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Phytoconstituents and antioxidant Activity of extracts of Glycine Max Seeds

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ABSTRACT

Glycine max is a medicinal and economical plant, belonging to the genus fabacea widely used in Mediterranean countries. In this study, we have evaluated the phytochemical constituents and its antioxidant i.e. DPPH, Hydrogen Peroxide, Reducing Peroxide, total polyphenols and flavonoids contents of methanolic and aqueous extracts of seeds. The total polyphenols content of the methanolic extract was 769 and aq 1139 mg/ml. µg GAE/ mg extract and flavonoids was 107, 120 mg/ml. In the DPPH assay, Aq extract showed the higher scavenging capacity (IC50 followed by aqueous extract with IC50 value 69.17 mg/ml and methanolic extract 4.17 mg/ml.

Keywords: Glycine, Phytoconstituents, IC_{50}, DPPH

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INTRODUCTION:

Free radicals balanced cell growth in human body. It prevents cell damage and or death. Anything which acts adjacent to is known “anti" and "to oxidize" is to unite with oxygen is called antioxidants. Antioxidant is play important role for maintain our body free radicals for preventing ant cellular damage[1,2] . The oxidation induce by Reactive oxygen species in cell membrane crumbling, membrane protein injure and DNA mutation, which can auxiliary instigate or promulgate the development of various diseases [3, 4] i.e. diabetes, inflammation, genotoxicity, , cancer, alzheimers disease and cataracts , rheumatism , retinopathy, skin disease, senile dementia stroke, porphyria [5, 6].

Plant rich Antioxidant such as flavonoids, tannins, coumarins, curcumanoids, xanthons, lignans and terpenoids. These are obtained from various plant parts i.e. roots, bark, steam, leaves, fruit and seeds. Therefore, there is upward interest in unraveling these bioactive compounds and with them as natural antioxidants [7].

Herbal medicine is a very important form of medicine for healthcare professionals which are mostly used for mankind. Herbal medicine is being worn by people belonging to all the cultures. It is an indissoluble part of the maturity of modern evolution. The information on herbs was compiled and stored in to precise herbal pharmacopoeias. In the 20thcentury majority of the pharmacopoeia of scientific medicine was derived from the traditional knowledge of natives regarding medicinal uses and health benefits of various plants. Present era, herbal drugs have a chief share amongst the usually used drugs. Approximately 70% of drugs marketed now-a-days have their derivation in natural products[8].

Glycine max commonly known as Soyabean is a shrub of the family of Fabaceae. It is native from the Israel, Mediterranean region, north of central Europe and Eastern Central Asia. It has been found wide range of soya protein.

Scientific name: Glycine max (L.) Merr [9,10]

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Magnoliopsida
Order: Fabales
Family: Fabaceae
Subfamily: Faboideae
Genus: Glycine
Species: G. max

MATERIALS AND METHODS

1. **Soyabean**

a) **Plant collection**

Seeds of *Soyabean* were collected from our farm, Dungla-Barisadri region of Chittorgarh district of Rajasthan. Seeds of *Glycine max* washed and dry it. Using mixer seeds by converted into powder.

b) Preparation of Aqueous extract

The aqueous extract of the soybean powder was prepared by soaking 20 g of the powder in 400 ml of distilled water for 12 hours. The aq. extract was then filtered using filter paper, concentrated and stored in an airtight container[11].

Phytochemical Screening

Phytochemical broadcast of the extracts was conceded out according to the standard procedures[12,13]. The Aqueous and methanolic extracts were subjected to preliminary phytochemical screening to identify the various phyto-constituents present in them i.e. alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins and tannins. For Detection of Phytochemical various solvent extract was used i.e. Aqueoue, Methanol, Petroleum ether, Ethyl Acetate etc.

A. Chemical test for alkaloids

Little quantity of dried extract with alcohol was shaken with dilute hydrochloric acid and filtered. The acidified filtrate was used to detect the presence of alkaloids by the following tests.

Mayer's test

The acidified filtrate (2 ml) was treated with Mayer's reagent (1 ml), shaken well and observed for the presence of creamy precipitate.

