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Evaluation of phytochemical, antioxidant and cytotoxic potential of *Sesbania grandiflora* Linn.

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ABSTRACT

Sesbania grandiflora Linn. is perennial branching, small erect quick-growing short-lived soft-wooded tree, mostly cultivated in southeast Asian countries including India and grows primarily in hot, humid environments. This study investigated on phytochemical values- total phenolic flavonoid, tannins and saponin contents for *Sesbania grandiflora* leaf extracts. The total antioxidant capacity, DPPH, reducing power, superoxide scavenging and Iron chelating effects were examined by established methods. In-vitro cytotoxicity by brine shrimp bioassay. Both the extracts of *Sesbania grandiflora* were found to possess moderate to high amounts of phytochemical contents. The total phenolic, flavonoid and saponin contents were found significantly higher in EQSG whereas tannins were more in AQSG. The EQSG and AQSG possessed low IC₅₀ for H₂O₂ Scavenging (32.9, 27.3µg/ml), TAC (35.6, 41.8µg/ml) and moderate for DPPH (114.9, 113µg/ml) and reducing power (176.3, 181.6µg/ml) respectively. The Brine shrimp lethality bioassay showed a significantly high LC₅₀ value with EQSG (10313µg/ml) and AQSG (12773µg/ml) compared to cyclophosphamide (110µg/ml) in cytotoxic assay. The results evidenced the potential of *Sesbania grandiflora* Linn. as potent drug with antioxidant and cytotoxic activity and could be useful for preparation of nutraceuticals for pharmaceutical use in the treatment of various human diseases and its complications.

Keywords: *Sesbania grandiflora*, cytotoxicity, Antioxidant, Oxidative stress, flavonoids.

INTRODUCTION

Medicinal and nutritional properties of plants are known since a long time, as an integrated part of treatment. Despite the recent advances in modern medicine, the therapeutic effects of plant products still have important contribution to health care. Free radicals or reactive oxygen species (ROS) are generated during metabolism and oxidative stress in living systems as a part of the normal physiological process [1, 2]. The evidences suggest that the ROS and free radicals mediated reactions are involved in various pathological conditions such as anaemia, asthma, arthritis, inflammation, neurodegeneration, cancer, mutagenesis, Alzheimer's, AIDS, ageing process and perhaps dementia, malaria [3, 4]. These reactive radicals and oxidants may injure cells and tissue directly via oxidative degradation of essential cellular components and injure cells indirectly by altering the protease/antiprotease balance that normally exists within the tissue interstitium. Thus, maintaining adequate antioxidant status may provide a useful approach in attenuating the cellular injury and dysfunction observed in some inflammatory disorders [5].

Large number of medicinal plants has been investigated for their antioxidant and potent pharmacological properties. Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells. Though the toxicity of most medicinal plants have not been evaluated thoroughly, but the medicines derived from plant products are safer than their synthetic counterparts for health management. Primary sources of natural antioxidants are the compounds majorly belongs to the higher plants such as vitamins, carotenoids, phenolics, flavonoids, tannins, and proanthocyanidins [6]. Recently, it has been reported that there is an inverse relationship between antioxidative status and incidence of human diseases such as cancer, aging, neurodegenerative disease, and atherosclerosis [7]. The plant derived compounds, having potent antioxidant activity, have also been shown to possess anti-inflammatory activity [8].

Sesbania grandiflora (L.) Pers. (Leguminosae), is an Indian medicinal plant, also known as 'sesbania', 'agathi', 'humming bird tree' and listed Ayurvedic drug in Indian Materia Medica [9]. All parts of *Sesbania grandiflora* are utilized in the treatment of various disorders as: bruises, catarrh, dysentery, eyes, fevers, headaches, smallpox, sores, sore throat, anemia, bronchitis, ophthalmia, nasal catarrh, inflammation, leprosy, gout and rheumatism, stomatitis, antiulcer, swellings and tumors. Classically, it is

