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Spectroscopic Determination of total Phenolic and Flavonoid Contents of *Sesbania Grandiflora* (Linn) Flower

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ABSTRACT

Oxidative stress caused by free radicals is implicated in the pathogenesis of many diseases. In order to continue the other plants with potential benefits with free radical scavenging properties, researchers start looking forward for exploring new plants with rich in flavonoids and polyphenolics. These constituents possess not only free radical scavenging properties but also they are highly safe in the treatment of many diseases/disorders. In one of the field survey, it was identified that *Sesbania grandiflora*(Linn) was extensively used by folklore practitioner for treating many human ailments such as flatulent-colic, astringent, cooling, bitter, tonic, anthelmintic, febrifuge, dyspepsia, diarrhea and gastralgia, pain, nyctalopia, anaemia, emaciation. Even the plant is a rich source of antioxidants such as phenolics and flavonoids. However, scientific information about the plant and concentration of these constituents was not updated. Hence it was planned to undertake the plant of the present study for its spectrophotometric determination of total phenolic and flavonoid contents from 70% alcoholic extract of flowers of *Sesbania grandiflora* using Catechol and Quercetin as reference standard. It was observed that, 70% alcoholic extract of flowers of *Sesbania grandiflora*(Linn) showed 64.0mg/G of total phenol equivalent to catechol and 28.80mg/G of flavonoidal content equivalent to quarcetin standard. Hence it may be concluded that the plant many be potential source of antioxidant principles such as phenolic and flavonoid. Furthermore, the plants possessing these antioxidants can be highly beneficial for the treatment of many common human ailments.

Key-Words: Catechol, Flavonoid, Phenolic, Quercetin, *Sesbania grandiflora*(Linn).

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INTRODUCTION:

Herbs/plants are the good source of antioxidants and they play a vital role in combating generated free radicals. Free radicals have been claimed to play an important role in affecting human health by causing several diseases including cancer, hypertension, heart attack and diabetes. These free radicals are generated during body metabolism. Exogenous intake of antioxidants can help the body scavenge free radicals effectively¹. Flavonoids and Phenolics are the bioactive phytoconstituents having an important role in control and prevention of tissue damage by activated oxygen species and hence herbal drugs or herbal preparations containing such phytoconstituents are gaining importance in the prevention and treatment of various organ toxicities due to xenobiotic/environmental challenges². In one of the field survey, we found a plant namely *Sesbania grandiflora* (Linn.) belongs to family Fabaceae, it is an ornamental plant and popularly known as “Basna” found in plains of western Himalayas and Sri Lanka³. It was indicated in the literature that the plant is used as astringent, tonic, anthelmintic, febrifuge, diuretic, nyctalopia, cephalalgia, anxiolytic, stomatalgia and antipyretic⁴. Also the plant is useful in the treatment of ulcer, dyspepsia, diarrhea, gastralgia nyctalopia and cephalalgia, Fever, anaemia and emaciation⁵. The literature survey of this plant revealed that this plant possess arginine, cystine, histidine, isolucine, phenylalanine, tryptophan, valine, threonine, alanine, asparagine, aspartic acid, leucocyanidine, cyanidine, saponin yielding oleanolic acid, methyl ester, kaemferol-3-rutinoside, galactose, rhamnose, glucuronic acid⁶⁻⁸. Many herbs containing these phytoconstituents have been investigated for their antioxidants and organ-protective properties. Hence, in order to continue the plants with antioxidant potential, efforts are made not only to identify the type of phytoconstituents present in 70% alcoholic extract but also to determine their concentration spectrophotometrically.

MATERIALS AND METHODS

Collection of plant material and preparation of extracts

The flowers of *Sesbania grandiflora* (Linn) (Figure I) were collected from Neelgunda, Davanagere (Dist) in the month of September 2009. The plant was identified (herbarium specimen No. SCSCOP.Ph.Col Herb.No.008/2010-2011) and authenticated by Prof. K. Prabhu Department of Pharmacognosy, S.C.S College of pharmacy, Harapanahalli. The dried powder of the flowers was defatted with pet ether, Chloroform and then extracted with 70% alcohol using soxhlet apparatus. The extracts was concentrated under reduced pressure using rota flash evaporater and stored in air tight container in refrigerator below 10°C.



