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Phytopharmaceuticals and *In-Vitro* Antioxidant Potentials of Soyabean Methonolic Extract

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ABSTRACT

Soyabean methanolic extract were used for investigation of phytopharmaceuticals and antioxidant potentials. The extract was analyzed for total phenolic compound, total flavonoid compound, reducing power, hydrogen peroxide and DPPH assay. The results depicted that the methonolic extract have broad range of antioxidants present in it.

Keywords: phytopharmaceuticals, *Soyabean* methanolic extract, antioxidant

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INTRODUCTION

In human body reactive oxygen species and free radical are responsible for cellular damage. These free radicals may be involved in various diseases like cardiac disease, cancer and various type of naturopathy pain [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 scavenger [2]. 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 [3]. In short, all plants have antioxidant activities. These may be rich or low in antioxidant potential. antioxidant doings of in nature stirring substance into privileged plant life, awareness have improved under the defending movement of these natural antioxidants not in favor of unremitting disorder cause via oxidative progression[4].

Useful opinion for maintain health by using healthy diets that may endorse health and endurance that may be include the daily utilization of at slightest three serving of fruits or vegetables. Plants should belong to different botanical families [5]. Soybean has been a vital food resource in several part of the ancient world[6]. Soybeans are of meticulous significance to Asian country. The bigger insist for food-grade soybeans have generated significance in rising soybeans[6].

MATERIAL AND METHOD

Soyabean seeds were collected from our farm. These were authenticated by Botanist

Preparation of plant extract: Soyabean Seeds were powdered by using mixer. Powder transfer into 70 % Methonolic container.

Plant Profile[7]

Kingdom : Plantae
Unranked : Angiosperms
Unranked : Eudicots
Unranked : Rosids
Order : Fabales
Family : Fabaceae
Subfamily : Faboidae
Genus: Glycine

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[8]

Identification of active phyto chemicals which are present according to kokate et 2006 al procedure. In this category

performed phyto chemicals detection of carbohydrates, proteins, amino acids, alkaloids, flavonoids etc.

CARBOHYDRATE TEST:

a. Molish test – 2ml of soyabean extract was treated with molish reagent.

b. Fehling test - 1ml of Soyabean extract, it was treated with Fehling A and Fehling B. This mixture was heated in water bath for 5 minutes.

c. Benedict's test – 1 ml of Soyabean extract and 1ml of Benedict's reagent was added in a test tube. This mixture was heated at water bath for 7 minutes observed that red colour was formed.

d. Barfoed's test – 2ml of Soyabean extract was taken in test tube. This mixture added 1ml of barfoed's reagent red precipitate was observed.

PROTEIN AND AMINO ACID TEST :

a. Biuret's test: 1ml of c treated with 1ml of 10% sodium hydroxide solution in a test tubeand heated. In this solution added 0.7% copper sulphate solution. Million's test – 3ml of fresh juice was treated with 5ml of millions reagent.

b. Ninhydrin test – 2ml of extract was treated with 4-5 drops of 5% ninhydrin solution. These was heated in water bath blue colour was observed.

GLYCOSIDES TEST:

a. Borntrager's test – took 3ml of extract, diluted sulphuric acid was added. This mixture was boiled for 5-6 minutes and filtered. Leave it for chilling, after these 3ml of benzene was added and shake it.

b. Legal's test – 1ml of extract was dissolved in pyridine. In these mixture added 1ml of sodium nitroprusside solution. It was made alkaline using 10% sodium hydroxide solution.

c. Keller – Killiani test – 2ml of extract was took. In this sample added 3ml of glacial acetic acid and a drop of 5% ferric chloride were added in test tube. 2-5 drops of concentrated sulphuric acid was added the side of test tube.

SAPONIN TEST:

a. Froth test: Extract was diluted with distilled water and shake regularly in graduated cylinder for 20 minutes. No change observed in this juice.

TEST FOR ALKALOIDS:

Extract was diluted with dilute hydrochloric (HCl) shake it well and filtered

a. Mayer's test – 2 ml Extract, added few drops of Mayer's reagent.

b. Dragendroff's test – 2ml of extract added few drops of Dragendroff's reagent in a test tube.

c. Hager's test – 3ml of extract was took added few drops of Hager's reagent in test tube. Yellow precipitate was formed.

d. Wagner's test – 2ml of extract was took, added few drops of Wagner's reagent in test tube. A reddish brown precipitate was formed.

TEST FOR FLAVONOIDS:

a. Lead Acetate Test – Extract was treated with few drops of lead acetate solution. Yellow precipitate was formed.

b. Alkaline Reagent test – Extract was treated with few drops of sodium hydroxide (NaOH) in test tube yellow colour is formed. In these mixture added few drops of concentrated sulphuric acid (conc. H2SO4)

c. Shinoda test - Extract was treated with small amount of 95% of ethanol. This mixture was treated with 2-3 fragments' of magnesium turning (lob chemical), regularly added drop wise concentrated hydrochloric acid (HCl).

