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IN VITRO ANTIOXIDANT ACTIVITY OF *GLYCINE MAX.* AND ITS PHYTOCONSTITUENTS IN METHANOLIC EXTRACT OF FRUIT AND AQUEOUS JUICE

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ABSTRACT

Glycine max fruits methanolic extract and juice were used for investigation of antioxidant. Methanolic extract and juice have posse wide range of phenolic and flavonoid compounds. For investigation performed various parameters in which total phenolic compound, total flavonoid compound, reducing power, hydrogen peroxide and DPPH assay. After completion of all protocol with synthetic antioxidant and resulted that these fruits have broad range of antioxidant are present.

INTRODUCTION

In human body reactive oxygen species and free radical are responsible for cellular damage. It may be involved in various diseases like as cardiac disease, cancer and various type of naturopathy **[1]**. The antioxidants are able to hamper by the rust progression via react through free radicals, chelating gratis catalytic metals among moreover with stand-in as oxygen scav. A bulky add up in the direction of curative flora with their purify constituents encompass publicized valuable restorative potentials. Natural obtained plant sources have great number of antioxidant properties. If we started to write name of all of plant. Than these article is not enough. In shortly all of plants have antioxidant activities. These may be rich or low in antioxidant potential **[2-3]**.

Present era is therapeutic era. Working is best policy so they do not take care of his health. At lastly if he suffered from any of untreatable disease than he want to treat. If disease is easily identical or say that not serious than he want to treat used allopathic medicine. If cases is serious or untreatable disease. He thinks that allopathic medicines are more costly or more toxic effect for bodies. Then he used this herbal medicine. Herbs are present in any part of the world. Wide range of herbal drugs family are present in earth in which like leguminosae, febaceae, Rutaceae, Meliaceae, Apocynaceae, Euphorbiaceous, Zygophyllaceae, Rosaceou **[4]**. Lot of review of article concluded that most people want to treat by using herbal medicine. Its wide range of application less side effects, less toxic, wide range of antioxidant is present. These are may be vitamins, minerals and other phytoconstituent constituent **[5]**. It helps against damage caused by free radical causing oxygen species Antioxidant are molecule work as donor if body molecule create problem or lack of its efficacy than it give in human bodies as outer side like it is plants leaf, fruit, flower, stem, bark etc. its application depend on its working of bodies. It worked properly or not for identification performed in vitro antioxidant assay. In these categories mostly referred DPPH, H_2O_2 scavenging activity, total phenolic content, total flavonoid content estimation.

Glycine max has been s commonly cultivated in northern Rajasthan. These plants all constituent are important like leaf, bark, root, fruits etc.

MATERIAL AND METHOD

Glycine Max. was collected from locally market. These were authenticated by Dr. Gautam Kukda Head of Botany Department Maharana Pratap Postgraduation College Chittorgarh (raj.). These plants were preserved for further reference.

Preparation of plant extract: Glycine maxm seeds were sun dried for 8 days. After these help of grinder these were grinded and prepared methanolic extract of these powder.

Materials: all chemicals were used analytical grade. Synthetic antioxidant follin – ciocalteu reagent, 2 – 2 diphenyl -1-picrylhydrazyl (DPPH), trichloroacetic acid (TCA) were purchased from merck specialities PVT. LTD. Mumbai and sigma alorich.

Methods

Phytochemical screening:

Preparation of Aqueous extract

The aqueous extract of the *glycine max* powder was prepared by maceration. 500 g of the powder in 5000 ml of distilled water

for 7 days. The aq. extract was then filtered using filter paper. Filtrate was kept in 25 Petri dishes and dried in sun light. Dried extract was packed in airtight container **[6]**.

A. Chemical test for alkaloids

2gm 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

2gm 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:** 2 ml 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:** 2 ml 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:** 2 ml of extract was mixed with water and treated with dilute ferric chloride solution (5%) and observed for the presence of a blue color.
- **Gelatin test:** 2 ml of extract was dissolved in the water and 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:** 2ml of extract was dissolved in the water and treated with a 10% lead acetate solution and observed for the presence of bulky white precipitate.
- **Decolorization test:** 2ml of extract was dissolved in water and treated with dilute potassium permanganate solution. 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 1 ml of extract and observed for the yellow coloration of the solution.
- **Ammonia test:** The filter paper wetted with 2 ml of alcoholic solution of extract was exposed to ammonia vapor and observed for the formation of yellow color.
- **Shinoda test:** 2ml of extract was mixed with alcohol. It 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

10 ml extract was mixed with water 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:** 2ml of extract was treated with millon's reagent (2 ml) and observed for the formation of white precipitate, which on warming turn into a red colored solution.
- **Biuret test:** 2ml of 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:** 2ml of 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:** 2ml 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

10 ml of extract was refluxed with alcoholic potassium hydroxide solution until the saponification was observed. The mixture was diluted and extracted with solvent ether. The 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:** 1gm of 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 **[7-9]**.

Total Phenolic content estimation

Total phenolic content estimation in methodology was according to Ainsworth EA *et al* 2007 and Alhakmani *et al* 2013. Determination of phenolic compound in Glycine max. It is equivalent to gallic acid calibration curve. Prepared different dilution of gallic acid 10,20,30,40,50,60,70,80,90 µg/ml. different concentration of these dilution taking aliquot of 0.5ml and added 2ml of follin – ciocalteu reagent (1: 10 deionized water). Now added 4ml of sodium carbonate solution (7.5% w/v). These solutions were leave for 30 minute for incubation with intermittent shaking. Spectrophotometer was leave for warming. Calibrate the instrument. After these was taking absorbance at 765nm (due to blue colour). Methanol was using as blank **[10]**.

