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Review Article

Antioxidant and Antimicrobial properties of *Glycine Max*-A review

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ABSTRACT

Vegetable soybean is rich in phytochemicals beneficial to the human being and is therefore considered a neutraceutical or a functional food crop. Soybean as a “functional food” that reduces the risk of range of hazardous diseases like atherosclerosis, osteoporosis, various types of cancer (breast, uterus cancer, and prostrate) has attracted people's attention across the globe. People in India are becoming increasingly aware about the health benefits of consuming soy food. Although isoflavones present in soy are believed to be major components responsible for the antioxidative activity, a recent study showed that anthocyanins present in black soybean had strong antioxidative potential. This review article focuses on both the antioxidant and antimicrobial activity of *Glycine max.*

1. Introduction

Polyphenols as antioxidant compounds are gaining a lot of importance due to their dual role in the food industry as lipid stabilizer and in prevention of oxidative stress-related disease. Antioxidants especially natural antioxidants are reported to inhibit lipid peroxidation and protect from damage due to free radical [1]. Edamame, also called maodou in China, is a large seeded (seed dry weight greater than 250 mg per seed) green vegetable soybean (Glycine max) cooked and served in pods as snacks like peanuts [2]. In Asia, where edamame is an important vegetable, farmers harvests stems with fresh green pods before full maturity when pods are fully filled, nearly 80% matured, and just before turning yellow [3]. Consumers rated some varieties of the edamame type soybeans higher than others and suggested that trained panelist relied on the texture and sweetness to differentiate edamame varieties. An opinion among older Japanese consumers is that the taste of vegetable soybeans has deteriorated as the product has become commercialized [4].

Vegetable soybean is rich in phytochemicals beneficial to the human being and is therefore considered a neutraceutical or a functional food crop [5]. Edamame (vegetable soy bean) is well established legume in the human diet in Asia . The positive health benefits of soy have greatly increased consumer awareness of soy products and created a market potential for soy products [6].

The green seeds shelled from the immature pods of soybeans bar great potential for human consumption in India, like the green pods of other legumes. Immature soybean pods are soft in texture and can be cooked just like sweet peas (Pisum sativum), chickpeas (Cicer artietinum), or lima beans (Phaseolus limensis L); their green seeds can be added to stews and soups, boiled in salt water, or roasted like peanut seeds. Furthermore, no other vegetable crop can match the nutritive value of the immature pods of soybeans pods are rich in vitamins (B1, B2), minerals (iron, calcium, phosphorous), and protein content [7].

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Consumption of soybeans and soy products has been associated with reducing the risks of various cancers, such as prostrate and mammary and several other chronic inflammatory diseases. The health promoting activity associated with soy consumption is attributed to the presence of isoflavone [8]. The structural similarities of isoflavones to naturally occurring estrogens may protect hormone dependant cancer by modulating activity of estrogen cholesterol levels. Soybean isoflavones have reportedly increased HDL cholesterol and lowered LDL cholesterol [9].

The mimicry of estrogen by the phytoesterogens in soybean like genistein and daidzein has introduced a controversy over whether such a replacement is harmful or helpful to the brain. Because most naturally occurring estrogenic substances show weak activity, normal consumption of foods that contain these phytoestrogens should not provide sufficient amounts to elicit a physiological response in humans .

Isoflavones present in soybean are in the aglycone, beta glucoside, 6-o-maloyl-beta-glucoside or 6-o-acetyl-beta-glucosideforms. The biologically active components of soy isoflavones include genistein, daidzein and biochanin A. Genstein inhibits protein tyrosine kinase activity, topoisomerases l and ll, ribosomal 6S kinase and alters cell proliferation. It also has antioxidant properties and suppresses skin tumorigeneses [10].

The content of isoflavones in soy are as much as 3mg⁄g dry weight. Isoflavones belongs to the class of polyphenols. The phenolic constituents of the diet act as antioxidant by virtue of free radical scavenging properties of the constituent hydroxyl group allowing them to act as reducing agents, hydrogen-electron-donatingagents or singlet oxygen scavengers.

Genistein acts as an oxidant (stimulating nitrate synthesis) and it blocks the formation of new blood vessels (antiangiogenic effects). It also act as an inhibitor of the activity of substances that regulate cell division and cell survival . Soybeans are a significant source of mammalian lignan precursor secoisolariciresinol [11].

increasing popularity of soybean as a nutraceutical is currently driving the demand for this vegetable and it is estimated that by the year 2005 US could be importing about 25000 tonnes of edamame a year [12].

The vegetable soybean (Glycine max. (L.) Merrill) is a specialty soybean harvested when the seeds are immature. Due to the narrow window of time available to harvest this crop, freezing is essential for year-round availability of vegetable soybeans. Investigation into vegetable soybean cultivation and processing in the U.S. is ongoing. A flavor lexicon, a set of terms which identify and define the associated aromatic, tastes and feeling factors of a product, for frozen vegetable soybeans was created This lexicon will provide a standard flavor language for vegetable soybean producers and researchers [13].

Edamame,also called maodou in China, is a large seeded (seed dry weight greater than 250 mg per seed) green vegetable soybean (Glycine max) cooked and served in pods as snacks like peanuts [14].

Edamame (vegetable soybean) is well established legume in the human diet in Asia). The positive health benefits of soy have greatly increased consumer awareness of soy products and created a market potential for soy products. Research on edamame quality in Asia has focused on the processing of both shelled and in-pod products. Taiwan is one of the largest producers of edamame. Early methods to evaluate vegetable soybean flavour lacked descriptive detail and the sensory characteristics were often vague. Indistinct statements such as soybeans are characterized by a mild flavour and superior eating quality compared to field soybean [15].

Consumption of soybeans and soy products has been associated with reducing the risks of various cancers, such as prostrate and mammary and several other chronic inflammatory diseases. The health promoting activity associated with soy consumption is attributed to the presence of isoflavones.The structural similarities of isoflavones to naturally occurring estrogens may protect hormone dependant cancer by modulating activity of estrogen cholesterol levels .Soybean isoflavones have reportedly increased HDL cholesterol and lowered LDL cholestero [16].

Proximate analysis of seed nutritional composition of edamame (vegetable soybean) in Colorado US and Japan indicated that edamame has superior nutritional content than green peas. The calorific value (energy) of vegetable soybean is about 6 times that of green peas. The vegetable soybean contains 60% more Ca and twice the P and K of green peas. The Na and carotene content of soybean is about one third that of green peas and has similar quantities of iron vitamins B1 and vitamin B2.It is rich in ascorbic acid but low in niacin [17].

In a study to investigate the potential of green immature soybean among Nigeria soybean varieties as human food, five Nigeria soybean varieties were harvested at 90 days old and were evaluated for chemical composition, physical and sensory characteristics. The physical characteristics looked into seed size (breadth and length), weight, seed colour, hull thickness and percentage of hydration. The chemical composition was compared to mature soybean seeds while the sensory attributes were compared to fresh green peas. The raw mature soybean was high in chemical composition and low in anti nutritional factors.The moisture content ranges from 62.8 per cent 65.4 per cent where as the protein content varied form 15.3 per cent to 20.3 percent among the different varieties studied. The hull thickness of the seeds was within 0.01 to 0.05 and the percentage of hydration ranges within 5.5 to 6.8 per cent. All varieties had a green colour for the seed coat. Overall sensory acceptability compared favorably well with green peas used as control for sensory evaluation [18].

Fifty Six genotypes of grain-type soybean and 17 genotypes of vegetable-type soybean collections were analyzed for protein and oil content, trypsin inhibitor, and lipoxygenase activities. Protein and oil contents ranged from 36.9 to 47.9% and from 13.3 to 23.0% for different accessions in grain- and vegetable-type soybeans, respectively. Trypsin inhibitor and lipoxygenase activities ranged from 22.0 to 47.0 trypsin inhibitor units/mg meal and from 482 to 6265 lipoxygenase units/min/mg meal for grain- and vegetable-type soybeans, respectively. Several genotypes of grain-type soybean and vegetable-type soybean showed good nutritional potential and may be useful in a breeding programme to improve the nutritional quality of soybean [19].

