 1000 µg/ml, 750 µg/ml and 500 µg/ml. the *Bacillus* showed 9 mm, 9 mm and 7 mm at 1000 µg/ml, 750 µg/ml and 500 µg/ml, *Staphylococcus aureus* showed 8 mm at 500 µg/ml, *Salmonella* showed 9 mm, 9 mm and 8 mm at 1000, 750, 500 µg/ml and *Pseudomonas aurtginea* showed 9 mm, 7 mm and 7 mm at 1000, 750, 500 µg/ml respectively (Table 1, Fig 1).

Table 1 Antibacterial activity of soybean methanol extract on bacterial strains

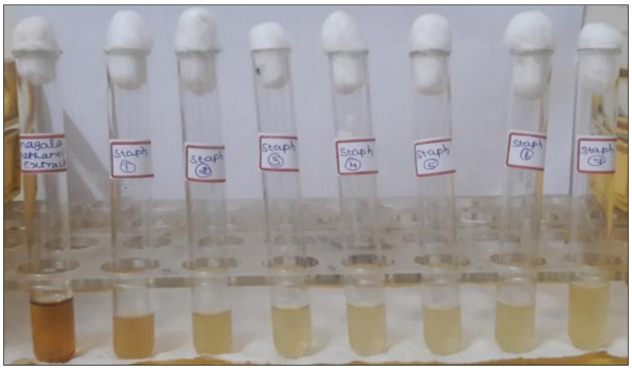
Organisms	Zone of inhibition (mm)			Antibiotic (1mg/ml)
	1000 µg/ml	750 µg/ml	500 µg/ml	
Escherichia coli	9	7	7	12
Bacillus subtillis	9	9	7	14
Stphylococuss aureus	10	10	8	11
Salmonella typhi	9	9	8	16
Pseudomonas aeruginase	9	7	7	12

Table 2 Antibacterial activity of *Glycine max* (soybean) chloroform extract on bacterial strains

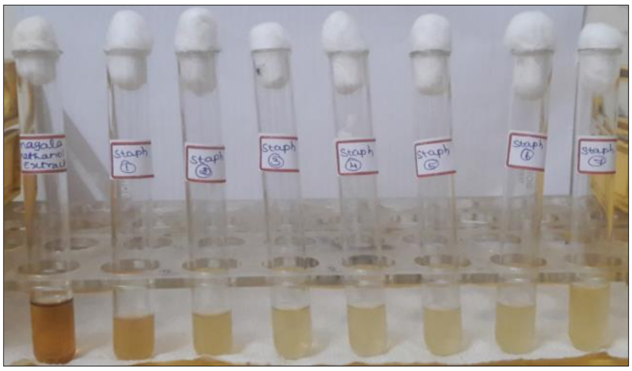
Organisms	Zone of inhibition (mm)			Antibiotic (1mg/ml)
	1000 µg/ml	750 µg/ml	500 µg/ml	
Escherichia coli	10	9	6	14
Bacillus subtillis	9	9	7	14
Stphylococuss aureus	8	7	6	11
Salmonella typhi	9	7	7	17
Pseudomonas aeruginase	14	13	10	18



