PHYTOCHEMICAL ANALYSIS, PROTEIN CONTENT & ANTIMICROBIAL ACTIVITIES OF SELECTED SAMPLES OF GLYCINE MAX LINN

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Abstract

Two seed samples of Glycine max Linn. (S1, S2) were purchased from two retail stores of local market. Non-sprouted and sprouted seed powder were extracted separately with methanol (100%, 50%) by cold maceration to obtain methanolic and hydroalcoholic extract of Glycine max Sample 1 was designated as MES1 and HES1 and sample 2 as MES2 and HES2 respectively. Phytochemical analysis indicated the presence of various phytoconstituents viz. phytosterols, flavonoids, phenolic compounds, tannins, carbohydrates, proteins, amino acids, fixed oils and fats etc. Thin layer chromatography study on extracts revealed the presence of a number of compounds. The protein content of these samples were studied. The protein content of samples MES1, HES1, MES2 and HES2 with respect to BSA was found to be 90.6 2µg/ml, 82µg/ml, 94.5µg/ml and 79.1µg/ml respectively. The highest among these were found to be in MES2. Sprouting enhanced the protein content of the two samples. The samples have shown antimicrobial activity at selected concentration and microbial strains (26mm) for gram negative bacteria (27mm) for gram positive bacteria.

Keywords: *Glycine max Linn, phytochemical constituents, TLC, antimicrobial activity, protein, methanolic extract, hydroalcoholic extract.*

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1. INTRODUCTION

Soybean is classified in family leguminosae and it is a good source of protein and used in several industries, especially for oil extraction and animal feed industry (1). An antimicrobial is a substance which inhibits the growth of microorganisms such as bacteria, protozoan's or fungi. Antimicrobial drugs either prevent or kill the growth of microorganisms. Soy products act as antimicrobial agents against the bacteria and the fungus (2) (8).

2. MATERIALS AND METHODS

2.1 Collection of Material:

Two seed samples Glycine max Linn. (S1, S2) purchased from two retail stores of Roopnagar in the month of january 2013. The seeds were washed with water, then it was dried. The dried samples were grinded properly using a grinder, to obtain the powdered form. The powder of the seeds were stored in air tight containers (9).

2.2. Seed Germination

Soybean seeds were selected and washed with water. A cotton bed was used to spread and germinate seeds. 150 gram of seeds was spread on wet-cotton bed at room temperature. Seeds were covered with similar type of cotton covering. Water was sprinkled as and when required to keep bedding wet. Sprouts with 1.5-3.0 cm germinate length were picked up for analysis. It took about 72 hours for seeds to germinate up to this length. Fine powder of raw clean seeds and their sprouts were prepared for extraction. Sprouting are better techniques to improve the protein content (3).

2.3 Preparation of Extracts

2.3.1. Cold Maceration

Sprouted and non-sprouted seed powder of samples was extracted separately with methanol (100%, 50%) by cold maceration to obtain methanolic and hydroalcoholic extract of Glycine max Sample 1 (MES1 and HES1 respectively) and for sample S2 (MES2 and HES2). Seed powders (30g) of each was extracted trice with 280 ml of methanol for 3 hour in an electrical shaker at 40ºC. The extracts were filtered through Whatman No.1 filter paper and evaporated. Yield of the extracts is weighed. Each extract were transferred to glass vials and kept at 4º C (4).