Genetic variations in the nutrient composition and anti-nutritional factors of 17 vegetable soybean genotypes were determined and a wide variation in protein %, total phosphorus (TP) and available phosphorus (AP) was found (Mohamed A.I et al., 1991) Variations in Ca, K, Fe, Mn, and Cu were also observed. Variation was also found for trypsin inhibitor (TI) activity and phytate (PA) content. High protein %, TP and minerals are desirable qualities for vegetable-type soybeans; they result in a food with a high nutrient density [20].

A study to evaluate vegetable soybean (Glycine max) for green pod yield and individual and total sugar when harvested at green pod stage suggested that because pod length reaches the maximum size 30 days after flowering, it could be used as an indirect selection criterion to identify genotypes with high sucrose and total sugar contents [21].

Local soybean with various seed coat colours were evaluated for chemical composition (sucrose content), physical properties (springiness, gumminess, adhesiveness, chewiness and hardness), and sensory properties (taste, sweetness, and chewiness). Vegetable soybean lines with green seed coat were better than those with black, brown, mixed and yellow seed-coats [22].

Analysis of protein content and trypsin inhibitor (TI) activity in (i) 16 grain-type and (ii) 16 vegetable-type soybean varieties showed considerable variation where the specific TI activity units/mg protein ranged from 33.46 to 86.37 and protein ranged from 36.41 to 43.13% [23].

Polyphenols as antioxidant compounds are gaining a lot of importance due to their dual role in the food industry as lipid stabilizer and in prevention of oxidative stress-related diseases. The synthetic compounds like a butylated hydroxy toluene (BHT), tertiary butyl hydroquinone (TBHQ), butylated hydroxy anisole (BHA) and propyl gallate (PG) are widely used antioxidant in food industry. Their toxicological aspects together with consumer preference for natural products have popularized the use of natural antioxidants. Antioxidants especially natural antioxidants are reported to inhibit lipid peroxidation and protect from damage due to free radical [24].

Phenolic profiles and antioxidant properties of a total of 30 soybean samples, including 27 grown in the North Dakota-Minnesota region and three soybeans from the other regions, were reported.The total phenolic content (TPC), total flavonoids content (TFC), phenolic acids, flavonols, anthocyanins, and isoflavones were quantified. Antioxidant properties of soybean extracts were assessed using 2-diphenyl-1-picryhydrazyl free radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) methods. Results showed that black soybean cultivars possessed significantly higher TPC, TFC, DPPH, FRAP, and ORAC values than all yellow soybean cultivars. However, black soybean cultivars did not exhibit significantly higher individual phenolic contents (except for anthocyanins), such as phenolic acids and isoflavones, than the yellow soybean cultivars. The isoflavone profiles of North Dakota soybean cultivars were similar to those of South Dakota, but average values of total isoflavone (TI) contents were higher than soybeans grown in the other states and Korea and Japan according to the U.S. Department of Agriculture-Iowa State University Database on the isoflavone contents of foods. Correlation assays showed that TPC, TI, total phenolic acids, daidzin, genistin, malonyldaidzin, daidzein, genistein, and trans-cinnamic acid significantly ($r = 0.73, 0.62, 0.49, 0.68, 0.59, 0.59, 0.56, 0.47$, and 0.76, respectively, $p < 0.0001$) correlated with ORAC values of yellow soybeans. Both isoflavones and phenolic acids contributed to the ORAC values of yellow soybeans. These data suggest that some selected soybean cultivars may be used as high-quality food-grade soybeans for providing high phenolic phytochemicals and antioxidant activities [25].

Fermented soybean extract is produced by symbiotic fermentation of organic soybean with 20 types of Lactobacillus and yeast. In vitro and in vivo models are used in this study to evaluate the antioxidant effect of fermented soybean extract [26].

Several in vitro models are used to detect the antioxidant capacity of the fermented soybean extract, which is compared to vitamin C and Trolox. The results demonstrate that the fermented soybean extract has strong antioxidant activity against unsaturated fatty acid peroxidation compared to vitamin C and Trolox. By the means of the test system developed) it is shown that the fermented soybean extract can function both as an antioxidant and as a free radical acceptor that can convert free radicals into harmless substances through an energy-decreasing procedure [27].

The effects of fermented soybean extract on the activities of the antioxidant enzymes (AOE) such as total superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) in liver, kidney, and brain from male Sprague–Dawley rats reports that the activities of CAT, SOD, and GPX are increased in the liver. However, the SOD activity is decreased in the kidney. SOD and GPX activities are decreased in the brain. These results lead to the conclusion that fermented soybean extract not only has antioxidant activity but also has an effect on the activity of antioxidant enzymes in liver [28].

The antioxidant activity of the soybean extract (Isoflavin Beta®) and of formulations added with this extract were evaluated using stable free radical 2,2- diphenyl-1-pycrylhydrazyl (DPPH) and deoxyribose as well as the lipid peroxidation inhibition assays (For all the assays the extract showed a dose-dependent activity. The IC50 values of the extract for lipid peroxidation inhibition, DPPH radical scavenging and hydroxyl radical scavenging assay, were 21.03 µg/ml 161.8 µg/ml and 33.5µg/ml respectively. The antioxidant activity of the extract suggests that the formulations containing soybean extract can protect the skin against free radicals, which can be generated by the ultraviolet radiation exposure [29].

There is ample evidence that soybean has antioxidative activity and protect tissues from oxidative stress-induced injury. Although isoflavones present in soy are believed to be major components responsible for the antioxidative activity, a recent study showed that anthocyanins present in black soybean had strong antioxidative potential. The authors investigated the antioxidant potential of black and yellow soybean in seven groups of mice, each comprising of 10 mice. Biomarkers for oxidative stress evaluated include superoxide dismutase, catalase,

glutathione peroxidase, phosphoglycerate kinase 1, TBARS, FRAP and were compared among tissues collected from experimental groups. Overall, high-isoflavone and black high isoflavone varieties of soy appear to effectively protect the animals from Fe-NTA-induced oxidative stress [30].

Contents of phenols in embryos, cotyledons and seed coats of 9 soybean varieties were studied and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was also measured to determine correlations between phenols contents and antioxidative activity.A total of 10 anthocyanins and 21 phenols were detected and quantified. In all 9 varieties, the seed coat and cotyledon contained the highest and lowest levels of phenolic compounds and anthocyanins, respectively. A strong correlation was observed between seed coat colour and contents of both phenols and anthocyanins. Brown and black soybean seed coats contained much higher levels of phenols and anthocyanins than yellow or green seed coats. Of the phenols, syringic acid (214 mug/g) and chlorogenic acid (31 mug/g) were present in the highest amounts in seed coats and embryos, respectively. Myricetin levels were highest both in whole seeds (16.7 mug/g) and cotyledons (16.0 mug/g), representing 20 and 30% of total phenols, respectively. Among the 10 anthocyanins, cyanidin-3-glucoside was found to accumulate at the highest level in the seed coat (1783 mug/g), whole seed (106 mug/g) and embryo (0.35 mug/g), corresponding to 95, 96 and 40% of the total anthocyanin contents, respectively. The cotyledon accumulated pelargonidin-3-glucoside (0.39 mug/g) at the highest level, equivalent to 62% of the total anthocyanin contents. DPPH activity was found to correlate strongly with contents of both phenols and anthocyanins [31].