Wagner's test

The acidified filtrate (2 ml) was treated with Wagner's reagent (1 ml) and observed for the presence of reddish-brown precipitate.

Hager's test

The acidified filtrate (2 ml) was treated with Hager's reagent (1 ml) and observed for the presence of yellow precipitate.

Dragendorff's test

The acidified filtrate (2 ml) was treated with Dragendorff's reagent (2 ml) and observed for the presence of orange-red precipitate.

B. Chemical tests for glycosides

Little quantity of dried extract was hydrolyzed with dilute hydrochloric acid on a water bath for a few hours, and the hydrolysate obtained was used to detect the presence of glycosides by following tests.

Legal test

The hydrolysate (2 ml) was dissolved in pyridine (2 ml). Freshly prepared sodium nitroprusside solution (2 ml) was added to it. Made the mixture alkaline with sodium hydroxide solution and observed for the formation of a pink color.

Baljet test

The hydrolysate (2 ml) was treated with sodium picrate solution (1 ml) and observed for the formation of a yellow to orange color.

Borntrager's test

A little quantity of the residue obtained from the evaporation of hydrolysate was mixed with water and shaken with an equal volume of chloroform. The chloroform layer was separated and equal quantity of dilute ammonia solution was added to it and shaken well and observed for the formation of pink color in the ammoniacal layer.

Modified Borntrager's test

A little quantity of the residue obtained from the evaporation of hydrolysate was treated with ferric chloride and dilute hydrochloric acid. Then, it was extracted with chloroform. The chloroform layer was separated, and an equal quantity of dilute ammonia solution was added to it and shaken well and observed for the formation of pink color.

C. Chemical tests for Phenolic compounds and tannins

Ferric chloride test

A small quantity of the dried extract was mixed with water and treated with dilute ferric chloride solution (5%) and observed for the presence of a blue color.

Gelatin test

The dried extract dissolved in the water was filtered. To the filtrate, a 2% solution of gelatin containing 10% sodium chloride was added and observed for the presence of milky white precipitate.

Lead acetate test

The dried extract dissolved in the water was treated with a 10% lead acetate solution and observed for the presence of bulky white precipitate.

Decolorization test

The dried extract dissolved in water was treated with dilute potassium permanganate solution and observed for the decolorization of potassium permanganate.

D. Chemical tests for Flavanones and flavonoids

Aqueous sodium hydroxide test

Aqueous sodium hydroxide solution was added to the little quantity of dried extract and observed for the yellow coloration of the solution.

Ammonia test

The filter paper wetted with a small quantity of an alcoholic solution of the dried extract was exposed to ammonia vapor and observed for the formation of yellow color.

Shinoda test

The dried extract mixed with alcohol was treated with magnesium or zinc and dilute hydrochloric acid and observed for the formation of orange-red or violet color.

E. Chemical tests for carbohydrates

A small quantity of ethanolic extract was mixed with water or alcohol and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates.

Molisch's test

The filtrate (2 ml) was treated with a few drops of Molisch's reagent and concentrated sulfuric acid (2 ml) was added through the side of the test tube without shaking and observed for the presence of violet ring at the junction of two solutions.

Fehling's test

The filtrate (1 ml) was treated with 1 ml each of Fehling's solution A and B and boiled in a water bath and observed for the formation of a reddish precipitate.

Benedict's test

The filtrate (2 ml) was treated with Benedict's reagent (2 ml). Then, the mixture was heated in a boiling water bath and observed for the presence of reddish precipitate.

F. Chemical tests for proteins and amino acids

Millon's test

Little quantity of dried extract was treated with of Millon's reagent (2 ml) and observed for the formation of white precipitate, which on warming turn into a red colored solution.

Biuret test

Little quantity of dried extract was treated with a few drops of 2% copper sulfate solution. To this excess of potassium hydroxide solution was added and observed for the formation of violet colored solution.

Ninhydrin test

Little quantity of dried extract was treated with few drops of ninhydrin solution and heated on a water bath and observed for the presence of a violet color.