2.3.2 Successive Extraction Method

The non-sprouted seed powder of Glycine max samples S1, S2 were subjected to successive extraction with solvents in increasing order of their polarity viz. hexane, chloroform, ethyl acetate, ethanol and water. In this method, powder materials were passed through sieve no. 40 and used for extraction. A weighed quantity of powder was extracted in Soxhlet apparatus for 16 h using twice the amount of solvent. The extract was evaporated to dryness at 40°C in rotary vacuum evaporator. The extracts were placed in dark bottles and stored in refrigerator at 4°C until use (5)

2.4 Phytochemical Screenings

The extracts obtained from cold maceration i.e methanol extract (GMME) and extracts obtained from successive extraction i.e hexane extract (GMHE), chloroform extract (GMCE), ethyl acetate extract (GMEAE), ethanol extract (GMEE) and residual aqueous extract (GMAE) were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as flavonoids, alkaloids, glycosides, phenolic compounds, steroids, tannins, amino acids, carbohydrates, proteins and fats. The various tests were carried out to identify the various phytoconstituents present in hexane, chloroform, ethyl acetate, ethanol and aqueous extracts (5, 9).

2.5 Thin Layer Chromatography

The spots of test samples were applied on TLC plates, keeping a minimum distance of 1 cm between the two adjacent spots. The spots of the samples were marked on the top of the plate to know their identity. Silica gel G was uesd as adsorbent in thin layer chromatography. Then, TLC plates are placed in chromatography chamber containing solvent solution. The experiments were carried out at room temperature in diffused daylight. Compressed air sprayer with a fine nozzle was used to detect the different constituents present on TLC plates. Air compressor was attached to a glass sprayer. After each spray, the sprayer was washed separately with water, chromic acid, distilled water and then with acetone. Other, UV chamber can be used for the substance exhibiting fluorescence nature. Maximally, the results were observed at 254 nm and 366 nm of UV light and at day light (6).

2.6 Estimation of Protein Content by Lowry Method

Take 0.2 ml of BSA working standard in 5 test tubes and make up to 1ml using distilled water.

Add 4.5 ml of Reagent I and incubate for 10 minutes. After incubation add 0.5 ml of reagent II and incubate for 30 minutes. Measure the absorbance at 660 nm and plot the standard graph. Estimate the amount of protein present in the given sample from the standard graph.

2.7 In Vitro Testing of Extracts for Antimicrobial Activity:-

2.7.1. Agar Cup Method:

The agar cup method was adopted for determination of antibacterial activity of the prepared extracts. 1.2 ml of standardized bacterial stock suspensions was thoroughly mixed with 50 ml of sterile nutrient agar. 40 ml of the inoculated Nutrient agar were distributed into sterile petri dishes. The agar was left to set and in each of these plates 4 cups, 4 mm in diameter, was cut using a sterile borer and the agar discs were removed. Alternate cups were filled with 0.1 ml of each extracts dissolving in DMSO (20 mg/ml) using microtiter-pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 h. After the incubation period formation of zones around the wells, confirms the antibacterial activity of the respective extracts. The same procedure was followed for each strain and extract. Each experiment was carried out in triplicates. Doxycyline (500 µg) was used as standard drug (7).

Table 1: Details of micro-organisms (bacteria) used for study

S. No.	Name of micro organism	Strain	Code
1	Staphylococcus aureus	Gram-positive bacteria	MTCC 87
2	Escherichia coli	Gram-negative bacteria	MTCC 40
3	Pseudomonas alcaligenes	Gram-negative bacteria	MTCC 493
4	Pseudomonas fluorescens	Gram-negative bacteria	MTCC 103

3. RESULTS

Table 2: Phytochemical screening of various extracts of two samples of Glycine max Linn

Phytochemical tests	Extracts				
	HE (S1,S2)	CE (S1,S2)	EAE (S1,S2)	EE (S1,S2)	AE (S1,S2)
Phytosterols					
Leibermann	-	+	-	+	-