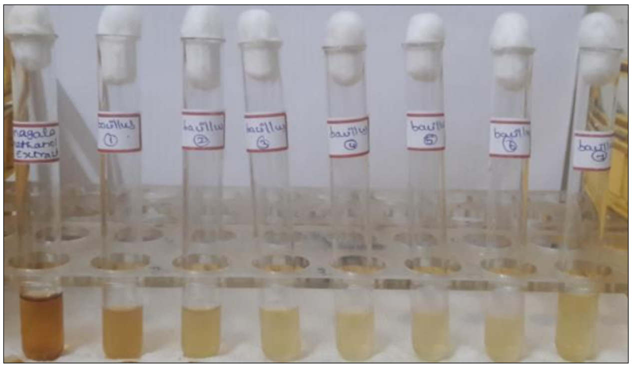
Escherichia coli



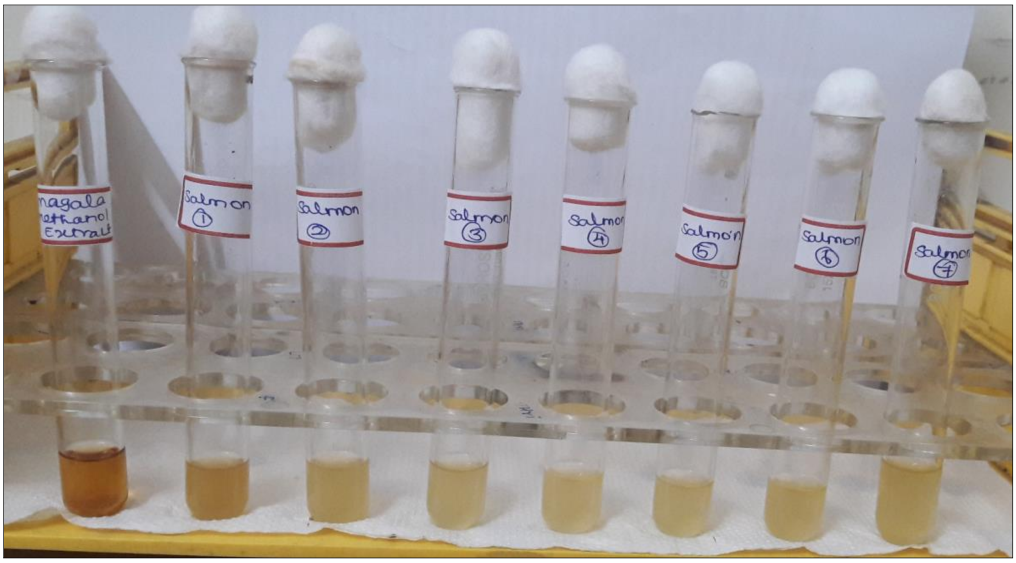
Staphylococcus aureus



Bacillus subtilis



Pseudomonas aeroginosa



Salmonella spp.

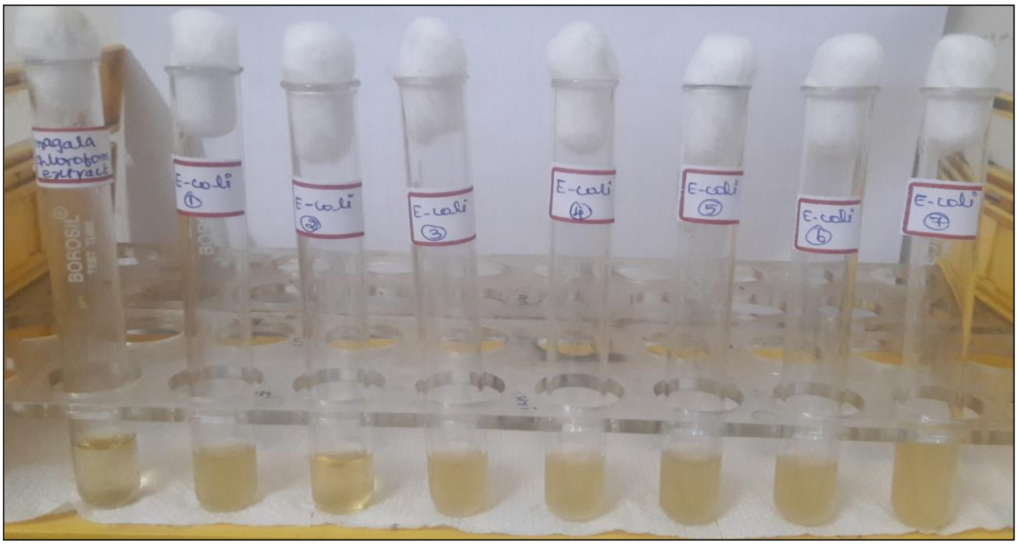
The chloroform extract showed highest zone of inhibition 10 mm at 1000 µg/ml in *Pseudomonas auraginasa* followed by 13 mm and 10mm at 750 and 500 µg/ml in the same bacteria. The *E. coli* bacteria showed 10, 9, 6 mm *Bacillus subtillus* showed 9, 9, 7 mm, *Staphylococus aures* showed 8, 7, 6 mm and *Salmonella typhi* showed 9, 7, 7 mm at 1000, 750 and 500 µg/ml concentrating the chloroform extract respectively (Table 2, Fig 2). From the (Table 2) it can be seen that chloroform extracts from soybean seeds indicated good inhibitory activity against all tested bacteria with inhibition zones between 7 to 14 mm in diameter.

The present work revealed that methanolic extract of *G. max* had significant antimicrobial effects on tested bacteria and especially staphylococcus aureus, *Escherichia coli*, *Bacillus sabtilis*, *Salmonella thphi* and *Pseudomonas aeruginosa* at concentration of 1000, 750 and 500 µg/ml showed 10, 9, 8, 7 mm zone of inhibition [16]. They reported that *Glycine max* is a rich source of Phenols, Saponins, Micronutrients, Flavonoids and Poly saccraides which may be responsible for its high antimicrobial effects. Phenolic compounds are well known to have a negative effect on the growth of bacteria through inhibitory their nucleic acid synthesis, enzymatic activity, cytoplasmic membrane function and energy metabolism [17]. The chloroform extracts of *G. max* showed (6-14mm) 14, 13, 10, 9, 8, 7, 6 mm zone of inhibition at 1000, 750 and 500 µg/ml of concentration. The highest zone of inhibition 14 mm was found in *Pseudomonas aeruginosa* at 1000 µg/ml of concentration [18]. They showed that soybean isoflavones inhibited the nucleic acid synthesis of *S. aureus*, in addition phenolic acids were found at significant concentrations and their antimicrobial activity has been widely investigated. The synergic, cousmetic, ferulic and vanilic acids which are formed in the *Glycine max* extracts exhibited significant antimicrobial activity against gram-positive and gram-negative bacteria [19].