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used in gynecological applications for leucorrhoea, amenorrhea, anemia, emaciation, milk stimulation after child birth [9, 10] and gonorrhoea in males. *S. grandiflora* possess rich source of phytoestrogens including flavonoids (catechin, epicatechin, luteolin, kaempferol-3-rutinoside, myricetin, naringenin and quercetin,) and other phyto-compounds such as beta-carotene, cyanidin, glycosides, grandiflorol, leucocyanidin, neoxanthin, oleanolic acid, sesbanimide, tannin, violaxanthin, vitamins, zeaxanthin and saponins which on hydrolysis gave an acid sapogenin [11, 12]. The pharmacological properties, viz. anticancer, antiurolithiatic, hepatoprotective, anxiolytic, anticonvulsive, cardioprotective, anti-inflammatory, hypotensive, depressant, diuretic, hypoglycemic and hemolytic are scientifically proved [13-17]. The plant product is variedly used in traditional medicine and modern formulation to assuage leucorrhoea (Keva Leucorrhoea Care, Keva Industries, Bangalore) and fever relief (Ratnagiri rasa; Zandu Ayurveda, Mumbai; Imis Pharma, Andhra Pradesh). The objective of the current study was to systematically evaluate the antioxidant activity through DPPH, total antioxidant capacity, reducing power, superoxide scavenging effect and Iron chelating effects. This study also reported *in-vitro* anti inflammatory and brine shrimp activities of the leaf extracts using reference standards in each case.

MATERIALS AND METHODS

Chemicals Reagents and Diagnostic kits

All chemicals and reagents of analytical grade were purchased from Sigma–Aldrich (St Louis, MO, USA). Ascorbic acid; aluminum chloride, ferric chloride (FeCl₃); Folin–Ciocalteu; 2,20-diphenyl-1-picrylhydrazyl (DPPH); nitroblue tetrazolium; Sulphuric acid; sodium carbonate; sodium hydroxide; sodium nitrite hydrogen peroxide were obtained from Sigma–Aldrich (St Louis, MO, USA).

Plant material and preparation of extract

Sesbania grandiflora is widely available tree in India. The leaves were procured from Bhugaon, Pune, Maharashtra, in the month of August-October 2014. It was identified and authenticated at Botanical Survey of India, Pune, Maharashtra through voucher specimen (BSI/WRC/Cert./2014, ATP15), submitted in Botanical Survey of India, Pune, Maharashtra, under Ministry of Environment and Forest, Government of India. The fresh, shade dried leaves were subjected to coarse powdering and passed through #40 mesh to get a uniform size. The powder (1kg) was extracted with petroleum ether for defatting and subsequently subjected for 24h Soxhlet extraction with 99% ethanol (EQSG) and distilled water (AQSG) (3×1500mL) at 50-60°C. Extract was concentrated under rotary evaporator at 50°C. The dry extract obtained with respective solvent was weighed and the percentage yield was calculated in terms of air dried weight of plant material [18].

Determination of Total Phenolic content (TPC)

Total phenolic content was measured by Folin–Ciocalteu colorimetric method as described previously with a minor modification [19]. The calibration curve of gallic acid was prepared by diluting stock in distilled water (1 ml; 5-50 µg/ml) with 5 ml Folin–Ciocalteu reagent (0.2N). After 5 min, 4 ml of saturated sodium carbonate (75 g/l) were added and incubated for 2hr. The absorbance of blue colored solution was measured at 765 nm. Briefly, 1ml of 250µg/ml of plant extracts (EQSG, AQSG) were dissolved in respective solvent, mixed with the

above reagents and after 1.5 h the absorbance was measured. The total phenolic content in plant extracts was expressed as gallic acid equivalents (GAE) mg/g of the dry extract. All tests were conducted in triplicate.

Determination of Total Flavonoid content (TFC)

Total flavonoid content was measured by Dowd method as described by *meda et al* [20]. Briefly, 5 ml of 2% aluminium trichloride in methanol was mixed with the same volume of EQSG and AQSG (250 µg/ml). The absorbance was measured at 415 nm after 10 min against a blank sample consisting of a 5 ml EQSG and AQSG with 5 ml methanol without AlCl₃. The total flavonoid content was evaluated by using a standard curve with quercetin (1-15 µg/ml). The total flavonoid content in plant extracts was expressed as quercetin equivalents (QE) mg/g of the dry extract. All tests were conducted in triplicate.

Determination of Total Tannin content (TTC)

Total tannin content was measured by Folin–Ciocalteu colorimetric method as described previously with a minor modification [21]. The calibration curve of tannic acid was prepared by diluting stock in distilled water (1.5 ml; 12.5-500 µg/ml) with 2.5 ml Folin–Ciocalteu reagent (0.2N). After 5 min, 1 ml of saturated sodium carbonate (75 g/l) were added and incubated for 30 min. The absorbance of blue colored solution was measured at 760 nm. Briefly, 1.5 ml of plant extracts (250µg/ml) were dissolved in respective solvent, mixed with the above reagents and after 30 min the absorbance was measured. The total tannin content in plant extracts was expressed as tannic acid equivalents (TAE) mg/g of the dry extract. All tests were conducted in triplicate.