Whole dried soybean seeds were investigated to determine the nature of their antioxidant activity and were measured by rates of carotene bleaching in the coupled oxidation of linoleic acid and β-carotene. Methanolic extracts of the soybeans were found to possess potent antioxidant ability. Paper and Sephadex column chromatography gave incomplete resolution of individual components. Therefore, thin-layer chromatography of the whole methanolic extract rather than individual column fractions was used in isolating this component. Three fluorescent bands were found to possess the major activity. The most potent complex was further analyzed. Thin-layer chromatography was used to separate the complex into four fluorescent components. One compound was determined to be responsible for the antioxidant activity exhibited by the parent band. Further analysis of this component by paper chromatography, chromogenic spray reagent, and ultraviolet spectral analyses indicated that the antioxidant was an iso pentoside [32].

Several phytochemicals and micronutrients that are present in fruits and vegetables are known to exert cancer chemo preventive effects in several organs, including the colon. Among them, the soybean isoflavonoid genistein received much attention due to its potential anticarcinogenic, antiproliferative effects and its potential role in several signal transduction pathways. A study designed to investigate the effect of genistein on azoxymethane (AOM)-induced colon carcinogenesis and to study its modulatory role on the levels of activity of 8-isoprostane, cyclooxygenase (COX), and 15-hydroxyprostaglandin F2 dehydrogenase (15-PGDH) in the colonic mucosa and colon tumors of male F344 rats reports that administration of genistein significantly increased noninvasive and total adenocarcinoma multiplicity ($P < 0.01$) in the colon, compared to the control diet, but it had no effect on the colon adenocarcinoma incidence nor on the multiplicity of invasive adenocarcinoma ($P > 0.05$). Also, genistein significantly inhibited the 15-PGDH activity (>35%) and levels of 8-isooprostane (50%) in colonic mucosa and in tumors. In contrast, genistein had no significant effect on the COX synthetic activity, as measured by the rate of formation of prostaglandins and thromboxane B2 from [14C] arachidonic acid. The inhibition of 8-isoprostane levels by genistein indicates its possible antioxidant potential, which is independent of the observed colon tumor enhancement, yet this agent may also possess several biological effects that overshadow its antioxidant potential. The exact mechanism(s) of colon tumor enhancement by genistein remain to be elucidated; it is likely that its colon tumor-enhancing effects may, at least in part, be related to inhibition of prostaglandin catabolic enzyme activities [33].

The antibacterial activity of the methanol and aqueous extract of Camellia sinensis on Listeria monocytogenes were investigated using Agar-gel diffusion, paper disk diffusion and micro broth dilution techniques. The results obtained showed that methanol and water extract exhibited antibacterial activities against Listeria monocytogenes. The leave extract produced inhibition zone ranging from 10 .0 – 20.1mm against the test bacteria. The methanol extracts of the test plant produces larger zones of inhibition against the bacteria than the water extract. The minimum inhibitory concentration (MIC) for the methanol and water leave extract was 0.26mg/ml and 0.68mg/ml respectively [34].

A previously unrecognized phytoalexin has been isolated from soybean cotyledons that had been infected with bacteria or exposed to ultraviolet light. The phytoalexin has been purified to homogeneity by silica gel flash chromatography and high pressure liquid chromatography. It has been structurally characterized by its ultraviolet, circular dichroism and nuclear magnetic resonance spectra, polarimetry, and its mass spectrometric fragmentation pattern. The phytoalexin, (6aS, 11aS)-3, 6a, 9-trihydroxypterocarpan, is a compound that had previously been detected in $CuCl_2$-treated soybeans and is structurally related to the previously identified soybean phytoalexins glycerollins I to IV. It is proposed that the trivial name glycinol be used for this phytoalexin. Glycinol is a broad spectrum antibiotic capable of prolonging the lag phase of growth of all six bacteria examined namely Erwinia carotovora, Pseudomonas glycinea (races 1 and 3), Escherichia coli, Xanthomonas phaseoli, and Bacillus subtilis. Glycinol also inhibits the growth of the fungi Phytophthora megasperma f. sp. glycinea (race 1), Saccharomyces cerevisiae, and Cladosporium cucumerinum. Glycinol is a static agent against the six bacterial species listed above and against S. cerevisiae, and appears to be static against the other fungi examined. As with other phytoalexins, there is no correlation between the pathogenicity of a microorganism and its sensitivity to glycinol [35]

2.Materials and methods

2.1.Collection of sample

The sample was collected from Botanical garden, Trivandrum,Kerala and washed thoroughly to remove extraneous material and then dried at 40°C in a tray drier and packed in airtight polyethylene bags until further use.

2.2. Preparation of methanolic extract

The dried plant sample was powdered to a size of approximately 20-40 mesh size. 15 g of this was extracted with methanol in a soxhlet apparatus. The extracts were filtered through a filter paper and concentrated in a rotary evaporator at below 45°C. Each of the extract was made up to 100 ml, and stored as stock solution at 4°C in a refrigerator.

2.3. Determination of dry weight

One ml of methanol extract of sample (MES) was taken in a previously weighed petriplate to constant weight. The extract was dried at 105°C till constant weight. Dry weight was calculated by the following formula

Dry weight (1ml) = A-B.

Where, 'A' is the weight of petriplate with dry sample. 'B' is the weight of petriplate.

2.4.Proximate composition

Proximate composition ie., amount of moisture, total fatty matter, carbohydrate, ash content and protein of the Glycine max were analysed according to the AOAC procedures . Moisture content of the plant was determined by oven dry method. About one g of the material was accurately weighed in a pre weighed Petri dish and placed in hot air oven and dried for 2 hrs at 100ºC. The sample was cooled in a desicator and weighed. Heated again at 100ºC in air oven for 30 minutes, cooled and weighed. This process of heating for 30 mins was repeated till the difference in weight between two successive weighing was less than 1 mg. From the loss of weight during the drying amount of moisture was calculated.

$$\text{Total moisture content} = \frac{W_2 - W_1}{W} \times 100$$

W_1 = weight of petriplate with sample – weight of empty petriplate

W_2 = weight of sample .

2.4.1.Total Fatty Matter

Total fatty matter in the dried samples was extracted by soxhlet apparatus using hexane as solvent. The solvent was evaporated and the amount of residue represented the total fatty matter. The nonpolar extract or crude fat of plant parts or foods represents besides the true fat (triglycerides), other materials such as phospholipids, sterols, essential oils, fat soluble pigment etc. A known amount of dried sample was taken in a thimple and placed in the soxhlet fitted with pre-weighed round bottom flask and filled over 1/4 part of it with hexane or petroleum ether. Extraction with solvent is carried out for 4-6 hours. The solvent containing the fat fraction obtained in the round bottom flask was evaporated. From the weight of the residue total fat soluble matter was determined.

$$\text{Percentage of fat} = \frac{W - W1}{W\,2} \times 100$$

W = weight of sample W_1 = weight of fat + flask, W_2 = weight of empty flask

2.4.2.Total minerals

Ash content represents inorganic residue remaining after destruction of organic matter. It may not necessarily be exactly equivalent to mineral matter as some losses may occur due to volatilization.

