Isolation, Characterization and Antibacterial Activity of Crude and Purified Saponin Extract from Seeds of Soyabean (*Glycine Max*)

T. Kumaran[1, 2]* and T. Citarasu[1]

[1]Centre for Marine Science and Technology
Manonmaniamsundaranar University, Rajakkamangalam
District: Kanyakumari-629502

[2]PG and Research Department of Zoology,
Muslim Arts College, Thiruvithancode, District: Kanyakumari-629174
*Corresponding author: kumaranmac@gmail.com

Abstract

In the present investigation, the inhibitory effect of crude and purified saponin extract from Glycine max was evaluated against some pathogenic bacterial strains (gram positive and gram negative) using agar disc diffusion method. Agar well diffusion method was adapted to determine antibacterial activity against all the test microorganisms. Zone of inhibition of the crude and pure extracts were tested. Among all the test pathogens Vibrio harveyi was found most susceptible with zone of inhibition 16 mm. to the crude and purified saponin extract. The present study successfully isolated and characterized the single unit of Glycine max saponin with 27-kDa units. It also reveals the least susceptibility of the microorganisms towards the saponin isolated from seeds of Glycine max.

Keywords: Saponin, Glycine max, Antibacterial activity.

1. Introduction

Antibiotics are undeniably one of the most important therapeutic discoveries of the 20thcentury that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products. This is because of the emergence of resistant pathogens that is beyond doubt the consequence of years of widespread indiscriminate use, incessant misuse and abuse of antibiotics [8]. Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs [9].

For thousands of year's natural products have played a very important role in healthcare and prevention of diseases. The ancient civilizations of the Chinese, Indians and North Africans provide written evidence for the use of natural sources for curing various diseases [4]. However, it was not until the nineteenth century that scientists isolated active components from various medicinal plants. According to recent studies conducted by the World Health Organization (WHO), about 80%of the world's population relies on traditional medicine WHO, 2002. About121 drugs prescribed in USA today come from natural sources, 90 of which come either directly or indirectly from plant sources [2].

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 obsolete in the treatment of infections. The antibacterial compounds produced by plants are active against plant and human pathogenic microorganisms [3].The active principles isolated from plants appear to be one of the important alternatives when compared with many sub standard 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 [7].

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.

2. Materials and Methods

2.1 Plant Material Collection and Extraction

Seeds of Glycine max were purchased from the commercial market at Marthandam, kanayakumari district, Tamilnadu, India. The collected seeds were washed and shade dried. The dried seeds were disinfected with 15%H_2O_2 and stored at room temperature in sterile sealed bottled until its extraction. Dried powder plant seed materials were boiled at above 100°C with two hour. After filtered the extracts, the supernatant was collected and the residue were discarded. The supernatant was condensed in the water bath and the condensate was extracted again by methanol. The methanolic extract was concentrated in rotatory evaporator under reduced pressure at the room temperature of 45°C to 50°C in order to avoid the evaporation of plant materials. Aqueous extract was concentrated using Lyophilizer and stored at 4°C. The final powder crude were dissolved in sterile buffer and used for antibacterial studies Andrea et al., 2012.

2.2 Protein characterization by SDS-Page

The protein profiles of Glycine max saponin were analyzed by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) as described by Laemmli in 1970. The antibody was separated with a 4% stacking and 12% separating gel. Glycine max extract were taken (10µl) and treated with an equal amount of sample treatment buffer. The samples were loaded into sample wells along with a known molecular weight markers (Genei Pvt. Ltd, Bangalore) and the electrophoresis was performed at 100_V current. When bromophenol blue dye reached at the bottom of the gel, the electrophoresis was stopped. The gel was removed and was stained with coomassie brilliant blue R-250 for 30 minutes. The gel was rinsed with distilled water and destained. The molecular weight of the proteins resolved was estimated in comparison to the molecular weight markers.

2.3 Pathogens tested for antibacterial activity

The five bacterial strains used in the present study were the clinical isolates obtained from Scadar laboratory and CMST Rajakkamangalam. The bacterial strains used were Bacillus subtilis, Escherichia coli, Vibrio harveyi, Aeromonas hydrophila and Vibrio parahaemolyticus. The effect of plant extract on the several bacterial strains was assayed by agar well diffusion method.