Burchard's test					
Leibermann's reagent	-	+	-	+	-
Salkowaski test	-	+	-	+	-
Fixed oils and fats					
Strain test	+	-	-	-	-
Alkaloids					
Mayer's reagent	-	-	-	+	+
Dragendorff's reagent	-	+	-	+	+
Wagner's reagent	-	-	-	-	+
Hager's reagent	-	+	-	+	+
Carbohydrates					
Molish's test	-	-	-	-	+
Fehling's test	-	-	-	+	+
Benedict test	-	-	-	-	_
Legal test	-	-	-	+	+
Proteins and amino acids					
Millon's test	-	+	-	+	+
Biuret test	-	-	-	+	+
Phenolic compounds and Tannins					
Lead acetate solution	-	-	+	+	+
Dilute iodine solution test	-	-	+	-	+
Fecl3 solution	-	-	+	+	+
Flavonoids					
Lead acetate test	-	-	+	+	+
Shinoda test	-	+	+	+	+
Glycosides					
Legal's test	-	-	-	-	+
Liebermann-Burchard test	-	-	-	+	+

-: absent, +: present; HE: Hexane extract; CE- Chloroform extract; EAE-Ethyl acetate extract; EE-Ethanol extract; AE-Aqueous extract

3.1 Phytochemical Study

Phytochemical study shows the presence of phenolic, flavonoids and tannins in greater amount mainly in EAE, EE and AE. Other types of phytoconstituents are also present like phytosterols, fixed oils ans fats, alkaloids, carbohydrates, protein and amino acids, glycosides.

3.2 Thin Layer Chromatography:

The table represents the various coloured spots observed with their Rf values in respective extracts using different solvent systems and detecting agents.

Table 3: TLC profile of various extracts of Glycine max Linn seed samples

Extracts	Solvent system	Ratio	No. of spots	Detecting agent	Rf
EE	Toluene: Acetone: CHCl3	4: 2.5: 3.5	S1 = 5	A.S.	0.05,0.23,0.28 ,0.74, 0.95
			S2 = 5		0.05,0.15,0.56 ,0.75, 0.81
CE	Chloroform : Methanol	9.7: 3	S1 =4	A.S.	0.330.37, 0.41, 0.75
			S2 =4		0.24,0.41,0.47 , 0.75
EAE	Chloroform : Methanol	9.7: 3	S1 =3	A.S.	0.06, 0.56, 0.2
			S2 =2		0.03, 0.5
CE	Toluene: Ethyl acetate: formic acid: chloroform	3:3:0 .8: 0.2	S1 =3	A.S.	0.6, 0.9, 0.8
			S2 =3		0.66, 0.8, 0.92
HE	Toluene: Acetone:C HCl3	4: 2.5: 3.5	S1 =3	A.S.	0.68, 0.73, 0.81
			S2 =3		0.66, 0.69, 0.8
EE	Toluene: Ethyl acetate: formic acid: chloroform	3:3:0 .8: 0.2	S1 =3	A.S.	0.21, 0.74, 0.9
			S2 =3		0.23, 0.72, 0.87

EE- Ethanol extract, CE- Chloroform extract, EAE- Ethyl acetate extract, HE- Hexane extract, (S1, S2), AS- Anisaldehyde sulphuric acid.

3.3 Total Protein Content:

The protein content of samples MES1, HES1, MES2 and HES2 with respect to BSA was found to be 90.6 2µg/ml, 82µg/ml, 94.5µg/ml and 79.1µg/ml respectively. The highest among these were found to be in MES2. Sprouting enhanced the protein content of the two samples.

3.4 In Vitro Testing of Extracts for Antimicrobial Activity:

Antibacterial activity of various extracts of Glycine max Linn. was determined by agar cup method. Zone of inhibition were measured in mm (including bore diameter 4mm).