G. Chemical test for terpenoids

Salkowski test

Little quantity of dried extract was dissolved in chloroform. An equal volume of concentrated sulfuric acid was added to it and observed for the appearance of red color in the chloroform layer and greenish-yellow fluorescence in the acid layer.

H. Chemical tests for sterols

A little quantity of the alcoholic extract was refluxed with alcoholic potassium hydroxide solution until the saponification was observed. The mixture was diluted and extracted with solvent ether. The ethereal extract was evaporated, and the residue obtained was used in the tests for sterols.

Liebermann–Burchard test

The residue was taken with dry chloroform (1 ml), and then it was mixed with 2 ml of specially distilled acetic anhydride followed by a few drops of concentrated sulfuric acid through the sides of the test tube and observed for the formation of green color in the upper portion which changes to bluish violet.

Salkowski test

The residue was dissolved in chloroform, and an equal volume of concentrated sulfuric acid was added to it and observed for the red color in the lower layer.

I. Chemical tests for saponins

Foam (froth) test

A small quantity of dried extract was diluted with distilled water (20 ml) in a graduated cylinder. The suspension was shaken for 15 min and observed for the formation of froth.

Hemolysis test

A drop of blood was placed in a slide and mixed with a small quantity of dried extract and observed for hemolysis.

J. Chemical tests for gum and mucilage

Absolute alcohol (25 ml) was added with an aqueous extract (10 ml) with constant stirring. Filtered and the precipitate formed was dried in air and examined for swelling properties.

K. Chemical test for volatile oil

Powdered material (50 g) was subjected to hydro-distillation in volatile oil estimation apparatus (Clevenger apparatus). Collect the distillate and observed for the presence of volatile oil layer.

***In vitro* antioxidant assay**

Determination of antioxidant activity of the extract

Living cells may engender free radicals and other reactive oxygen species as by-products as a results of physiological and biochemical processes. Free radicals can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases, such as cancer, diabetes, ageing, and other degenerative diseases in humans[14]. Antioxidants exert protective effects against oxidative stress in biological systems. They terminate chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves[15]. Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity[16].

The various metabolic reactions occurred in the body of organisms produce oxidants or free radicals. Free radical is any species capable of independent existence that contains one or more unpaired electrons which reacts with other molecule by taking or giving electron and involved in many pathological conditions. The various secondary metabolites produced by plants may act as anti-oxidant by scavenging these free radicals. The methanolic extracts and juice were screened for anti-oxidant activity by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and Hydrogen Peroxide method.

All above mentioned extract was used for in *vitro* determination of antioxidant capacity.

DPPH radical scavenging activity

These are most identification part to identify which types of antioxidant are present in plant[17]. The antioxidant bustle of all mine was exact in requisites of hydrogen donate or free radical scavenging movement, via the sure radical DPPH. DPPH's (Di – phenyl – Picryl hydrazine) scavenging activity. According to these methodologies DPPH solution was prepared 40 microgram/ml solution. Now prepared different dilution of extract as .02,.04,.06,.08,.1,.2 µg/ml. in these solution were added 2ml of DPPH solution. These solutions were leave for incubation at room temperature at 10 minutes. After these spectrophotometer was leave for warming. Absorbance was measured at 517nm. Calculate percentage inhibition and IC_{50}.

Percentage inhibition = Ac – At*100/Ac

Ac = Absorbance of control

At = Absorbance of test

Hydrogen Peroxide assay

Hydrogen peroxide assay was performed according to Jayaprakash G.K. et al 2013 and Ruch R.J. et al 198917. According to both author prepared different dilution of extract. This dilution was 5 to 25 µg/ml. 2ml of test sample and 1ml of 20mMHydrogen peroxide solution in phosphate buffer saline 7.4. Spectrophotometer was calibrated by using same solvent. Wavelength was set 230nm of

spectrophotometer. Measured the absorbance of these samples and calculate the percentage inhibition.

