Preliminary Studies on Glycine Max Ethanol Extract (GMEE)

Deepa Gopinath*

IAPMO Plumbing Codes and Standards India Pvt. Ltd., Bangalore-560100, India.
Senior Scientist, Microbiological Research and Developmental Lab, Bangalore-560100, India.
E-mail: deepa.microfamily@gmail.com*

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ABSTRACT

Present study deals with the preliminary study of Glycine Max Ethanol Extract (GMEE). GMEE stacks more macro and micro nutrients with many pharmacological and nutraceutical standards. GMEE was preliminary screened by simple test methods and instrumentation methods such as RP-HPLC, IR and GC-MS. The obtained results from IR predicted the presence of different functional groups such as OH, CH₂, C=O, C-O and cyclic ring. While, the RP-HPLC and GC-MS profiles of GMEE predicted the presence of lipids, polyphenols, alkaloids and flavonoids in the extract.

Keywords: Glycine Max Ethanol Extract (GMEE), GC-MS, RP-HPLC and IR.

Introduction

Glycine Max is very rich in several beneficial phytoconstituents [1], it is one of the most common crop cultivated in European countries due to its wide range of pharmacological and nutraceutical properties [2]. Glycine max is also commonly called as soybean and it falls under legumes family [3]. The great value of glycine max in health sector industries, increases the value of its consumers day by day [4]. Especially is Asian continent the vegetable soy bean is well established legume in human diet [5]. Genistein, biochanin and daidzein are the major biological active iso-flavonoids are present in the glycine max [6].

Moreover, due to its increase in the popularity of glycine max as a nutraceutical product, USA importing soy bean 25000 tones every year [7]. Regular consumption of soybean reduces the risks factors associated with various cancers such as prostate, mammary and many other chronic inflammatory diseases [8]. Furthermore, iso-flavonoids present in the glycine max reported that it increase the level of HDL and lower the level of LDL cholesterol [9]. Numerous phytochemicals are present in the glycine max which may exert many pharmacological and nutraceutical properties which may be still unknown [10]. Thus, to evolve the unknown phytochemicals present in the glycerin max this preliminary study of glycine max ethanol extract was carried out as described further below in this manuscript. I believe this research study may help in one in another way to new emerging bud researcher to evaluate the unknown phytochemicals from glycine max.

Materials and Methods

All the chemicals used were of analytical grade.

Glycine Max Ethanol Extract (GMEE) preparation

Glycine max were purchased from local market of Bangalore. From the glycine max, GMEE was extracted by the solvent ethanol using Soxhlet extraction method. The finally obtained extract was termed as GMEE (Glycine Max Ethanol Extract) and it utilized for further assays.
Karl fisher titration method

Moisture content of GMEE was identified by Karl fisher (Kf) titration method.

Briefly, 10mL of GMEE (in conical flask) was titrated against Kf reagent containing free iodine using Kf instrument (light brown end point) and note down the burette reading.

\[
\text{Calculation: } \% \text{ of moisture} = \frac{\text{Burette reading} \times 5.8 \text{ Kf value}}{\text{Weight of sample}} \times 100 \quad (1)
\]

Residue on ignition

To quantify the amount of inorganic metal ions in the extract, 1g of GMEE was added to pre-weighed crucible and weight was recorded. Then it was kept in muffle furnace at 350°C for 2hr. After the incubation period crucible was again weighed and record the weight.

\[
\text{Calculation: } \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad (2)
\]

Where,

\(W_1\)- Weight of empty crucible
\(W_2\)-Weight of GMEE + crucible (before incubation time)
\(W_3\)-Weight of GMEE + crucible (after incubation time)

Test for Carbohydrates

GMEE (100µg) was mixed with few drops of Benedict’s solution and boiled in water bath. Observed for reddish brown precipitation.

Test for Proteins

GMEE (100µg) was treated with 10% NaOH solution and add 2 drops of 0.1% CuSO\(_4\) solution. Observed for violet pink color.

Test for Lipids

GMEE (100µg) was treated with 0.5N alcoholic K\(_2\)OH and add 1 drop of phenolphthalein as indicator. This solution was heated in water bath for 1 hr. Observed for white color foam.