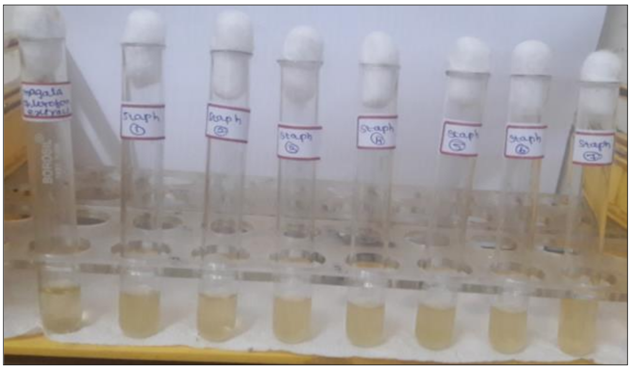
In the present study the gram-positive bacteria were more susceptible against *G. max* than gram-negative bacteria and the chloroform extracts showed high antibacterial activity than methanol extracts [20]. They reported that among tested bacteria the highest antimicrobial activity was observed against *L. monocytogenus, B. cereus* and *E. faccalis* with values around 50% inhibition even at the lowest concentration [21]. This is may be due to the assumption that gram-negative bacteria are more resistant to antibacterial compounds due to their outer lopopoly saccharide membrane.

MIC values of chloroform extract

Name of the organism	Minimum inhibitory concentration (µg/ml)
Escherichia coli	500
Staphylococcus aureus	250
Bacillus subtilis	250
Pseudomonas aeroginosa	500
Salmonella spp.	500



Escherichia coli



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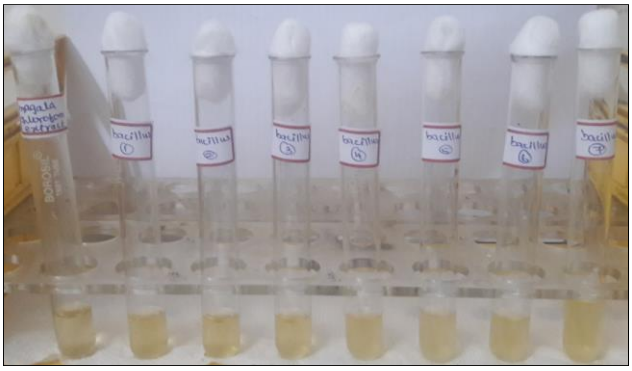

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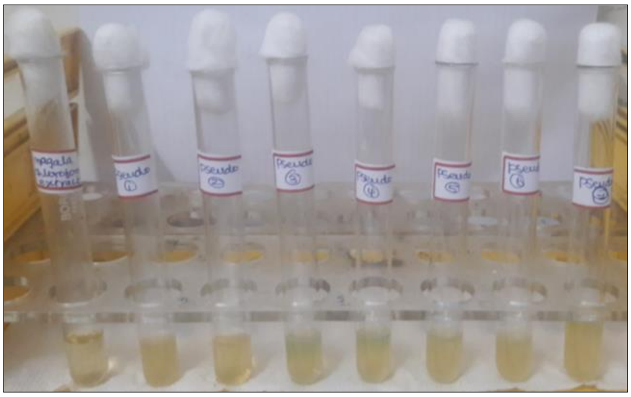
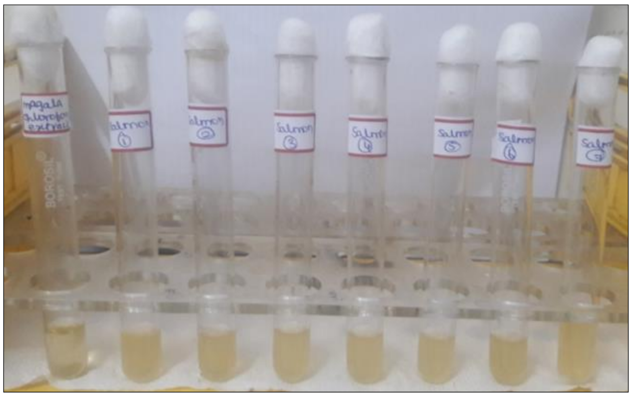


Pseudomonas aeroginosa



Salmonella spp.

CONCLUSIONS

The present study indicated that the Methonolic and Chloroform extracts of *Glycine max* (Soybean) showed high antibacterial activity against both gram negative and positive bacteria such as *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*, the gram-positive bacteria such as *Basillus subtilis* and *Staphylococcus aureus.* This antibacterial activity may be due to presences of various phyto-constituents present in the plant seed extracts. So, from the study we concluded that the seed extracts of *Glycine max* could be used as alternative to anticancer, antibiotics, vaccines, and chemotherapeutic agents.

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