About one g of sample was accurately weighed into a pre-weighed, clean crucible. The crucible heated to the point of charring of the sample on a hot plate. The crucible with the carbon residue obtained as a result of ignition, was placed in muffle furnace at temperature of 650º C until the carbon residue disappears. Allowed to cool and then weighed.

$$\text{Percentage of fat} = \frac{W - W1}{W\,2} \times 100$$

W=weight of sample,W_1= weight of empty crucible, W_2 = weight of ash + crucible

2.4.3.Total Protein content

Nitrogen content in the plants mainly appears as proteins and amino acids. So total amount of nitrogen indicates the amount of total proteins and amino acids. Nitrogen content was estimated by Kjeldahl method, which was based on the determination of the amount of reduced nitrogen (NH_2 & NH) present in the sample. The various nitrogenous compounds were converted into ammonium sulphate by boiling with concentrated H_2SO_4 .The ammonium sulphate formed was decomposed with an alkali (NaOH), and ammonia liberated was absorbed in excess of neutral boric acid solution and then titrated with standard acid.

$$\text{Nitrogenous organic compound} + H_2SO4 \longrightarrow \text{digestion}$$
$$(NH_4)_2SO_4 + 2\ NaOH \longrightarrow Na_2SO_4 + 2NH_3 + 2H_2O$$
$$NH_3 + H_3BO_3 \longrightarrow H_2BO_3 + H_3HBO_3$$

About one g sample and 0.5 g of digestion mixture were weighed into a Kjeldahl flask. Added about 10 ml concentrated H_2SO_4 and heated on a mantle (in slanting position) until colour of solution changes to blue green (pale). This clear solution was made up to 100 ml under cold conditions. The Kjeldahl apparatus was set up for protein estimation. A round bottom flask half filled with water was connected to the apparatus and heated on a Twenty ml of boric acid and 1 ml indicator is taken in a conical flask and placed under condensor. Five ml of sample with 20 ml of 40% NaOH and three ml water were added to distillation tube through funnel. When water boils inside the round bottom flask, steam produced is passed into distillation tube. NH3 evolved in distillation tube is trapped in boric acid. Upon ammonia evolution, colour of boric acid changes to blue. For maximum ammonia evolution, the process is continued for 20 mins. Titrated with standard HCl till blue colour of the solution disappeared.

Amount of nitrogen in the samples was calculated by the following relation

$$\%\ \text{of nitrogen} = \frac{14 \times 100 \times N\ HCl \times 100 \times \text{volume of HCl}}{}$$

Where, 'N' and 'W' represents the normality of HCl used and weight of the sample respectively.

2.4.4.Determination of crude fiber

Crude fibre was the organic residue which remains after the sample has been treated under standardized condition with petroleum spirit, boiling dilute sodium hydroxide solution and alcohol.

The crude fibre consists largely of cellulose together with a little lignin. As the recovery of cellulose using the specified procedure seldom exceeds four by fifth of that actually present, the crude fibre content does not represent a measure of specific group of substances.

2.4.4.1. Preparation of Gooch crucible

Cleaned , dried gooch crucible was filled with ¾ of its volume with dried asbestos powder and was fixed on to a filteration device. Then it was washed with distilled water. While adding distilled water, suction was applied to facilitate the asbestos to settle tightly on gooch crucible. The packed volume should not be more than ¼ of total volume of gooch. The packed gooch crucible was washed with distilled water until the washings were clear. It was then dried at 120ºC for 1 hour and was stored in dessicator until further use.

2.4.4.2. Determination of crude fibre

Residue from crude fat determination was used. Take twog of sample and reflex with 200ml of 1.25% sulphuric acid. The mixture, while hot, was filtered through wet muslin cloth. The residue was transferred quantitatively over muslin cloth back with the help of 200ml of 1.25% sodium hydroxide solution and it was refluxed again for 30 mins. The digested material was filtered under suction through prepared gooch crucibleThe residue was washed with hot distilled water several times until the washings were free of alkali and later washed with 25ml 95% alcohol and dried. Transferred the gooch crucible into hot air oven at 110 C and dry to a constant weight. It was allowed to cool in a desiccators and weighed. The gain weight represents the crude fibre.

$$\text{Percentage of fat} = \frac{W_2 - W_1}{W} \times 100$$

W = weight of sample, W_1 = weight of crucible, W_2 = weight of crucible with sample.

2.5.Evaluation of Antioxidant activity

The antioxidant property was assessed in terms of total phenolic content, total reducing power, DPPH radical scavenging activity, metal chelating activity, ABTS radical cation scavenging assay, hydroxyl radical (OH^-) scavenging activity and antioxidant activity in linoleic acid emulsion system .

2.6.Thin-layer chromatography

Three thin-layer chromatography (TLC) plates, coated with silica gel G (Fluka Chemie, Switzerland) to 0.25 mm thickness, were spotted with methanolic extract of soybean. All plates were then developed in a solvent system of ethyl acetate/methanol/water (10:2:1; v/v/v) .

2.7.Evaluation of antibacterial activity

The antibacterial activity of soybean methanolic extract was studied against gram positive and gram negative bacteria using disc diffusion method.

3.Results and discussion

Soybeans contain isoflavones that have several known biological activities. Hence the present study was carried out to investigate the antioxidant, antimicrobial properties of *Glycine max.*

Glycine max powder was taken for the present study and was extracted by refluxing with methanol and subjected to proximate analysis for the determination of moisture, ash, crude fiber, fat and carbohydrate content. The total phenolic content was also studied and antioxidant properties were investigated [36].

Similarly free radical scavenging activity of *Pterogyne nitens* have been analyzed and it may be concluded from the results of this study that *Pterogyne nitens* have potential antioxidant activity based on ABTS and DPPH radical scavenging assay [37]. Hence the previous and present studies concluded that the Glycine max extract contained compounds that conferred antioxidant properties.

3.1.Proximate composition

The results from the proximate analysis of Glycine max showed that moisture content was higher with 63.06±1.06 followed by total carbohydrate content 20.05±1.14%.The crude protein content was 1.04±0.09%,crude fiber content was3.47±0.50% whereas the total fat and ash content were 5.31±0.46 and 7.34±0.54% respectively (Table 1) [38].

Table 1. Proximate composition of vegetable soybean

Compositional Analysis	Values In %wet Weight Basis
MOISTURE	63.06 ± 1.06
ASH	7.34 ± 0.54
FAT	5.31 ± 0.46
PROTEIN	1.04± 0.09
CARBOHYDRATE	20.05± 1.14
FIBRE	3.47 ± 0.50

The results of proximate analysis showed a high content of moisture and carbohydrate (Table1).The moisture content analyzed was high (63.05±1.06) compared to that of a medicinal plant Nypa fructican as reported by Odomenaand Ekpo (2005) in which moisture content for leaf (50.19±0.33%), stem (63.51±0.54%) and root (29.19±0.94%) were obtained. High moisture content promotes susceptibility to microbial growth and enzyme activity .

3.2.Dry weight of extract

The dry weight of the sample was calculated to be 24.6 mg/ml which corresponds to a yield of 16.4% of the dry sample

3.3. Antioxidant activities

Antioxidant activities of soybean methanolic extract against DPPH radical, superoxide radical, hydroxyl radical, ABTS radical and metal chelation were evaluated using standard assay methods. In, addition, total phenolic content (TPC) and total reducing power (TRP) were also evaluated. The antioxidant activity of soybean extract in linolenic acid emulsion system was also evaluated. All the experiments were carried out in triplicates.

3.3.1.Total phenolic content (TPC)

Polyphenols are the large group of phytochemicals that are gaining acceptance as being responsible for the health benefits associated with fruits and vegetables. The antioxidant activity of plant materials was well correlated with the contents of their phenolic compounds . Total phenolic contents were determined using Folin-ciocalteu reagent and expressed as GAE (Singleton et al., 1999). Folin-ciocalteu reagent contains metals like polytungston. Phenolic content from the sample reduce the metal and change the colour from yellow to prussian blue. The intensity of the colour is directly proportional to the phenolic content.

The extracts were diluted with the same solvent used for extraction, to a suitable concentration for analysis and 0.5 ml of commercial Folin-ciocaltue reagent was added. The contents were mixed well and kept for 5 min at room temperature followed by the addition of 1 ml of 20% aqueous sodium carbonate. After incubation at room temperature for one and half hr, the absorbance of the developed blue colour was read at 760 nm (Shimadzu UV- 2450 Shimasu corporation, Kyots ,Japan.) against reagent blank and the results were calculated as GAE (mg/100 g) of sample.