Percentage inhibition = Ac – At*100/Ac

Ac = Absorbance of control

At = Absorbance of test

Reducing power assay

Reducing power assay performed according to R. Jain et al 2006 of extract. Prepared different dilution of substances 5, 10, 20, 30, 40 µg/ml. Take aliquot of these dilutions up to 0.5ml of sample. These mixture were diluted with 0.5ml of 0.2mMphosphate buffer 6.6.also added 0.5 ml of potassium ferric cyanide (1% w/v). These mixtures were incubated at 50 degree centigrade for 20 minutes. These mixtures were cool at room temperature. After these added 1.5 ml of trichloracetic acid (10 % w/v). And finally added 0.5ml of ferric chloride (.1% w/v). This entire procedure constant time interval is used. Spectrophotometer was calibrated by using same solvent. Wavelength was set 700nm of spectrophotometer.

Total Phenolic content

Determination of total phenol content was carried out by Folin- Ciocalteu reagent method[18]. 0.5 ml of extract (1:10 µg/ml) or Gallic acid was mixed with 5 ml Folin- Ciocalteu reagent (1:10 distilled water) and 4 ml of aqueous Na2CO3 (1 M). The mixtures were left at room temperature for 15 min and the total phenol content was determined by colorimetric method at 765 nm. Total phenol content was calculated from calibration curve and expressed as Gallic acid equivalent (mg GAE/g).

Total Flavonoid content

The total flavonoid content was estimated using $AlCl_3$ method[19]. 0.5 ml of each extract (1:10 µg/ml) in methanol were mixed with 1.5 ml of methanol, 0.1 ml of $AlCl_3$ (10%), 0.1 ml of KCH_3COO (1 M) and 2.8 ml of distilled water and left at room temperature for 30 min. Absorbance of the mixtures was measured at 415 nm. Total flavonoid content was calculated from calibration curve and expressed as Quercetin equivalent (mg QE/g extract).

RESULT AND DISCUSSION

Preliminary Phytochemical Analysis:

1. Soyabean

The Phytochemical analysis *Soyabean* were revealed the presence of various chemical constituents such as alkaloids, saponins, glycosides, tannins, flavonoids, carbohydrate etc.

DPPH Radical Scavenging activity

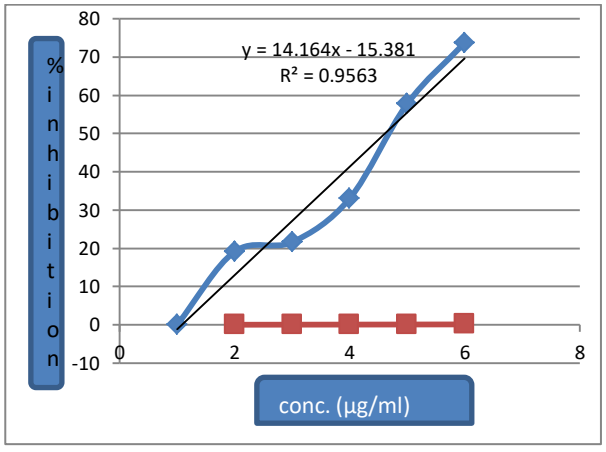
A number of research have resulted that free molecules in bodies are responsible for lot of rare disease originate[20]. These are as related to immunity, nervous system dysfunction, cardiovascular disease and may be carcinogenic etc. DPPH is a constant free radical at opportunity at room temperature. It have posses both properties in which accept an electron or hydrogen radical near suit a sure diamagnetic molecule[21]. The decrease potential of DPPH be indomitable in the shrink into its absorbance at 517 nm, which is induce via anti-oxidants. While the weird electron of DPPH become balancing by a hydrogen commencing a gratis radical scavenge antioxidant in the direction of variety the reduced DPPH-H. IC_{50} of DPPH is major role play in measuring antioxidant activity. *Glycinemax* aqueous and methanolic extract have IC_{50} value was 69.17µg/ml and 4.7 µg/ml. so that these compound have posse's good antioxidant activity.

For determining antioxidant potential used ascorbic acid as standard its IC50 value was 4.61 µg/ml.