TRITERPENOIDS AND STEROIDS TEST:

a. Salkowski's test – Extract was treated with chloroform and filtered. This filtrate was added few drops of concentrated sulphuric acid (Conc. H2SO4), shake it and allowed to stand. Two layers are turn red, result that steroid are present.

b. Liebermann – Bur chard's test – Extract was treated with chloroform. This solution added few drops of acetic anhydride boiled it and cooled. Few drops of concentrated sulphuric acid were added through side of test tube.

TANNINS AND PHENOLIC COMPOUND TEST:

a. Ferric chloride test – Extract was dissolved in water. This solution added 2ml of 5% ferric chloride. Violet colour was observed.

b. Lead Acetate test – Extract was dissolved in distilled water. These mixture added few drops of lead acetate solution.

c. Dilute Iodine test – Extract was took, added dilute iodine solution were added. Red colour was observed.

d. Gelatin Test – Extract was dissolved in distilled water. In these solution added 2ml of 1% gelatin solution containing 10% sodium chloride.

FATS AND OILS TEST –

a. Solubility test – Extract with alcoholic solution, added 1ml of chloroform. Observed that two layer was separated.

Total Phenolic content estimation[9]

Total phenolic content estimation in methodology was according to Ainsworth EA et al 2007 and Alhakmani et al 2013. Determination of phenolic compound in *Soyabean* methanolic extract. It is equivalent to gallic acid calibration curve. Prepared different dilution of gallic acid was 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.

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 *Soyabean* methanolic extract. 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[10].

Reducing Power

Reducing power assay performed according to R. Jain et al 2006 of *Soyabean* methanolic extract. 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[11].

Hydrogen Peroxide assay

Hydrogen peroxide assay was performed according to Jayaprakash G.K. et al 2013 and Ruch R.J. et al 1989. According to both author prepared different dilution of methanolic extract of fruit. This dilution was 5 to 25 and 2.5 to 15 µl/ml and 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[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 (Brand Williams et al 1995). DPPH's (Di – phenyl – Picryl hydrazine) scavenging activity we referred the method of Gulcin J. et al 2006 and R. Jain et al 2006. According to these methodologies DPPH solution was prepared 40 microgram/ml solution. Now prepared different dilution of methanolic 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].

Percentage inhibition = Ac – At*100/Ac

Ac = Absorbance of control

At = Absorbance of test

RESULT AND DISCUSSION

Table 1: Phytochemical investigation

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

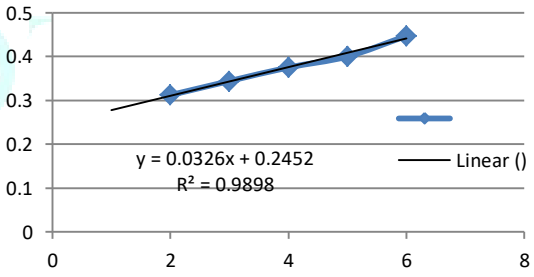
Total phenolic content

Total phenolic content assay presentation is main aim is plant has high amount of Phytochemicals are present[14]. Phenolic compound have posses high number of free radicals (Rathee et al 2007). Many of research resulted that natural phenolic compound are flavonoids. Many of reasons in which broad therapeutic activity [15], Total phenolic content in methanolic methanolic extract have high level of phenolic content is present, methanolic extract posses 1139mg/gm .

Reducing power assay

Reducing power capability of compound is estimated to reduce Fe^{3+} to Fe^{2+} (S.O. et al2010). Absorbance value is more than it showed more reducing capacity of extract[16]. At finally resulted that if compound is posses good reducing power than increase absorbance with concentration.

Methanolic extract



Hydrogen Peroxide

Hydrogen peroxide scavenging activities of methanolic extract were determined. Resulted showed that it is a concentration dependent activity against hydrogen peroxide with IC_{50} values of 22.95µg/ml.

Table 2: Hydrogen peroxide scavenging activity of methanolic extract

Sample	Concentration (µg/ml)	% inhibition	IC_{50}	R^2
Methanolic extract	2.5	2.35		
	5.0	4.26		
	7.5	9.59	22.95	0.944
	10.0	22.67		
	12.5	25.81		
	15.0	29.21		

DPPH Radical Scavenging activity

A number of research have resulted that free molecules in bodies are responsible for lot of rare disease originate[17]. 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 [18].The decrease potential of DPPH be indomitable in the shrink into its absorbance at 517 nm, which is induce via anti-oxidants[19]. 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[20]. IC_{50} of DPPH is major role play in measuring antioxidant activity[21,22]. Methanolic extract have IC_{50} value was 69.17µl/ml, these compound have posse's good antioxidant activity.

Table 3: DPPH Radical Scavenging activity of methanolic extract

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

The results of current work bare that methonolic extract of *soyabean* have good antioxidant potentials. The antioxidant potentials of these methonolic extracts are accredited to the phenolic and flavonoid, DPPH contents estimations. Viseversa , our data guided that the methonolic extract can be utilize as an valuable and secure and reachable spring of ordinary antioxidants.

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