Determination of Total Saponins content

Total saponins contents were estimated by prescribed colorimetric methods by *Goel et al* [22]. 1 ml of plant extracts (250 µg/ml) was mixed with 0.25 ml of vanillin reagent (8%, w/v in 99.9% ethanol) in ice-cold water bath and 2.5 ml of 72% (v/v) sulphuric acid was added slowly on the inner side of the wall. The contents were mixed thoroughly and left for 3 min. The tubes were transferred to 60°C waterbath for 10 min and then ice cold for 5 min. Absorbance was measured at 544 nm against the reagent blank. Standard curve was prepared by mixing quillaja saponin (25-1000 µg/ml) as a reference with above reagents and the content of total saponins was expressed as Quillaja saponin equivalents (QS mg/g extract). All tests were conducted in triplicate.

In vitro antioxidant activity

Diphenylpicrylhydrazyl Radical Scavenging Activity

The free-radical scavenging effect of *S. grandiflora* was measured with the stable radical scavenger diphenylpicrylhydrazyl (DPPH) with minor modification [23]. Briefly, the concentrations (20–1000µg/ml) of EQSG and AQSG were prepared in respective solvent. Positive control ascorbic acid was prepared with different concentration (1-100 µg/ml). 1 ml DPPH solution (0.1 mM in methanol) was mixed with 1ml of extract and standard solution separately and kept in dark for 30 min. The absorbance was measured at 517 nm. The intensity of discoloration of DPPH-purple to DPPH-yellow indicated the scavenging efficiency of the extract. Lower absorbance indicated

higher free radical-scavenging activity. The antioxidant activity of the extract was expressed as IC₅₀, which was defined as the concentration (µg/ml) of extract that inhibits the formation of DPPH radicals by 50%. The percentage scavenging activity was determined by formula:

$$\% \text{ Scavenging} = [(A-B)/A] * 100$$

where, A was the absorbance of control (DPPH solution without the sample), B was the absorbance of DPPH solution in the presence of the sample (extract/ascorbic acid). All tests were conducted in triplicate.

Hydrogen Peroxide Radical Scavenging Activity

The scavenging capacity of *S. grandiflora* extracts for hydrogen peroxide was determined by [24]. 3.4 ml of each extracts (5–500 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40mM). Positive control ascorbic acid solution was made with the concentration (2.5–50 µg/ml). After 10 min, absorbance of the hydrogen peroxide was measured at 230 nm against a blank (phosphate buffer without hydrogen peroxide). Hydrogen peroxide scavenging ability was calculated by the formula:

$$\% \text{ Scavenging} = (1 - A_c/A_s) \times 100$$

where A_s is the Absorbance of ascorbic acid / plant extract with H₂O₂ after 10 min, A_c is absorbance of control. All tests were conducted in triplicate.

Iron chelating activity

Iron chelating activity of *S. grandiflora* extracts were evaluated as mentioned by [25]. 2.0 ml of extracts at various concentrations (10 to 400 µg/ml) were added to the reaction mixture consisted of 1.0 ml of O-Phenanthroline (0.05%), 2.0 ml of FeCl₃ (200µM) and incubated for 10 min at ambient temperature. The absorbance was measured at 510 nm against blank (without extract/standard). Ascorbic acid was taken as standard control ranging from 2.5 to 400µg/ml. The percentage of iron chelating activity by extract and standard compound ascorbic acid was calculated as follows:

$$\text{Percent of Iron chelating activity} = \frac{(\text{Test absorbance} - \text{Control})}{\text{Test absorbance}} * 100$$

Reducing power

The reducing power was determined by as mentioned in [19]. Compounds with reduction potential reacts with potassium ferricyanide (Fe³⁺) to form potassium ferrocyanide (Fe²⁺), which further reacts with ferric chloride to form ferric ferrous intense Prussian blue colored complex with an absorption maxima at 700 nm. Higher absorbance of the reaction mixture indicates higher reductive potential. 2.0 ml of extracts at various concentrations (10 to 500 µg/ml) were mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of potassium ferricyanide (10 mg/ml). The mixture was incubated at 50°C for 20 min followed by addition of 2 ml of trichloroacetic acid (100 mg/l), centrifuged at 3000 rpm for 10 min and the upper layer of the solution was collected. 2 ml of collected mixture was mixed with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) fresh ferric chloride. The absorbance was measured at 700 nm after 10 min incubation. Positive control ascorbic acid solution was

made with the concentration (1.0–50 µg/ml). All tests were conducted in triplicate.

$$\text{Percent of Reducing Power} = \frac{\text{Test absorbance} - \text{Control}}{\text{Test absorbance}} * 100$$