Total Flavonoid Content estimation

Flavonoid content determination was performed according to method developed by Zhishen *et al* 1999. According to these methods rutin was used for estimation of flavonoid content in*Glycinemax*. Rutin was used for preparing calibration curve. 5mg of rutin was weight it dissolved in 5 ml of methanol. These stock solution was 1000µl/ml. Then prepared different dilution of rutin 10 to 100µl/ml. in these mixture 0.5ml aliquot of appropriate diluted sample solution was take in different test tube. These were diluted by 2ml of distilled water. Consequently further 0.15ml of 5% NaNO2 solution was added. This reacting mixture was leave for 6minute then further 0.15ml of 10% AlCl3 solution was added. Repeated same process, these solutions was leave for 6 minute. In this sample solution was added 4% NaOH solution. Gradual added water to finalized volume up to 5ml. this mixture was mixed properly. These were leaved it for 15 minute for incubation at room temperature. Spectrophotometer was calibrated by using same solvent. Absorbance was set 510nm of spectrophotometer **[11]**.

Reducing Power

Reducing power assay performed according to R. Jain *et al* 2006 of Glycine max. Prepared different dilution of substances 5, 10,20,30,40 µl/ml and 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 **[12]**.

DPPH radical scavenging activity

These are most identification part to identify which types of antioxidant are present in plant (Raj *et al* 1999). 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 *Glycine max* extract as 1,2,3,4,5 µl/ml and .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} **[13-15]**.

Percentage inhibition = Ac – At*100/Ac

Ac = Absorbance of control

At = Absorbance of test

RESULT AND DISCUSSION

Phytochemical investigation

Results have been shown in **Table 1**.

Total phenolic content

Total phenolic content assay presentation is main aim is plant has high amount of Phytochemicals are present (Kuti *et al.,* 2004). Phenolic compound have possess high number of free radicals. Many of research resulted that natural phenolic compound are flavonoids. Many of reasons in which broad therapeutic activity (Vundac *et al.,* 2007). Total phenolic content in methanolic extract of *glycine max* fruits have 769mg/gm of total phenolic content of 100µg/mg **[14-16]**.

Total flavonoid content

Phenolic compound and flavonoids are mainly present in natural sources (Kuti *et al.,* 2004). 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 have possess good flavonoids

component. It has in methanolic extract 107mg/gm. These results show in minute concentration of sample that was 100µg/ml **[17-18] (Figure 1, Table 2)**.

Reducing power assay

Reducing power capability of compound is estimated to reduce Fe^{3+} to Fe^{2+} (S.O. *et al.,* 2010). Absorbance value is more than it showed more reducing capacity of extract (Y. Pan *et al.,* 2010). At finally resulted that if compound is possess good reducing power than increase absorbance with concentration **[19-20]**.

DPPH Radical Scavenging activity

A number of research have resulted that free molecules in bodies are responsible for lot of rare disease originate. 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 possess both properties in which accept an electron or hydrogen radical near suit a sure diamagnetic molecule .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. *Glycine max* extract have IC_{50} value was 69.17µl/ml and 4.7 µg/ml. so that these compound have posse's good antioxidant activity **[21-27] (Table 2)**.

Table 1: Phytochemical investigation

S. No.	Name of Test	Extract
1	Carbohydrate	+
2	Protein	+
3	Amino Acid	+
4	Glycosides	+ -
5	Saponins	+
6	Alkaloids	-
7	Flavonoids	+
8	Triterpenoids	+
9	Tannins	+
10	Fats and oils	+

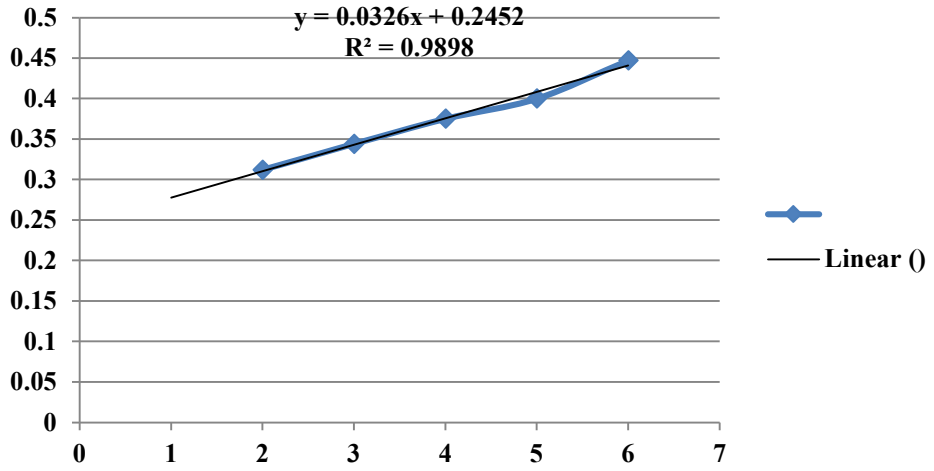

Figure 1: Glycine Max. (Methanolic extract)

Table 2: DPPH Radical Scavenging activity

S. No.	Concentration (µg/ml)	% inhibition	R^2	IC_{50}
1	0.02	25.23		
2	0.04	32.78		
3	0.06	50.23	0.985	4.17
4	0.08	63.2		
5	0.1	75.23		
6	0.2	86.76		

CONCLUSION

Aqueous extract of Glycinemax identification of antioxidant properties. These investigation confirmed by using synthetic antioxidant. These natural obtained compounds have posse's high number of phenolic and flavonoids components. In these investigations resulted that these component have much useful in various rare disease treatment. Such a good platform for identification of how many or how much quantity of antioxidant is present in any plant. Finally resulted that these *Glycinemax* have broad range of antioxidant are present which are therapeutically beneficial for human being.

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