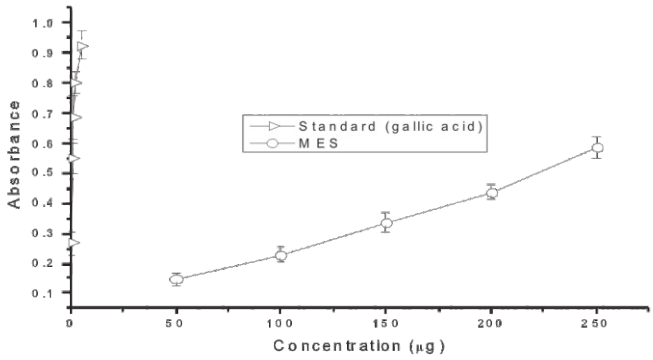
Because of their chemical structure; plant polyphenols can scavenge free radicals and other pro-oxidants, and also interact with a number of biological relevance. Total polyphenol present in the methanolic extract was determined by using Folin ciocalteau method and expressed as mg of gallic acid equivalents (GAE) per g of extract [39].

The total phenolic content in methanolic extract of Glycine max were 8.8% of the dry extract which corresponded to 1.4% of the dry sample.Overall,Glycine max revealed better antioxidant properties than *T.portentosum*,which was in agreement with the higher content of phenols found in the first species.

3.3.2.Total reducing power

The Fe3+- Fe2+ transformation in the presence of MES (sample) and gallic acid were investigated to measure reductive ability according to Oyaizu (1986).The presence of reducers (antioxidants) cause the reduction of Ferricyanide complex to the ferrous form. The absorbance was measured at 700 nm. Evaluation of total reducing power showed that gallic acid had reducing activity greater than that of soybean sample. A linear relation of reducing activity was observed between sample and standard (Fig.1). The reducing power of soybean extract might be due to the di and monohydroxyl substitutions in the aromatic ring which possess potent hydrogen donating abilities as described by Shimada, Fujikava and Nakamura (1992).

Fig.1.Total reducing power of MES and standard



Total reducing power (TRP) was estimated according to Oyaizu (1986) and Zhu et al. (2002). The principle lies on the reduction of potassium ferric cyanide to ferrous cyanide by antioxidants present in the sample. With the increase in antioxidant's concentration, there is an increase in the reducing power, thus increasing the absorbance.

The different concentration of the extracts (100-500 g) and the standard gallic acid, were mixed with 2.5ml phosphate buffer (0.2mM, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The reaction system was closed and incubated at 50C in a water bath for 30 min. Following incubation, 2.5 ml of 10% trichloroacetic acid was added to the assay system and the contents were mixed well. 2.5 ml sample was collected and mixed with 2.5 ml of distilled water and 0.5 ml of 0.5% ferric chloride. The color developed was read at 700 nm (Shimadzu UV-2450) against reagent blank.

The reducing power of soybean methanolic extract was compared with the reducing power activity of the potato peel which suggested that the reducing power did not contribute to the antioxidant effect of the plant extracts [40].

3.3.3. DPPH radical scavenging activity

Scavenging the stable DPPH radical model is another widely used method to evaluate antioxidant activity. DPPH is a stable free radical with characteristic absorption at 517 nm and antioxidants react with DPPH (purple colour) and convert it into 2, 2-diphenyl-1-picryl hydrazine (yellow). The degree of discoloration indicated the scavenging potential of the antioxidant extract, which was due to the hydrogen donating ability.

Free radical scavenging activity of the soybean extract were determined by using a stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (Brand William et al., 1995; Sindhu and Emilia, 2006). DPPH is a free radical of violet colour. The antioxidants in the sample scavenge the free radicals and turned it into yellow colour. The change of colour from violet to yellow is proportional to the radical scavenging activity.

The assay contained 1 ml of 0.05mM DPPH in ethanol and various concentrations of methanol extracts and standards. The volume of all the tubes were made up to 3.5 ml with methanol. The contents were mixed well immediately and then incubated for 30 min at room temperature (24 - 30 c). The degree of reduction of absorbance was recorded in UV-Vis spectrophotometer at 517nm (Shimadzu UV-Vis 2450).

The percentage of scavenging activity was calculated as:

$$\frac{(Ac - As)}{Ac} x100$$

where, Ac 'is the absorbance of control and 'As' is the absorbance of sample.

Fig.2. showed the scavenging activity of MES and standard on DPPH radicals at various concentrations. The scavenging activity on DPPH radicals increased with increasing concentrations (5gμ - 100gμ). A linear relation was obtained between radical scavenging activity and sample concentration.

Fig.2. DPPH scavenging activity of MES and standard

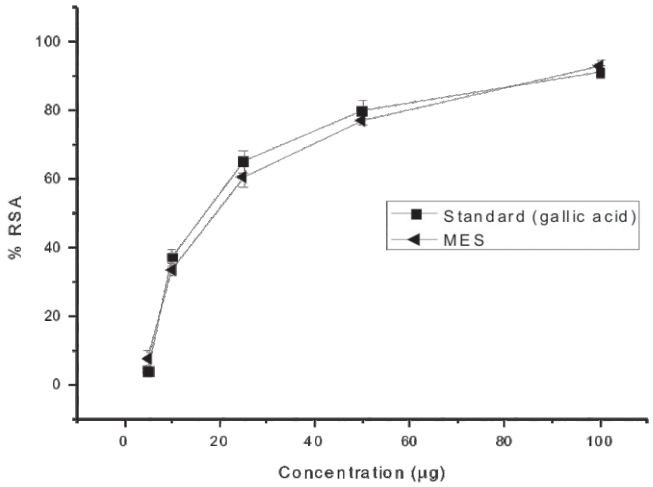


Table 2. gives the percentage DPPH scavenging activity of the extract and standard (gallic acid). There was an inverse relationship between IC50 and antioxidant activity. The IC5O value of sample was 16.48μg/ml and was high as compared to the standard (26.98μg/ml). This indicated antioxidant activity of sample was higher when compared to standard, indicating MES to be a potential antioxidant.

Comparative studies with butanol fractions from stem barks of Pterogyne nitens Tul. (Fabaceae) exhibited potent free radical scavenging activity on DPPH with IC50 value of 4.10±0.3μg/ml.It may be concluded that Glycine max have potential antioxidant activity based on scavenging ABTS and DPPH [41]

Table 2. Percentage DPPH radical scavenging activity and IC50 values of MES & standard

Standard			Sample		
Concentration (μg/ml)	% inhibition	IC50 (μg/ml)	Concentration (μg/ml)	% inhibition	IC_{50} (μg/ml)
5	3.83		5	7.66	
10	33.28		10	33.33	
25	47.13	26.98	25	60.48	16.48
50	79.9		50	76.99	
100	90.86		100	92.68	

3.3.4. Metal chelating activity

The chelation of ferrous ions by the extracts and standards was estimated by the method of Dinis et al. Metal chelating capacity is important since it reduces the concentration of the catalyzing transition metal in lipid peroxidation. Chelating agents may inhibit lipid oxidation by stabilizing transition metals. Ferrozine can quantitatively form complexes with ferrous (Fe2+). The absorbance of Fe2+- ferrozine complex was measured. In the presence of other chelating agents, the complex formation was disrupted with the result that the red colour of the complex gets decreased. As shown in Fig.3. the formation of the ferrozine-Fe2+ complex is not complete in the presence of the extracts indicating that MES can chelate ions. The absorbance of the complex decreased linearly in a dose dependant manner for the extracts (Table 3).