Superoxide Radical Scavenging Assay

Superoxide scavenging activity is assayed by the reduction of nitroblue tetrazolium (NBT) as described by [26]. 1.0 ml of extracts at various concentrations (10 to 500 µg/ml) were mixed Tris HCl buffer (3 ml, 16 mM, pH 8.0), 1 ml NBT (50 µM) solution and 1 ml NADH (78 µM) solution. The reaction was initiated by adding 1 ml of phenazine methosulfate (PMS) (10µM) to the reaction mixture and incubated at 25 °C for 5 min. Absorbance was measured at 560 nm against the corresponding blank samples. Ascorbic acid was used as the standard ranging (10-500 µg/ml). Decreased absorbance indicated increased superoxide anion scavenging activity. All tests were conducted in triplicate. The scavenging ability of the plant extract was determined by the following equation:

$$\text{Scavenging effect (\%)} = (1 - \text{absorbance of sample/absorbance of control}) * 100$$

Total Antioxidant Activity (Phosphomolybdic acid method)

The Total antioxidant capacity (TAC) of extracts was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex as described by [26]. 0.4 ml of extracts (50–1000 µg/ml) was mixed with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 min. The reaction was cooled to room temperature and absorbance was measured at 765 nm against a blank. The antioxidant activity was expressed relative to that of ascorbic acid as standard (10–1000 µg/ml). All tests were conducted in triplicate. The antioxidant capacity was estimated using following formula:

$$\text{Antioxidant effect (\%)} = (1 - \text{absorbance of sample/absorbance of control}) * 100$$

Brine shrimp lethality bioassay

The cytotoxicity of the extract was carried out by brine shrimp lethality bioassay according to to *Rahman et al.* (2013) [25]. The dried extract were dissolved in DMSO for stock concentration of 10 mg/ml for toxicity test. Serial dilutions ranging from 20-1000 µg/ml of EQSG and AQSG extract and 2-200 µg/ml of cyclophosphamide (reference control) were prepared in 5.0 ml of artificial seawater (prepared by using sea salt 38 g/L and adjusted to pH 8.5 using 1 N NaOH). *Artemia salina* (simple zoological organism) was used to monitor the cytotoxic screening. Brine shrimps eggs were purchased from Manish aquarium, Pune and hatched in artificial seawater under constant aeration for 48 hrs under the light. The hatched active shrimps now called nauplii were aliquoted into separate glass petridish containing artificial sea water and used for the assay. A suspension containing 20 nauplii was added into each test tube with extract or standard and incubated at room temperature (25±1°C) for 12 h under the light. At the end of 24 hrs, the number of surviving larvae was examined and counted under 3X· magnifying glass. All the experiments were conducted in 4 sets per dose along with control. The LC₅₀ value was estimated using the statistical method of Probit

analysis as well as linear regression equation. The percent mortality of brine shrimp was calculated using the formula:

$$\% \text{ Mortality} = (\text{Number of brine shrimp dead} / \text{Number of brine shrimp introduced}) * 100$$

Statistical analysis

All data were expressed as Mean \pm SD (n=3) and analyzed using student's t-test. Group means were considered to be significantly different at 5% level of significance, $P < 0.05$. All statistical analyses were performed using the "GraphPad Prism V-5.03".

RESULTS

Total phenolics, total flavonoids and % yield contents

The total phenolic, flavonoid, tannin, saponin and % yield content in different fractions of *S. grandiflora* are exhibited in (Table 1). The aqueous extract possess a significant higher % yield content as ethanolic. The value measured for phenolic, flavonoids and saponin content in ethanolic extract were significantly higher as 63.2 \pm 2.5 GAE/g, 73.8 \pm 2.4 quercetin/g and 532.5 \pm 3.5 QS mg/g dry extract. The aqueous extract significantly higher contents of tannins as 79.25 TAE/g.

Table 1: Total phytochemical content in *S. grandiflora* leaf extract.

Plant extracts	EQSG	AQSG
Total Phenolic Content (GAE mg/g of extract)	63.2 \pm 2.5*	61.4 \pm 3.1*
Total Flavonoids Content (QE mg/g of extract)	73.8 \pm 2.4*	60.1 \pm 1.6*
Total Tannin Content (TAE mg/g of extract)	47.53 \pm 0.1*	79.25 \pm 0.1*
Total Saponin Content (QS mg/g of extract)	532.5 \pm 3.5*	442.5 \pm 3.4*
% Yield Extraction	16.8 \pm 2.5*	25.8 \pm 1.9*

Each value represented as Mean \pm SD (n=3). Results were evaluated by student's t-test. Differences were considered to be statistically significant as $P < 0.05$.