2.4 Antibacterial activity by agar disc diffusion method

Antimicrobial activity of each plant extracts determined by using a modified Kirby Bauer (Bauer et al., 1966) disc diffusion method. Briefly, broth culture of test bacteria was spreaded on the Muller Hinton Agar media in petriplates and microbes broth culture were applied on media by swabbing, under lab condition. Petriplates containing 20 ml Muller Hinton medium were seeded with 24 h culture of bacterial strains. Well were cut and 20 ml of the plant extracts were added. The plates were then incubated at 37^0C for 24 h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around well. Chloramphenicol was used as positive control.

3. Results and Discussion

Saponin was isolated from the Glycine max seeds in crude and purified form. Saponin can be isolated in a good yield, with approximately 42±2 mg of protein per 500g seeds. Electrophoresis on non-denaturing gels indicates that the Saponin is quite pure, with only a single band detected on a Coomassiestained gel (Fig. 1). Molecular mass analysis of the protein as compared with the standard essentially all the protein was of the 27- kDa form.

The antibacterial activity of seed extracts of Glycine max were screened in vitro by agar well diffusion method using chloramphenicolas the standard positive control against selected bacterial strains. The results of seed extracts of Glycine max using extracts were shown in Tables 1respectively.Among the methanolic extract of Glycine max against Vibrio harveyi (16 ± 1) possess maximum zone and minimum inhibition was observed in methanol extract against E. coli (13 ± 0.2).Similar observations were made by Saad et al. [6] in which the methanolic extract of seed showed highest activity than leaf.

The antibacterial activities of crude and purified Glycine max extract**.** Saponin showed the moderate potency against major pathogens. All the purified Saponin from the seeds of the plant Glycine max tested showed highest activity against most of the pathogens .The susceptibility was exhibited by the crude and purified extract of Saponin from Glycine max against Vibrio harveyi with the average diameter of zone of inhibition as 9.11 mm (Table 1). All other microbial strains under the present investigation showed resistance towards both the crude and purified Saponin extracted from the seeds Glycine max plant respectively.

Table 1: Crude and pure Saponin from Soyabean extract against some selected microorganisms

S. No.	Test organisms	Zone of Inhibition (mm)		
		Chloramphenicol	Crude Saponin	Pure Saponin
1	*Bacillus subtilis*	14.32 ± 0.5	14.07 ± 1.2	15.54 ± 0.5
2	*Escherichia coli*	12.04 ± 0.6	9.05 ± 0.5	13.17 ± 0.2
3	*Vibrio harveyi*	11.08 ± 0.2	8.02 ± 0.6	16.07 ± 0.8
4	*Aeromonashydrophila*	13.04 ± 0.5	9.47 ± 0.2	14.32 ± 0.2
5	*Vibrio parahaemolyticus*	12.01 ± 0.8	10.84 ± 0.6	15.49 ± 0.7

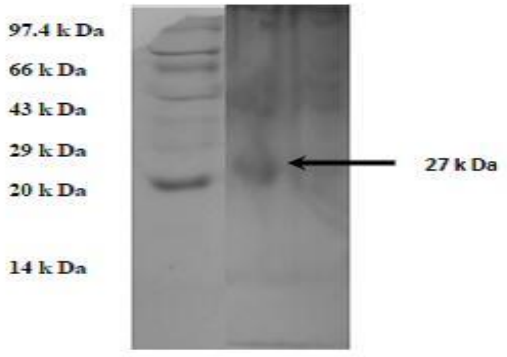

Figure1. Polyacrylamide gel electrophoresis of purified (Glycine max) saponin.

4. Conclusion

The result of the antibacterial assay showed promising evidence for the antibacterial effect of leaves and seeds of Glycine max. Traditionally plant parts, extracts and decoctions are used for treatment against microorganisms. Screening of in vitro antibacterial activity of the medicinal plant involves various approaches which contribute for the different degree of susceptibility (Zone of inhibition) against different micro organisms. We selected to study Saponin from soyabean (Glycine max**)** seeds because these seeds are among the highest sources of plants. However, results reveal that the crude and purified Saponin from Glycine max seed may contribute for the antibacterial activity. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

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