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Soyasaponin characterization and phytochemical investigation of the extract of soybean (*Glycine max*)

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Abstract
Phytochemicals from medicinal plants serve as lead compounds for drug discovery and design. Medicinal plants are the richest resource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. 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. Thin-layer chromatography (TLC) fractions were screening method was used to detect active components. The Fourier Transform Infra Red (FTIR) spectroscopic analysis revealed that the selected extracts might have the functional group of alkenes, carbonyl group, amide, aldehyde and ketonic functional group of the *G. max* respectively. The may due to the presence of the phytochemicals present in the plant. The results suggest that the phytochemical properties demostrated by the plant extract for curing various diseases and leads to the isolation of new and novel compounds.

Keywords: *Glycine max*, phytochemical, saponins, TLC, FTIR

1. Introduction
A knowledge of the biological activities and/or chemical constituents of plants is desirable, not only for the discovery of new therapeutic agents, but because such information may be of value in disclosing new sources of such economic materials such as tannins, industrial oils, gums, precursors for the synthesis of complex chemical substances, etc. A knowledge of the chemical constituents of plants would further be valuable to those interested in chemotaxonomy and to those interested in deciphering the actual value of folkloric remedies. Phytochemical screening and phytopharmacology are two main approaches used in the discovery of new biologically-active plant principles.

Many plants have been investigated in recent times and found to contain active substances that are medically useful, whereas many more are yet to be scientifically investigated. Recent studies have showed that plants with medicinal values with antibiotic resistant bacteria can be used in drug discovery which need of an hour phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening (Sonali nigam and shrivastava, 2013) [15]. 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 Harbone (1973) [11]. 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.

Phytochemical constituents such as alkaloids, flavonoids, tannins, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms. In India, different regions have specific features according to the climatic conditions (Kumaran & Citarasu 2015) [12]. 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% (Babu Shankar *et al.*, 2011) [1]. 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 (Francis *et al.*, 2002) [10]. Saponins are used antioxidant, antimicrobial, and anti-inflammatory etc. according to medical field. It is a bioactive antibacterial agent of plants Yoshiki (1998) [18]. The present study was designed to evaluate the fundamental phytochemical constituents of the *Glycine max*.

2. Materials and Methods
2.1 Collection and Extraction
Seeds of *Glycine max* shall do remained as one of the world's most important sources of oil and protein. Soybean (*G. max*) seeds were obtained from the local market at Nagercoil, Kanyakumari District, India. Shadow dried seed powder plant 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.

2.2 Phytochemical screening
(Sofowora, 1993; Trease, 1989) [14, 16]. This screening was carried out with the methanolic extracts using chemical methods and Thin-layer chromatography (TLC) according to the methodology given in Wagner and Bladt 1996 [17].

2.3 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 40^0C 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 100^0C for constant weight.

a. Calculation

$$\text{Percentage of total saponins} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \text{ X } 100$$

2.4 Fourier Transform Infra-Red (FTIR) spectroscopic analysis
The selected hot water extract such as *G. max* were analyzed qualitatively for the active compounds by Fourier transform infra red (FTIR) method described by Kemp (1991) [2]. AFourier Transform Spectroscopy is simply a technical variant, of a common infra-red spectrometer which can yield an intensity signal as a function of wavelength or spectral colour. The setup differs from a classical grating or prism spectrometer in that way that does not record the spectral intensity directly as a function of wavelength but an interferogram is taken instead.

2.4.1 Preparation of KBr discs
KBr discs were prepared by grinding the dried extract (0.1 – 2.0 by weight) with KBr by compressing the whole into a transparent water or disc. The KBr was dried, and it is an advantage to carry out grinding under an infrared lamp to avoid condensation of atmospheric moisture, which gives broad absorption at 3500/ cm.

2.4.2 Infra Red analysis
The frequency of the spectra set to analysis was between 400 – 5000/cm wave number and the vibration spectrum was recorded as graphical chart. The instrument used for FTIR analysis was Shimadzu, Japan.

2.4.3 FTIR Spectroscopy to organic molecules
Organic functional groups differ from one another both in the strength of the bond(s) involved, and in the masses of the atoms involved. For instance, the O – H and C = O functional groups each contain atoms of different masses connected by bonds of different strengths. According to equation (1), we therefore expect the O – H and C = O groups to absorb IR radiation at different positions in the spectrum. The presence of a strong, broad band between 3200 and 3400/ cm indicates the presence of an O – H group in the molecule, while the presence of a strong band around 1700/ cm confirms the presence of a C = O group.

For organic molecules, the infrared spectrum can be divided into three regions. Absorptions between 4000 and 1300/ cm are primarily due to specific functional groups and bond types. Those between 1300 and 909/ cm, the fingerprint region, are primarily due to more complex interactions in the molecules, and those between 909 and 650/ cm are usually associated with the presence of benzene rings in the molecule. Particularly some important regions are indicated below as vibrational frequencies for organic molecules.

3. Results
3.1 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: Phytochemical Analysis of *Glycine max* plant material extract.

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

Note: + = Present

3.2 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 0.87. And it shows on Fig (1).