Table 3. % Metal chelating capacity of standard and sample at varying concentrations

Standard			MSE		
Concentration (μg/ml)	% inhibition	IC50 (μg/ml)	Concentration (μg/ml)	% inhibition	IC_{50} (μg/ml)
2	0.75		50	6.1	
4	23.93		100	38.8	
6	28.63	7.27	150	72.06	116.83
8	50.60		200	74.27	
10	76.06				

The chelation of ferrous ions by the extract was estimated by the method of Dinis et al. (1994) and Decker et al. (1990) with slight modifications and compared with that of EDTA, BHT and ascorbic acid. The chelation test initially includes the addition of ferrous chloride. The antioxidant present in the sample chelates the ferrous ions from the ferrous chloride. The remaining ferrous combined with ferrozine and formed ferrous-ferrozine complex. The intensity of the ferrous-ferrozine complex formation depends on the chelating capacity of the sample and the colour formation was measured at 562 nm (Shimadzu UV- Vis 2450).

Different concentrations of standard and extracts (100-500 g/ml) were added to a solution of 100 l FeCl2 (1mM). The reaction was initiated by the addition of 200 l ferrozine (1 mM). The mixture was finally quantified to 1.3 ml with methanol, shaken vigorously and kept at room temperature for 10 min. After the mixture had reached equilibrium,the absorbance measured spectrophotometrically. The percentage inhibition of ferrous-ferrozine complex formation was calculated using the formula:

$$\text{Percentage of chelation} = \frac{(Ac - As)}{Ac} x100$$

where, Ac 'is the absorbance of control and 'As' is the absorbance of sample

Similar studies on the metal chelating ability of Plumeria acuminate leaves showed that antioxidant effect exponentially increased as a function of the development of metal chelating capacity, suggesting that the antioxidant properties be associated with the development of the metal chelating powers [42].

The IC_{50} values for metal chelating activity of MES were found to be 116.83 g/ml. However the chelating ability of the extract was much lower than that of EDTA (IC_{50} of 7.27 g/ml) which acted as a standard.

3.3.5.ABTS radical cation scavenging activity

Scavenging the stable ABTS radical model was widely used method to evaluate antioxidant activity. ABTS is a free radical with characteristic absorption at 734 nm (Shimadzu UV-Vis spectrophotometer model 2100). The experiment was carried out using an improved ABTS decolorisation assay (Abraham and Sindhu Mathew, 2006) and it involves the generation of ABTS+ chromophore by the oxidation of ABTS with potassium persulphate. It is applicable for both hydrophilic and lipophillic compounds.

The ABTS radical cations (ABTS+.) was produced by reacting 7 Mm stock solution of ABTS with 2.45 mM potassium persulphate (final concentration) and allowed the mixture to stand in the dark for at least 6 hrs at room temperature before use. The ABTS solution was diluted to an absorbance of 0.7 ± 0.06 at 734 nm (Shimadzu UV-Vis spectrophotometer, model 2450). Kinetic study was conducted to evaluate the free radical scavenging efficacy of the samples. Absorbance was measured 7 min after the initial mixing of different concentration of the methanolic soybean extracts (final concentration 10-40g/1.1 ml) with one ml of ABTS solution.

The ABTS+. scavenging capacity of the extract was compared with that of trolox. A standard curve was prepared by measuring the reduction in absorbance of the ABTS+. solution at different concentration of trolox and samples over a period of 7 min. The Trolox equivalent antioxidant capacity (TEAC) of an extract represents the concentration of trolox solution that has the same antioxidant capacity as the extract. The TEAC values were determined as follows:

$$\Delta A_{trolox} = (At{=}0_{min\ trolox} - At{=}6_{min\ trolox)} - \Delta A_{solvent(0\text{-}6\ min)} \quad (1)$$

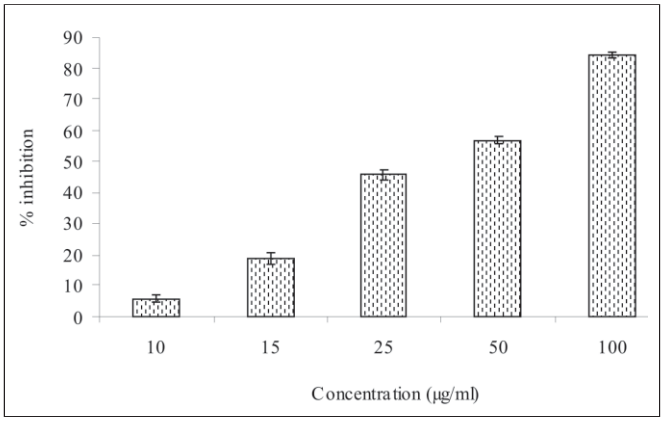
$$\Delta_{Atrolox} = m\ (\text{Trolox}), \quad (2)$$

$$TEAC_{extract} = (\Delta A_{extract/}m) \times d \quad (3)$$

where ΔA is the reduction of absorbance; A- the absorbance at a given time; m- slope of the standard curve; [Trolox], the concentration of trolox; d- the dilution factor.

This method measured the relative antioxidant ability to scavenge the radical ABTS+ as compared with a standard amount of Trolox, and was an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and of chain breaking antioxidants. Trolox, the water soluble analogue of vitamin E, was used as a reference standard. The degree of decolourisation indicated the scavenging potential of the extract/standard, which is due to the hydrogen donating ability. Fig.4.depicted a steady increase in the ABTS radical scavenging capacity of the extract with increase in concentration. The TEAC value for the extract at the maximum concentration studied (100 µg/ml) was found to be 47.96 which means that 47.96 µg of trolox will give the same scavenging capacity as that of 100 µg of MES.

Fig.4. ABTS radical scavenging capacity of methanolic extract of soybean



The methanolic fractions from stem barks and green fruits of Pterogyne nitens Tul. (Fabaceae) exhibited the best activity against ABTS, with IC50 values (µg/ml) of 2.10±0.1 and it may be concluded that Glycine max have potential antioxidant activity based on scavenging ABTS and DPPH [43].

3.3.6.Hydroxyl Radical Scavenging Activity

Hydroxyl radical generated through Fenton reagent in a buffered system can be used to evaluate the scavenging activity of antioxidant. The highly reactive _OH can cause oxidative damage to DNA, lipids and proteins . The sugar deoxyribose on exposure to hydroxyl radicals, generated by the Fenton reaction model system degrades in to fragments and generates a pink chromogen on heating with TBA at low pH (Halliwell, Gutteridge, and Aruoma, 1987). The hydroxyl radical scavenging activity was measured by the deoxyribose method . The reaction mixture, which contained CBE, deoxyribose (3.75 mM), H2O2 (1 mM), potassium phosphate buffer (20 mM, pH 7.4), FeCl3 (0.1 mM), EDTA (0.1 mM) and ascorbic acid (0.1 mM), was incubated in a water bath at 37 ± 0.5 C for 1 hr. The extent of deoxyribose degradation was measured by TBA method (Ohkawa, Ohishi, & Yagi, 1979). 1 ml of TBA (1% w/v) and 1 ml of TCA (2.8% w/v) were added to the mixture and heated in a water bath at 100°C for 20 min. The absorbance of the resulting solution was measured spectrophotometrically at 532 nm. All the analyses were done in triplicates and average values were taken. Inhibition (I) of deoxyribose degradation in percent was calculated according to the equation

$$\text{Inhibition (\%)} = (A0 - A1/\ A0) \times 100$$

where A0 is the absorbance of the control reaction and A1 is the absorbance of the test compound.

As is the case for many other free radicals, _OH can be neutralized if it is provided with a hydrogen atom. The sample exhibited hydroxyl radical scavenging activity in a dose dependent manner in the range of 300 to 1500 µg ml-1 in the reaction mixture (Fig.5). Overall, the scavenging activities of phenolic substances might be due to the active hydrogen donating ability of hydroxyl substitutions. Fig.5.showed the dose dependant curve for the radical scavenging activity of extract and standard. The IC 50 values for standard catechin and MES were found to be 452.2 & 1153.3 µg/ml respectively. MSE exhibited lower hydroxyl radical scavenging activity as compared to standard, but still possess scavenging capacity (Table 4).