In vitro antioxidant activity

DPPH Scavenging Activity

The results for DPPH radical scavenging of both the extracts are shown in Fig 1 and Table 2, indicated that there was a significant ($P < 0.05$) difference of mean percentage scavenging between all the tested concentrations of the extracts and standard. The antioxidant content in EQSG and AQSG extracts were revealed by the gradient decolorization of the purple DPPH radicals in a dose dependent manner. The highest radical scavenging activity was in observed in EQSG (83%) followed by AQSG (80%) at 500 μ g/ml as compared to that ascorbic acid (96%) at 100 μ g/ml. The inhibition concentration (IC₅₀) values of EQSG, AQSG, ascorbic acid was calculated as 114, 113 and 5 μ g/ml respectively, which were inversely proportional to the antioxidant capacity.

Hydrogen Peroxide Radical Scavenging Activity

The scavenging ability of both extracts with hydrogen peroxide is

shown in Fig 1 and Table 2 and compared with the ascorbic acid as standard. It is considered that EQSG and AQSG extracts are capable of scavenging hydrogen peroxide in a concentration-dependent manner. The percent inhibition with 100 μ g/ml of EQSG and AQSG exhibited 70% and 72% scavenging activity on hydrogen peroxide, while ascorbic acid shown 85% inhibition at 50 μ g/ml concentration. The IC₅₀ values of EQSG, AQSG and ascorbic acid on hydrogen peroxide scavenging activity were 32.9, 27.3 and 3.6 μ g/ml, respectively.

Iron Chelating Activity

As depicted from the data Fig 1 and Table 2, both the extracts interfered the formation of ferrous-O-phenanthroline complex, thereby suggested that the extract has metal chelating activity. EQSG and AQSG exhibited inhibitory activity in dose dependent manner at concentration with maximal inhibition of 31% and 27% at 400 μ g/ml with IC₅₀ value of 681.7 and 807.6 μ g/ml respectively. Ascorbic acid used as standard exhibited inhibitory activity up to 63.16% with same concentration and IC₅₀ value of 149.6 μ g/ml.

Reducing Power

The reducing power of herbal extracts is associated with potential antioxidant property. The reducing capability (electron donating ability) of both the extracts was increased with increased concentration, reflecting the presence of reductants in the extracts that causes the reduction of the Fe³⁺/ferricyanide complex to the ferrous form and enhanced color formation. As suggested from the data, EQSG and AQSG showed stronger reducing capacity in dose dependent manner of 68% and 72% at concentration of 500 μ g/ml with IC₅₀ value of 176.3 and 181.6 μ g/ml respectively. Ascorbic acid used as standard exhibited inhibitory activity up to 92.7% with 50 μ g/ml and IC₅₀ value of 4.09 μ g/ml (Fig 1 and Table 2).

Superoxide Radical Scavenging Assay:

The result from the superoxide scavenging assay suggested that the extracts and ascorbic acid have the abilities to quench superoxide radicals in the reaction mixture. EQSG and AQSG exhibited inhibitory activity in dose dependent manner at concentration with maximal inhibition of 69.5% and 91.0% at 500 μ g/ml with IC₅₀ value of 266.1 and 104.1 μ g/ml respectively. Ascorbic acid used as standard exhibited inhibitory activity up to 94.5% with same concentration and IC₅₀ value of 73.9 μ g/ml Fig 1 and Table 2.

Total Antioxidant Activity

As depicted from the data, Fig 1 and Table 2, both the extracts have strong antioxidant activity statistically similar to ascorbic acid which may be attributable to the presence of phenolic compounds. EQSG and AQSG exhibited antioxidant activity in dose dependent manner at concentration of 85.2% and 79.2% at 100 μ g/ml with same concentration of ascorbic acid used as standard up to 95.9% respectively. The higher absorbance value indicates the higher antioxidant activity.