Test for Alkaloids

GMEE (100µg) was treated with few drops of Hager’s reagent saturated picric acid solution. Observed for yellow precipitation.

Test for tannins

GMEE (100µg) was treated with gelatin solution. Observed for white precipitation.
Test for steroids

GMEE (100µg) was mixed with few-drops of acidic anhydride boiled and cooled. Then concentrated sulphuric acid was added by sides of the test tubes.

Observed the formation of brown ring at the junctions of two layers.

Test for Flavonoids

GMEE (100µg) was treated with sulphuric acid and observed for the formation of orange color.

Phenol test

GMEE (100µg) was treated with 5% ferric chloride and observed for the formation of deep blue or black color.

Glycosides Test

GMEE (100µg) was hydrolyzed with concentrated HCL for 2hr on a water bath and filtered. Few mL of above filtrate was shaken with chloroform and add 10% of ammonia. Formation of pink color indicated the presence of glycosides.

IR spectrum

Fourier Transform Infrared (FT-IR) spectrum was recorded on Agilent FT-IR-4100 spectrophotometer in the spectral range of 650-4000cm⁻¹ taking the sample in the form of ATR powder discs (Baker S et al., 2012)

Reverse Phase High Performance Liquid Chromatography analysis

GMEE (10µg) was subjected to RP-HPLC using C₁₈ column (150mm×4.60mm, particle size 5µm) with PDA detector in shimadzu LC-20AD prominence. The column was pre-equilibrated with 0.1% Trifluoroacetic acid (TFA) in water and it was eluted at the flow rate of 1ml/min in linear gradient mode.

GC-MS

GC-MS analysis of samples was analyzed on quadrupole mass spectrometers in the Electron Capture Negative Ion Chemical Ionization (ECNICI) mode with capillary column (30X0.25mm IDX1EM df, composed of 100% Dimethyl poly siloxane).

Helium (99.9%) gas was used as carrier gas at the flow rate of 1ml/min and the injection volume of 0.5 µl (split ratio of 10:1). Temperature program was set as follows, injector temperature 250°C; ion-illuminator temperature 280°C, oven temperature 110°C (isothermal for 3min) with an increase in temperature of 20°C/min to 220°C, thereafter 5°C/min to 300°C. Mass spectrum was taken at 80ev; a scan interval of 0.5s [8].

Results and Discussion

Physical and chemical Characterization of GMEE

The extracted GMEE was look like fairly gray color and pungent odor. To know the chemical composition of GMEE, simple test tube chemical analysis was done. Interestingly, GMEE shows positive response for lipid, alkaloid, phenols and flavonoid tests with trace amount of inorganic metal ions and 10% of moisture content.
Fourier Transform Infrared (FT-IR) spectroscopy

The IR spectrum of GMEE confirms that presence of stretching vibrational band for OH group, aromatic C-H group, coordinated carbonyl (C=O) stretching of CHO group and the bending form C-H aliphatic groups.

Fig. 1. FT-IR Spectra of GMEE

RP-HPLC

Further the presence of phytochemicals (Alkaloids, flavonoids, lipids and phenolic compounds) in GMEE was confirmed by RP-HPLC chromatogram. GMEE (10µg) was injected to C18 Column (5mm, 0.21X25cm) which was pre-equilibrated with 0.1% Tri-Fluoro Acetic Acid (TFA) in water and sample was eluted in gradient mode by increasing the concentration (0-100%) of 0.1% TFA in acetonitrile for 20min at the flow rate of 1mL/min and monitored at 280nm.

Fig. 2. RP-HPLC chromatogram
Quantification by GC–MS

Moreover, the presence of phytochemicals (Alkaloids, flavonoids, lipids and phenolic compounds) in GMEE was also adjudged by GC-MS chromatography technique. Interestingly, as like RP-HPLC chromatogram in GC-MS also 11 major peaks were obtained (Fig.03).

![GC-MS Chromatogram of GMEE](image)

**Fig.3. GC-MS Chromatogram of GMEE**

Conclusion

In conclusion, this study demonstrates the characterization of GMEE. Thus, isolation and purification of active compound sounds to be good.

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Declarations

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**Competing Interests Statement**

The author declares no potential conflict of interest with respect to the authorship and publication.

**Consent for publication**

Author declares that he/she consented for the publication of this research work.

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