Fig.5.Hydroxyl radical scavenging efficacy of MES and standard at varying concentrations.

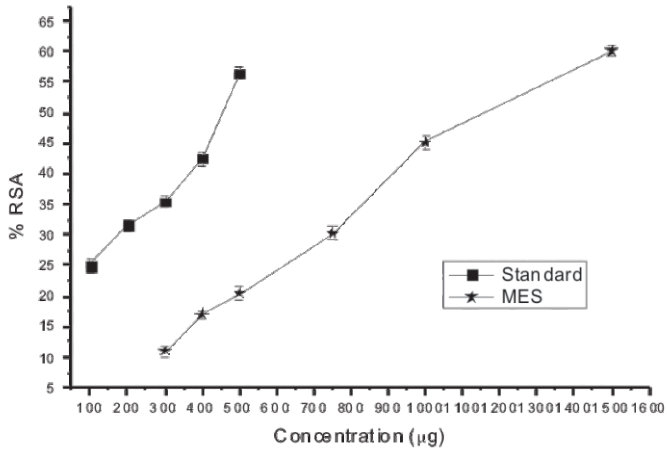


Fig.6. Superoxide radical scavenging efficacy of MES and standard at varing concentration

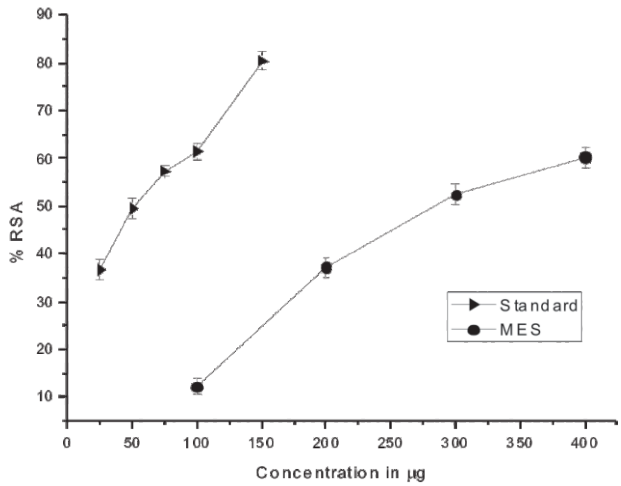


Table 4. % Hydroxy radical scavenging activity of standard and sample at varying concentrations

Standard			MSE		
Concentration (µg/ml)	% inhibition	IC_{50} (µg/ml)	Concentration (µg/ml)	% inhibition	IC_{50} (µg/ml)
100	25		300	11.8	
200	31.5		400	17.1	
300	35.5		500	20.4	
400	42.53	452.2	750	30.32	1153.3
500	56.57		1000	45.23	
			1500	60.1	

In a similar study for the free radical scavenging activity of Plumeria acuminate leaves the hydroxyl radicals generated attack the deoxyribose and result in a series of reactions that cause the formation of malondialdehyde [44].

3.3.7.Superoxide radical scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals (Korycka-Dahl & Richardson, 1978). In the PMS/NADH-NBT system, superoxide anions derived from dissolved oxygen by PMS/NADH coupling reaction reduce NBT. The decrease of absorbance at 560 nm with antioxidants thus indicated the consumption of superoxide anion in the reaction mixture.

Superoxide radical scavenging activity study was performed according to the method of (Parejo et al., 2002) using the xanthine-xanthine oxidase system. Xanthine is converted to uric acid by the enzyme xanthine oxidase with superoxide as a by product. This superoxide combines with Nitro blue tetrazolium (NBT, 5 mg/ml buffer) and results in blue colour. If the tested sample contained antioxidant, may scavenge the superoxide and thus the formation of formazine blue colour is reduced.

The reduction of colour is proportional to the antioxidant content in the sample and the blue colour developed was measured at 560 nm (Shimadzu UV-2450).

Briefly, 50 l xanthine and 20 l of NBT were added to varying concentrations of extracts and standard (5-30 g extracts and 8-50 g concentration of standard gallic acid). Final volume was made up to one ml with phosphate buffer (50 mM, pH 7.5). 50 l of xanthine oxidase was added to system and mixed well to start the reaction and incubated at 37 C for 30 min in water bath. The reaction was stopped after 30 min by adding 100 l of 0.1N HCl. Absorbance of blank prepared without sample and standard was considered as 100% radical. A decreased NBT reduction in the presence of added soybean extract and standard compound was monitored. Percentage radical scavenging activity (RSA) was calculated using the formula and represented in RSA was percentage.

$$RSA\% = \frac{OD \text{ of control} - (OD \text{ of sample} - OD \text{ of sample control})}{OD \text{ of control}}$$

Similar studies were carried out for the evaluation of superoxide radical scavenging activity of Plumeria acuminata leaves where the superoxide radical reduces NBT to a blue colored formazan which is measured at 560 nm [45].

The investigations in the present study on the superoxide radical scavenging capacities, showed that the MES inhibited superoxide radicals in a dose dependent manner and the extract exhibited super oxide scavenging activity at all the concentrations studied (100-300) µg/ml. IC_{50} value of standard was found to be 52.96 µg/ml and the same for MES was 284.1 µg/ml (Table 5).

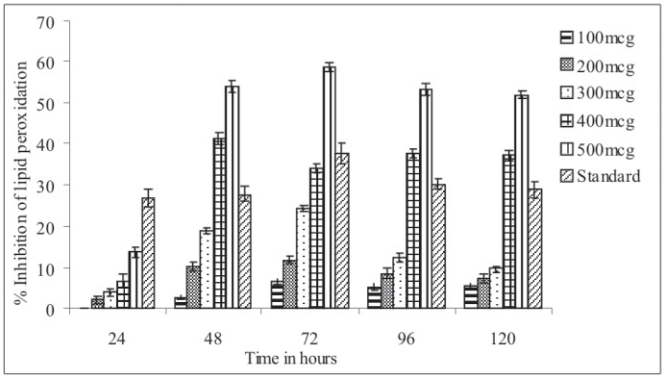
Table 5. % Superoxide scavenging activity of standard and sample at varying concentrations

Standard (Ascorbic acid)			MSE		
Concentration (µg/ml)	% inhibition	IC_{50} (µg/ml)	Concentration (µg/ml)	% inhibition	IC_{50} (µg/ml)
25	36.7		100	12.46	
50	49.5	52.96	200	37.12	284.1
75	57.3		300	52.30	
100	61.4		400	60.13	
150	80.4				

3.3.8.Total antioxidant capacity determination in linoleic acid emulsion system

The sample exhibited effective and powerful antioxidant activity at all the concentrations tested. The effect of various concentrations of MES (100–500 µg ml-1) on peroxidation of linoleic acid emulsions are represented in Fig.7.However, the maximum inhibition of catechin, at concentration of 500 µg ml-1 was found to be only 37.02% after 72 hours of incubation. As can be seen, sample at concentrations of 400 and 500 µg ml-1 was found to have better inhibition on linoelic acid peroxidation than the standard at the maximum concentration studied (500 µg ml-1).

Fig 7. Antioxidant activity of sample at different concentrations and catechin (500 µg ml-1) in the linoleic acid emulsion system using the thiocyanate method. Results are expressed as means ± SD of three parallel measurements.