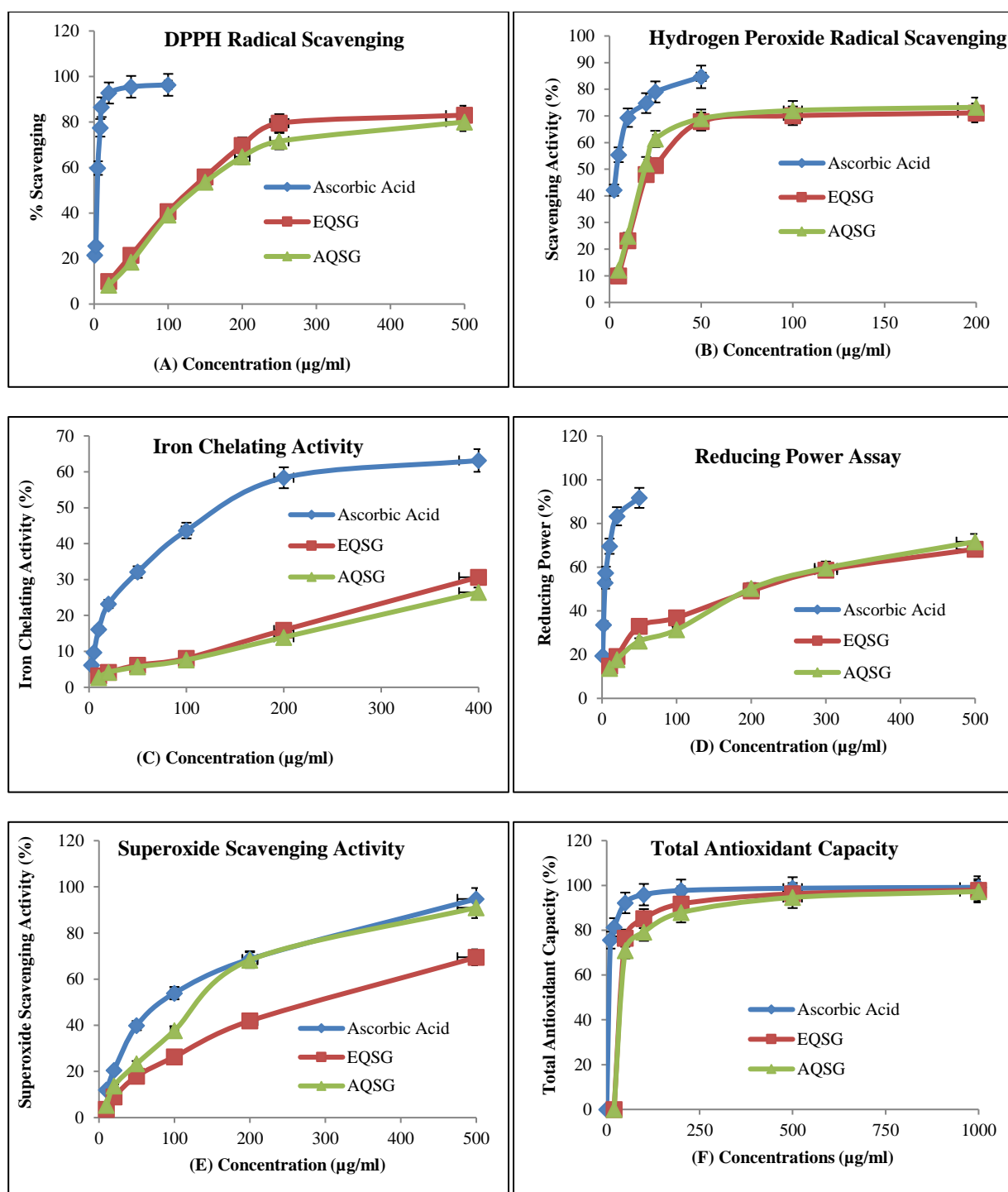


Figure 1: Comparative antioxidative activity (A-F) of *S. grandiflora* extracts. Each value represented as Mean \pm SD (n=3). Results were evaluated by student's t-test. Differences were considered to be statistically significant as $P < 0.05$.

Table 2: IC₅₀ values of the different extracts of the leaf of *S. grandiflora*

Assays (IC ₅₀ µg/ml)	EQSG	AQSG	Ascorbic Acid
DPPH Radical Scavenging	114.9 \pm 1.8*	113.5 \pm 13.1*	5.9 \pm 0.04*
Hydrogen Peroxide Radical Scavenging	32.9 \pm 2.8*	27.3 \pm 1.5*	3.6 \pm 0.06*
Iron Chelating Activity	681.7 \pm 14.7*	807.6 \pm 17.6*	149.6 \pm 3.9*
Reducing Power Assay	176.3 \pm 11.6*	181.6 \pm 8.9*	4.1 \pm 0.2*
Superoxide Scavenging Activity	266.1 \pm 12.2*	107.1 \pm 9.2*	73.9 \pm 6.2*
Total Antioxidant Activity	35.6 \pm 6.9*	41.8 \pm 3.9*	10.3 \pm 0.4*

Each value represented as Mean \pm SD (n = 3). Results were evaluated by student's t-test. Differences were considered to be statistically significant as $P < 0.05$.