Fig 1: Thin layer Chromatography (TLC) for the steroidal saponin from *Asparagus racemosus*

3.3 Functional group analysis

Fourier Transform Infrared Spectroscopy analysis for the *G. max* extract active fractions is given in the table 2. The possible functional groups of active principles were analyzed between in wave number 500- 4000 /cm

Table 2: Molecular stretches of active principles isolated from the active fraction of ethanolic extract of *G.max* through FTIR-Spectroscopic analysis

S. No	Peak value	Bond Type	Specific Context
1	559.16	C-X	C-Br
2	571.00	C-X	C-Br
3	878.53	Alkenes	1,2,4,5
4	1042.49	C-X	C-F
5	1272.34	C-C	C-C
6	1378.94	N-O	R-S(=O)$_2$-OR'
7	1643.97	C-C	C=C
8	2925.89	C-H	C_{SP} 3-H
9	2973.54	C-H	C_{SP} 3-H
10	3329.86	O-H	ROH

3.3.1 *G. max*

From the above spectral data (figure 2), the ethanolic extract of the active fraction, *G. max* showed the broad peak around 3329.86 /cm, which was due to O-H stretching. The presence of this peak revealed the fact that the sample contained a primary or secondary amine or an amide or substituted amide group in the sample. The peak at 2973.54 /cm and 2925.89 /cm, which was due to C-H stretching presence of C-H groups. The presence of C=C (olefinic band) was confirmed by the peak at 1643.97 /cm. The peaks at 1378.94 /cm, 1272.34 /cm and 1042.49 /cm corresponded to the presence of N-O, C-C and C-X respectively. The presence of the peak at 878.53 /cm confirmed the presence of Alkenes. The peaks 571 /cm, 559.16 /cm corresponded to the C-X stretching presence of C-Br bond respectively. The presence of these bonds strongly supports the fact that the sample was a highly halogenated one.

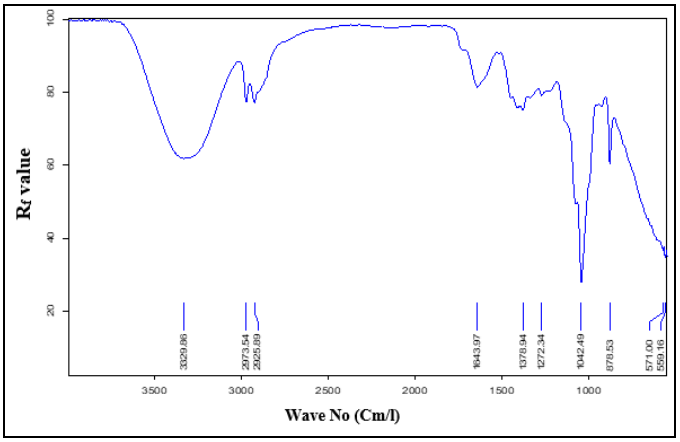

Fig 2: Characterization of the functional groups of saponin extracted from *G.max* by FTIR analysis

4. Discussion

Plants are the storehouses and rich sources of safer and cheaper chemical compounds. These natural plant products have been reported to have various activities like antistress, growth promoters, appetiser, tonic, immunostimulants and antimicrobials (Citarasu *et al.*, 2002) [8]. Moreover, the substances are obtained from natural sources, besides possessing other interesting properties like non-toxic, biodegradable and biocompatible (Citarasu *et al.*, 2003) [9].

Saponins may beconsidered a part of plants' defence systems, and as such have been included in a large group of protective molecules found in plants (Morrissey and Osbourn, 1999) [13].

The present study focuses on the phytochemical analysis 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 FTIR study revealed that, the ethanolic extract of *G. max* had alkenes, primary or secondary amine or an amide or substituted amide, olefininc band, C-X and C-Br bond. The peaks of 571 /cm, 559.16 /cm and 2973.54 /cm corresponded to the presence of C-X, C-Br and C-H bond respectively. The presence of C=C (olefinic band) was confirmed by the peak of 1643.97 /cm. It was believed that the adjuvant activity of saponins could be related to branched sugar chains or aldehyde groups (Bomford *et al*., 1992) [3] or to an acyl residue bearing the aglycone (Kensil, 1996). Latter, soyasaponins and lablabosides were found to show strong adjuvant activity despite lacking acyl residues and possessing only un-branched sugar chains (Oda *et al*., 2000) [5]. Oda *et al*. (2000) [5] concluded that not only the functional groups themselves, but the overall conformation of such functional groups, affected adjuvant activity of saponins.

Soyasaponins, lablabosides and purified *Quillaja* saponin-21, which possess adjuvant action, have only two to four O atoms, equally distributed around the aglycone, and may retain the typical amphipathic features. On the other hand, escins without adjuvant activity have seven O atoms, with five localized around one side of the aglycone, thus reducing its hydrophobic and adjuvant nature. Saponins from soybean was separated into six (Khalil and El-Adawy, 1994) [7] or eight (Oda *et al*., 2003) [6] fractions of soyasapogenol and soyasaponins groups. Oda *et al*. (2000; 2003) [5, 6] found that the soyasaponins exhibited high adjuvant activity. Finally, the extract of *G. max* showed that the spectral bands were prominent at 571 /cm, 559.16 /cm, 1249.97 /cm and 2973.54 /cm (amide, olefininc band, C-X, C-O, and C-Br bond). In the present study the active fraction of hot water and methanol extracts of two different herbal active fractions were analyzed by FTIR and the peaks represent various functional groups in the samples. The results of the present study, will be useful in the commercial utilisation of *G. max* plant as sources of saponins, steroids, triterpenoids and tannins. The findings may provide useful information with regard to its identification and standardization in future.

5. References

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