The antioxidant activity of the CAE extract was determined by the thiocyanate method (Duh et al., 1997). Different concentrations of the sample in methanol were mixed with linoleic acid emulsion in potassium phosphate buffer (0.02 M, pH 7.0). 50 ml linoleic acid emulsion was prepared by mixing and homogenizing 155 µl linoleic acid, 175 µl Tween 20 as emulsifier and 0.02 M phosphate buffer. The reaction mixture was incubated at 37°C. Aliquots of 0.1 ml were taken at various intervals during incubation. The degree of oxidation was measured by sequentially adding ethanol (5 ml, 75%), ammonium thiocyanate (0.1 ml, 30%) and ferrous chloride (0.1 ml, 0.02 M in 3.5% HCl) to sample solution (0.1 ml) and the absorbance was read at 500 nm. Solutions without added extracts were used as blank samples. The degree of oxidation was measured every 24 hr and the data used were the average of triplicate analysis. The inhibition of lipid peroxidation (LPI) in percent was calculated by the following equation:

$$LPI\ (\%) = 100\text{-}[(A1/A0) \times 100].$$

where A1 was the absorbance at 500 nm in the presence of sample and A0 was the absorbance of the control at 500 nm.

Similar studies were carried out in cinnamon bark extract where the auto oxidation of linolenic acid took place and the rate of oxidation increased with increasing concentration of MES. The percentage inhibition of peroxidation in linoleic acid system by 100, 200, 300, 400 and 500 µg ml-1 of MES was found to be maximum in 48 - 96 hours of incubation and the percentage scavenging activity after 72 hrs were 6.4 %, 11.6 %, 24.28, 34.27% and 58.66 % respectively [46].

3.3.9.Thin-layer chromatography

Three thin-layer chromatography (TLC) plates, coated with silica gel G (Fluka Chemie, Switzerland) to 0.25 mm thickness, were spotted with methanolic extract of soybean. All plates were then developed in a solvent system of ethyl acetate/methanol/water (10:2:1; v/v/v) (AppendixIV). After drying, one of the developed plates was first observed under UV light at a wavelength of 365 nm and sprayed with 0.4 mM DPPH radical in methanol (Espin, Soler-Rivas, & Wichers, 2000). Remaining two plates were sprayed separately with Spray 1 solution [1% solution of iron(III) chloride in water mixed immediately before use with an equal volume of a 1% solution of potassium hexacyanoferrate(III) in water (Barton's Reagent)] which gave a blue colour in the presence of phenolic compounds, and Spray 2 solution [2% iron(III) chloride in ethanol]gave either a blue colour, indicating the presence of phenolics with trihydroxy groups or a green colour, indicating phenols with dihydroxy groups or a red/ brown colour indicating the presence of other phenolics when heated at 105°c for five to ten minutes.

The chemical components, especially the phenolic compounds in the sub fractions, of the methanol extracts were detected by TLC with the use of UV absorption and specific spraying reagents. A solvent system, ethyl acetate: methanol: water (EMW) (10:2:1, v/v/v) was used to separate the chemical components in MES. With the EMW solvent system, each fraction

was separated into three to six UV-distinct spots (Fig.8).The results of further testing of these UV-positive TLC spots for their antioxidation activity (DPPH radical-scavenging) and phenolic compound identification (spray tests) are shown in Table 6. MES had two TLC spots that showed antioxidant activity in DPPH test. The presence of phenolic compounds were confirmed by the spray 1 test (Bartons test) where 5 distinct spots of phenolic compounds were separated. Spray 2 test, which indicated the presence of trihydroxy/dihydroxy phenolic compounds or indicated the presence of both trihydroxy (green colour with Rf 0.6 cm) and dihydroxy phenolic compounds (brown colour with Rf 1.5 and 3.1 cm) in the MES.

Similar studies were carried out on the methanolic extract of soybean and analysis was done by Thin Layer Chromatography and it exhibited a chromatographic profile corresponding to phenolic compounds [47].

Table 6: Rf values of the chemical compounds of the methanolic extracts of vegetable soybean which showed positive results to the tests of antioxidant activity and presence of phenolics in TLC under a mobile phase of ethyl acetate:methanol:water

Antioxidant Test	MSE				
	R_{f1}	R_{f2}	R_{f3}	R_{f4}	R_{f5}
DPPH test	1.1	2.5			
Spray 1	0.6	1.2	6	6.3	6.7
Spray 2	0.6 (green)	1.5 (blue)	3.1 (blue)		

Spray 1 solution: 1% solution of iron(III) chloride in water mixed immediately before use with an equal volume of a 1% solution of potassium hexacyanoferrate (iii) in water (Bartons reagent).
Spray 2 solution: 2%iron(III) chloride in ethanol.

3.4.Evaluation of antibacterial activity

It is well known that phenolic antioxidants act as inhibitors for radical chain reactions on autoxidation of organic substrates, and the sulfuric antioxidants act as decomposers for hydroperoxides (Pospisil, 1983). Moreover, some phenolic compounds were known to show antimicrobial activities in addition to their antioxidant effects. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant [48].

The antibacterial activity of soybean methanolic extract was tested against bacteria namely Bacillus subtilis and Psuedomonas aeruginosausing disc diffusion method.The bacterial cultures were swabbed over the nutrient agar plates,sterile discs were dipped into soybean methanolic extract,dried and placed over the agar.The plates were incubated at 37°c for 24 hrs.After incubation,the zone of inhibition was measured.

Studies have shown that some of the extracts from basil,clove,guava,pomegranate and thyme were active against Bacillus subtilis.On the other hand Pseudomonas aeruginosa, which was also resistant to different antibiotics, had its growth inhibited by the extracts from clove,jambolan,pomegranate and thyme.This shows plant extracts have great potential as antimicrobial compounds against microorganisms [49].

The antibacterial activity of methanolic extract of soybean was tested against Gram positive and Gram negative bacteria using disc diffusion method. The organisms used were Bacillus subtilis and *Psuedomonas aeruginosa*. The antibacterial activity was tested using Kirby Bauer method. The paper discs were treated with known concentration of plant sample (24.6mg/ml) and then placed in agar plates inoculated with the respective organism. Methanol was kept as the control. The methanolic extract of soybean showed more inhibition against *Pseudomonas* (Plate III) and growth inhibition was less in the case of Bacillus subtilis (Plate II). The results of the antibacterial activity are shown in Table 7. As can be seen there is a clear zone of inhibition in both the cases. The inhibition was higher for G-ve *Psuedomonas aeruginosa*.

Table 7. Zone of inhibition of MES in mm against the bacterial strains

Bacterial strain	Zone of inhibition in mm
Bacillus subtilis	0.2
Pseudomonas aeruginosa	0.5

As the present study was very preliminary in nature, the antioxidant activity can be systematically studied using different concentration of the extracts against different strains of microbial organisms including pathogenic bacteria and fungi. Such a study will establish the actual antimicrobial potential of the extract and its use as a potential anti-microbial agent. However, the current study revealed that the MES possess antimicrobial activity.

4. Conclusion

The main objective of the present study was to investigate the antioxidant and antimicrobial potential of vegetable soybean using various in-vitro studies. The total phenolic content (TPC) and total reducing power (TRP) were evaluated for the methanolic extract of the dried sample. DPPH radical scavenging activity, metal chelating activity, ABTS radical cation scavenging assay, hydroxyl radical (OH-) scavenging activity, superoxide radical scavenging activity and antioxidant activity in linoleic acid emulsion system carried out for the methanolic extract of the sample (MES) to assess the in-vitro antioxidant activities.

The presence of phenolic compounds was confirmed by TLC, by using three different spray tests as reported in the literature. A detailed study on chemical characterization is required to identify the active compounds in the MES.

Further studies where carried out to evaluate the antioxidant potential of MES as some phenolic compounds are known to show antimicrobial activities in addition to their antioxidant effects.MES exhibited zone of inhibition against both Gram positive and Gram negative bacteria. The inhibition was higher for Gram negative *Psuedomonas aeruginosa.* Thus the methanolic extract of soyabean is found topossess both antioxidant and antimicrobial activity.

5.References

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