Brine shrimp lethality bioassay

The results of the brine shrimp lethality assay are shown in Fig 2 and Table 3. According to the Clarkson's toxicity criterion for the toxicity assessment of plant extracts - extracts with LC_{50} above 1000 $\mu\text{g/ml}$ are non-toxic, 500 - 1000 $\mu\text{g/ml}$ are low toxic, extracts with LC_{50} of 100-500 $\mu\text{g/ml}$ are medium toxic, while extracts with LC_{50} of 0-100 $\mu\text{g/ml}$ are highly toxic [27]. Both the extracts exhibited very low toxicity, resulting median lethality dose for EQSG and AQSG to be 1031 $\mu\text{g/ml}$ and 1277 $\mu\text{g/ml}$ respectively, hence are safe for further pharmacological use compared to cyclophosphamide. Both the extracts have produced concentration dependent increment in percent mortality of brine shrimp nauplii. As depicted from the data EQSG have produced 90% lethality at 2500 $\mu\text{g/ml}$ dose whereas AQSG shown 82% mortality at >5000 $\mu\text{g/ml}$ concentration compared to cyclophosphamide.

Table 3: Results of different extracts of leaf of *S. grandiflora* in Brine Shrimp lethality bioassay.

Sample	LC_{50} ($\mu\text{g/ml}$)
EQSG	1031.5 \pm 13.5*
AQSG	1277.5 \pm 12.5*
Cyclophosphamide	110.5 \pm 1.5*

Each value represented as Mean \pm SD (n = 3). Results were evaluated by student's t-test. Differences were considered to be statistically significant as $P < 0.05$.

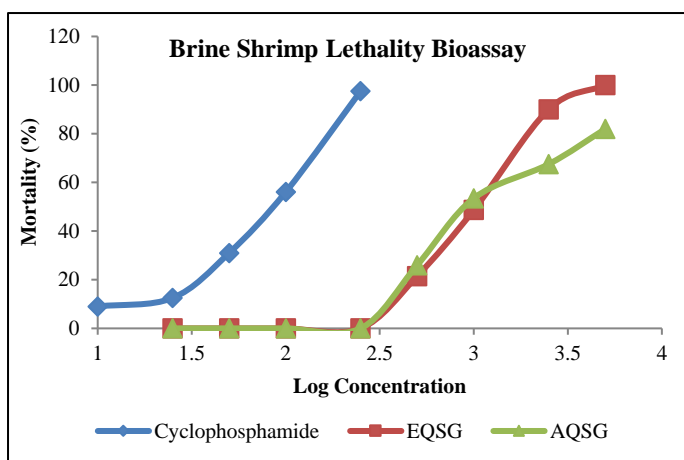


Figure 2: Concentration Dependent Lethality

Percent mortality of different extracts of the leaf of *S. grandiflora*. Both extracts produced concentration dependent increment in percent mortality of Brine Shrimp nauplii.

DISCUSSION

Sesbania grandiflora (SG) is variedly used ethnopharmacologically for the treatment of various ailments. The therapeutic advantage of medicinal plants is usually contributed to their antioxidant properties. The phytochemical investigation reported that SG constitute of antioxidant compounds such as carotenoids, catechin, rutin, quercetin and other phenolics [10, 28]. Moreover, SG have exhibited good antioxidant activities against oxidative stress, antibacterial and antitumor [13, 29]. Quantitative analysis proved that the EQSG possesses higher concentration of phenolics, flavonoids and saponins compared to AQSG, whereas tannins were more in AQSG.

Polyphenols constitute major source of antioxidant, have inhibitory effect on mutagenesis and carcinogenesis in humans when ingested in daily diet [30]. Natural antioxidants can be phenolic compounds (flavonoids, phenolic acids and tannins), nitrogen containing compounds (alkaloids, chlorophyll, derivative amino acids, peptides and amino acids, peptides and amines), carotenoids, tocopherols or ascorbic acids and its derivatives. Natural polyphenols are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, reducing α -tocopherol radicals and inhibiting oxidases. The biological functions of flavonoids include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcer, hepatoxins, viruses and tumors [30]. The mechanism of action of plant antioxidants occurs via prevention of free radicals, neutralizing or scavenging free radicals generated during metabolic processes or reducing and chelating the transition metal composition of foods. Excessive generation of reactive oxygen species and imbalance of antioxidant mechanism cause oxidative stress which provoke oxidative damage of biological macromolecules such as proteins, lipids, and DNA. Eventually results in lipid peroxidation leading to various pathological conditions such as cancer, atherosclerosis, cardiovascular diseases, osteoporosis, premature ageing and inflammatory diseases [31]. The observed differential scavenging and reducing activities of both the extracts against various assay systems may be referred to the different mechanisms *in vitro* to allow rapid screening of extracts. The free radical scavenging of the extracts were assessed by DPPH-stable free radical method [32]. The extent of decolorization of DPPH solution correlates significant scavenging activity and potency of the antioxidants in the EQSG and AQSG in dose dependent manner. EQSG showed slightly higher scavenging effect attributed to its stronger proton-donating abilities and higher concentrations of flavonoids and phenols.