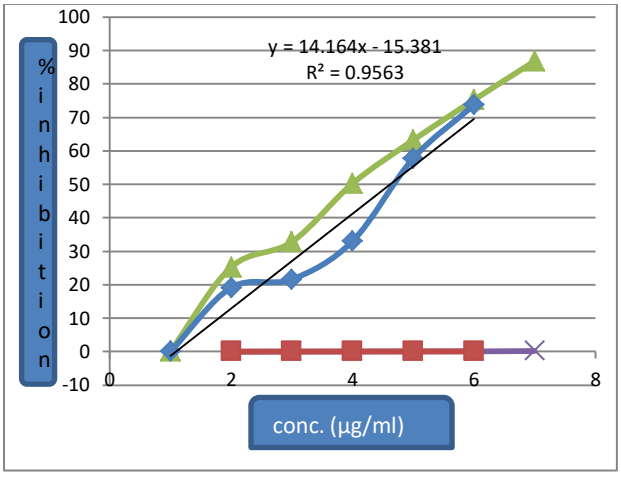
Table No. 5: Phytochemical Analysis

Phyto-constituents	Chemical tests	Aqueous	Methanol
Alkaloids	Dragendroffs	+	+
	Hager's test	-	+
	Mayer' test	+	-
	Wagner's test	-	-
Carbohydrate	Molish test	+	+
	Fehling's test	+	+
	Benedict's test	+	+
	Barfoed's test	+	+
Proteins	Biuret's test	+	+
	Milion's test	+	+
	Precipitation test	+	+
Amino tests	Ninhydrin test	+	-
	Xanthorproteic test	+	+
Steroids	Salkowski's test	-	-
Flavonoids	Shinoda test	+	+
Glycoside	Brontrager's test	+	+
	Legal's		
Tannins and Phenolic	Zinc HCl test	+	+
	With 5% ferric chloride	+	+
	With KMnO4	+	+
	With lead acetate	+	+

Curve of Ascorbic Acid



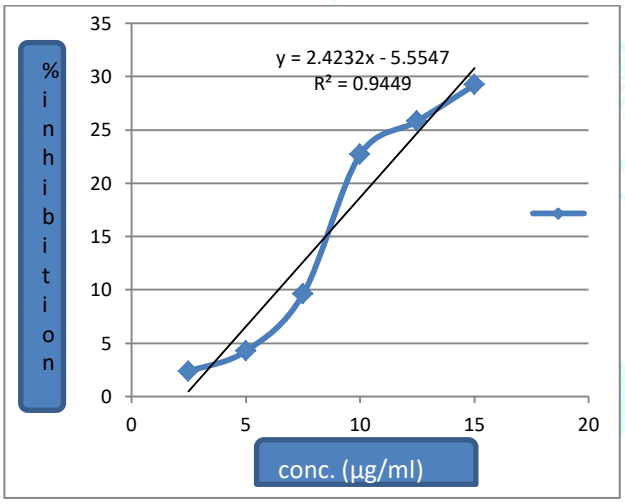
Curve of methanolic extract of *Glycine Max*



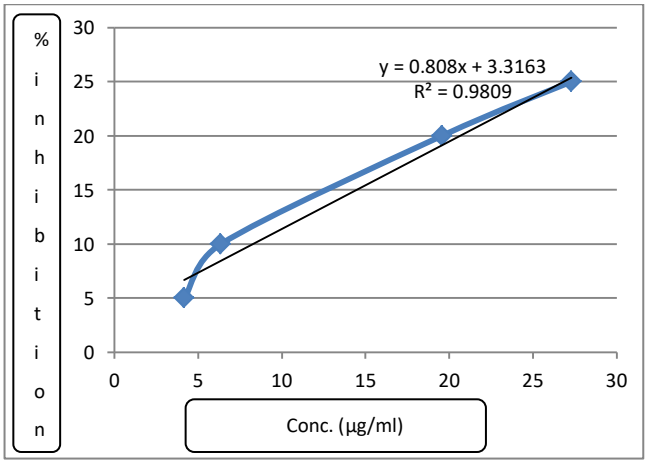
Hydrogen Peroxide scavenging assay

Hydrogen peroxide scavenging activity of *Glycine max* Aq and methanolic extract of seeds were determined. Resulted showed that it is a concentration dependent activity against hydrogen peroxide with IC_{50} values of 42.34µg/ml and 68.513µg/ml. ascorbic acid used as standard its inhibition value was 22.73 µg/ml