Table 4: Effect of antimicrobial activity of MES1 containing different concentrations against different microorganisms

Effect of MES1				
Microorganism→ Conc.↓ µg/ml	S. aureus	E. coli	P. alcaligenes	P. fluorescens
50	7	8	8	-
100	12	13	10	-
200	18	19	14	-
Doxycyline (100 µg/ml)	28	29	18	-

Table 5: Effect of antimicrobial activity of HES1 containing different concentrations against different microorganisms

Effect of HES1				
Microorganism→ Conc.↓ µg/ml	S. aureus	E. coli	P. alcaligenes	P. fluorescens
50	10	9	7	--
100	17	15	9	--
200	26	27	17	--
Doxycyline (100 µg/ml)	32	31	28	--

Table 6: Effect of antimicrobial activity of MES2 containing different concentrations against different microorganisms

Effect of MES2				
Microorganism→ Conc.↓ µg/ml	S. aureus	E. coli	P. alcaligenes	P. fluorescens
50	9	7	8	--
100	15	11	11	--
200	25	22	21	--
Doxycyline (100 µg/ml)	30	29	30	--

Table 7: Effect of antimicrobial activity of HES2 containing different concentrations against different microorganisms

Effect of HES2				
Microorganism→ Conc.↓ µg/ml	S. aureus	E. coli	P. alcaligenes	P. fluorescens
50	8	7	9	--
100	11	12	15	--
200	23	23	28	--
Doxycyline (100 µg/ml)	30	30	31	--

Values are mean inhibition zone (mm), (--) No zone of inhibition; Inhibition zone including 4 mm bore diameter.

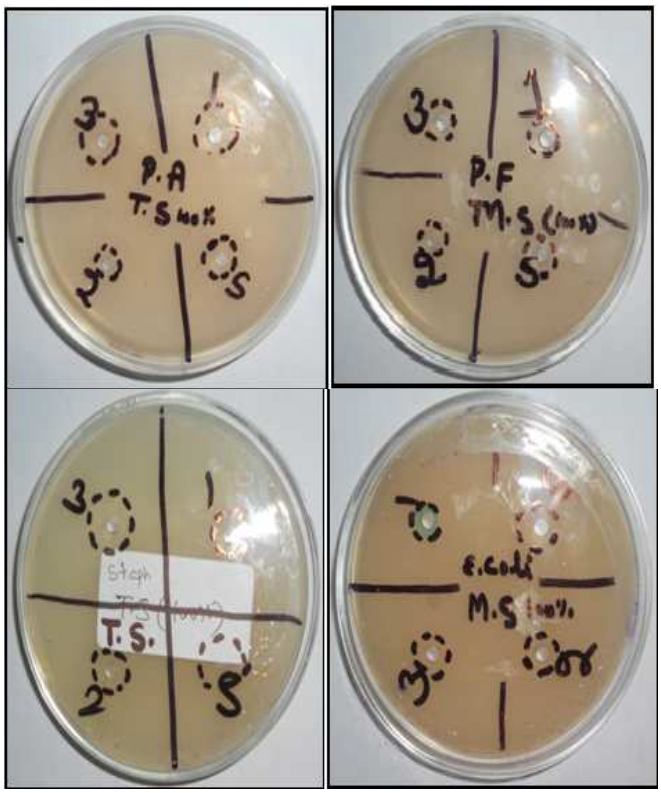

Figure 2 a, b, c, d: Antimicrobial Activity of Glycine max Linn. seed sample

CONCLUSIONS

Phytochemical analysis indicated the presence of various phytoconstituentsviz.phytosterols, flavonoids, phenolic compounds, tannins, carbohydrates, proteins, amino acids, fixed oils and fats etc. Thin layer chromatography study on extracts revealed the presence of a number of compounds. The protein content of samples MES1, HES1, MES2 and HES2 with respect to BSA was found to be 90.6 2µg/ml, 82µg/ml, 94.5µg/ml and 79.1µg/ml respectively. The highest among these were found to be in MES2. Sprouting enhanced the protein content of the two samples.

There was significant enhancement of antibacterial activity of all extracts with increasing concentration for selected microbial strains. At higher concentration i.e. 200 µg/ml the results were comparable with the standard Doxycycline. The study showed that HES1 show more potent activity against Staphylococcus aureus and Escherichia coli, where as HES2 show more potent activity against Pseudomonas alcaligenes among others extracts.

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