Hydrogen peroxide is a weak oxidizing agent that rapidly decomposed into oxygen and water to generate hydroxyl radicals ($\bullet\text{OH}$) that can initiate lipid peroxidation and cause DNA damage. It is reported that oxidative stress induced via hydrogen peroxide, inhibits the osteoblastic differentiation by reduced Runt-related transcription factor 2 gene expression and stimulation of apoptosis in MC3T3-E1 osteoblastic cells [33]. EQSG and AQSG scavenged hydrogen peroxide by donating electrons to hydrogen peroxidase, thereby neutralizing it into water. The superoxide scavenging activity of the extracts was assessed by reduced formazan formation. Both the extracts inhibited formazan formation in concentration dependent manner, with IC_{50} value of EQSG and AQSG as 266.1 $\mu\text{g/ml}$ and 104.1 $\mu\text{g/ml}$ compared to ascorbic acid as 73.9 $\mu\text{g/ml}$. These results demonstrated that EQSG and AQSG are capable of non-enzymatically inhibit the superoxide radical, produced in biological system, which is a precursor of many ROS.

The reducing power of a bioactive molecule depends upon the presence of reductants which can be ascribed by its hydrogen donating ability to convert free radicals into more stable metabolites. As depicted from the data both the extracts have strong reduction potential by reducing Fe^{3+} /ferricyanide complex to the ferrous form in a dose dependent manner. This may be due presence of higher concentration of reductants in the extracts which may stabilize the free radicals and terminate the radical chain reaction. The reducing power value of AQSG was significantly higher than EQSG but lower than ascorbic acid. Presence of high content of flavonoids and ortho substituted phenolic compounds were found to be more active than unsubstituted phenol and may exert pro-oxidant effect by interacting with Iron. The O-phenanthroline quantitatively forms complexes with

Fe²⁺ which get disrupted in the presence of chelating agents [34]. This ferrous-O-phenanthroline complex was disrupted by the alcoholic and aqueous extract, thereby suggesting that the extract has metal chelating activity.

Various synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole are primarily added to food products especially lipid containing food to increase the shelf life of foods [18]. But they are suspected to cause toxicity and may lead to cancer and liver damage. Thus there has been increased interest in natural antioxidants, especially those of plant origin. Phenolic compounds are ubiquitously found in plants, demonstrated to have potent antioxidant activity via redox reaction by act as reducing agents, singlet oxygen quenchers, hydrogen donors, and chelating agents of metal ions [11]. Brine Shrimp Lethality Assay (BSLA) has been widely accepted alternative bioassay technique, to screen the toxicity of plant extracts, pesticides, heavy metals and metal ions, toxicity of cyanobacteria and algae, mycotoxins, pollutants, cytotoxicity of dental materials, toxicity of nanoparticles and marine natural products [35]. The test has several advantages such as rapidness, simple, economical, less experimental requirement, no aseptic techniques, robustness and relatively small amount of test sample is utilized. Due to ethical issue in toxicological tests, substituting animals with BSLA, is alternative approach as a large number of test organisms of exactly the same age and physiological condition can be procured and high degrees of repeatability. The data from the lethality assay concluded that EQSG and AQSG extracts are nontoxic on brine shrimps. The degree of lethality depicted by the extracts was found to be directly proportional to the concentration of the extractives ranging from the lowest concentration (500µg/ml) to the highest concentration (5000µg/ml). These values were significantly different from the cyclophosphamide, an anticancer agent with dose concentration range from 10-250 µg/ml. This concentration dependent increment in percent mortality of brine shrimp nauplii exerted by the extracts indicated the presence of physiologically active phytochemical constituents.

CONCLUSION

From the current study, EQSG and AQSG extracts of *Sesbania grandiflora* clearly demonstrated the potent antioxidant and reduction capacity, justified by its high content of phenols and flavonoids. Bioactive molecules present in the extracts can be further used as a prototype for development of new drugs and/or as a source of antioxidant pharmaceutical raw material.

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