Hydrogen peroxide scavenging activity of Ascorbic acid

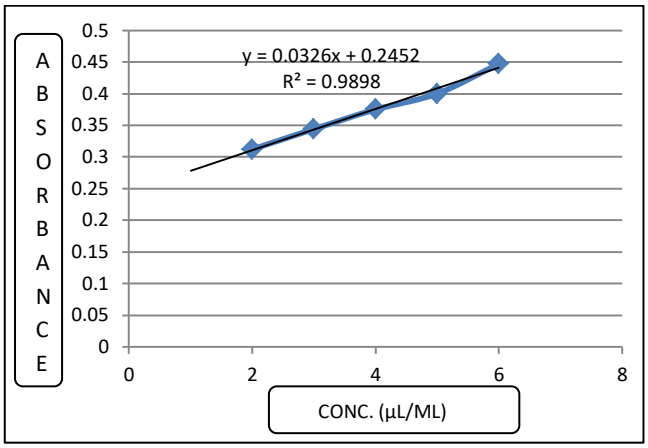


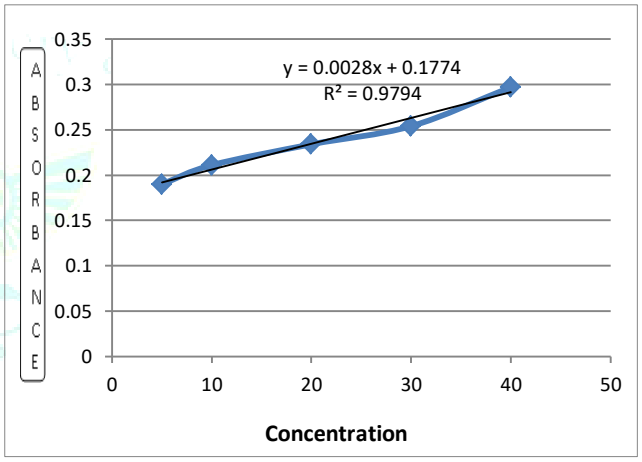
Hydrogen peroxide scavenging activity of Aq.



3. Reducing power assay

Reducing power capability of compound is estimated to reduce Fe^{3+} to Fe^{2+}. Absorbance value is more than it showed more reducing capacity of extract. At finally resulted that if compound is posses good reducing power than increase absorbance with concentration.





4. Total phenolic content

Total phenolic content assay presentation is main aim is plant has high amount of Phytochemicals are present[22]. Phenolic compound have posses high number of free radicals. Many of research resulted that natural phenolic compound are flavonoids. Many of reasons in which broad therapeutic activity. Total phenolic content in *Glycine max* methanolic extract possess 1139mg/gm and aqueous have 769mg/gm of total phenolic content of 100µg/mg.

5. Total flavonoid content

Phenolic compound and flavonoids are mainly present in natural sources. After reading many of research article resulted that in natural plant obtained sources. Phenolic compound is higher number than flavonoids because it is part of phenolic component. In these series it may be flavonoid, isoflavonoids, terpenes etc. *Glycine max* methanolic and aqueous have possess good flavonoids component. It has in methanolic extract 107mg/gm and juice 120mg/gm. These result shows in minute concentration of sample that was 100µg/ml.

CONCLUSION

The present study aimed to evaluate the *in vitro* Antioxidant activity of extracts prepared from the Seeds of Glycine max. The results showed that The extracts exhibited antiradical activities toward 2,2′- diphenyl-1-picrylhydrazyl (DPPH) and inhibiting lipid peroxidation. It possess wide range of antioxidant compounds.

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