Figure I: Photograph of *Sesbania grandiflora*(Linn) flower

Table I: Qualitative Photochemical Tests

Tests for phytoconstituents	Petroleum ether	Chloroform	70% alcoholic extract
Test for carbohydrates:			++
Molish's test	-	-	
Test for reducing sugar			++
Fehling's test	-	-	++
Benedict's test	-	++	
Test for proteins			+++
Biuret test	++	-	++
Xanthoprotein test	-	-	++
Million's test	-	-	
Test for amino acid			+++
Test for tyrosine	-	-	
Test for fats and oil:-			-
Solubility test	+++	+	-
Saponification test	++	-	
Test for Steroid:			++
Salkowski reaction	-	++	++
Liebermann's-burchard reaction.	++	-	
Test for cardiac glycoside			+++
Baljet's test	++	-	
Test for flavonoids			++
Shinoda test	-	-	++
Lead Acetate test	-	-	++
Sodium hydroxide test	-	-	
Test for alkaloids			+++
Dragendroff's test	-	+++	++
Mayer's test	-	++	++
Hager's test	-	++	++
Wagner's test	-	-	
Tests for tannins & Polyphenolics			++
Acetic acid test	++	++	++
Bromine water	-	+	++
Pot. Dichromate	++	-	+
Dil. Iodine	-	+	+
Dil. HNO ₃	+	-	

Note: '+++' indicates highly significant, '++' indicates significant, '+' indicates presence, '-' indicates Absence

Preliminary phytochemical screening

The preliminary phytochemical investigation was carried out for pet. Ether (60-80), chloroform and 70% alcoholic extract of flowers of *Sesbania grandiflora*(Linn) for the detection of various phytoconstituents by following standard methods as described in practical Pharmacognosy by K.R.Khandelwal and C.K Kokate^{9, 10}. The results were shown in Table. I.

Chemicals

Catechol, Quercetin, Folin ciocalteau reagent, Sodium carbonate, 5% Sodium nitrate, 10% Aluminium chloride, 1M Sodium hydroxide were purchased from the Sigma chemicals Co. All other chemicals used in the study were of analytical grade.

Experimental

Determination of total Phenolic Content (TPC)¹¹

The total phenolic content of 70% alcoholic extract of *Sesbania grandiflora*(Linn) was determined by adopting the method as described in Malik E.P and Singh M.B et al . Aliquots of the extract was taken in a 10 ml glass tube and made up to 3 ml volume with distilled water. Then 0.5 ml Folin Ciocalteau reagent (1:1 with water) and 2 ml Na₂CO₃ (20%) were added sequentially in each tube. A blue color was developed in each tube and the intensity of the colour is directly proportional to the phenolic content. The blue coloration in the tube is due to the formation of molybdenum blue as a result of complex redox reaction between phenols and phosphomolibdic acid in Folin Ciocalteau reagent in alkaline medium. The test solutions were warmed for 1 min, cooled and absorbance was measured at 650 nm. The calibration curve was prepared using catechol. The phenolic content of the plant was expressed as a milligram equivalent of phenol per gram of extract.

Determination of total Flavonoid content (FC)¹²

The total flavonoid content of 70% alcoholic extract of *Sesbania grandiflora*(Linn) was determined by adopting the method as described in Heljima et al. Aliquots of each extract was pipetted out in series of test tubes and volume was made up to 0.5ml with distilled water; sodium nitrate (0.3 ml of 5%) was added to each tube & incubated for 5 min at room temperature. Then aluminium chloride solution (0.06 of 10%) was added and incubated for 5 min at room temperature; Sodium hydroxide (1M; 0.25ml) was added and total volume was made to 1ml with distilled water. Absorbance was measured at 510 nm against a reagent blank using Shimadzu model 1700 double beam spectrophotometer and concentration of flavonoids in the test sample was determined and expressed as mg equivalent of quercetin per gram of sample.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of plant showed that, the plant possesses polyphenols, flavonoids, tannins and saponins. The 70% alcoholic extracts of the flowers of *Sesbania grandiflora* (Linn) found to contain 64.0 mg/G of total phenol equivalent to Cathacol and 28.80 mg/G of flavonoidal (Table II) content equivalent to Quercetin. The standard curve of catechol and quercetin were depicted in figure II & III respectively.

Table II: Total phenolic and flavonoid contents of 70% alcoholic extract

Particulars	Phenolic content	Flavonoid content
Standard curve	Catechol	Quercetin
Absorbance	650 nm	510 nm
Amount of content in 70% alcoholic extract per gram	64.00 mg/G	28.80mg/G
R ² value	0.993	0.945

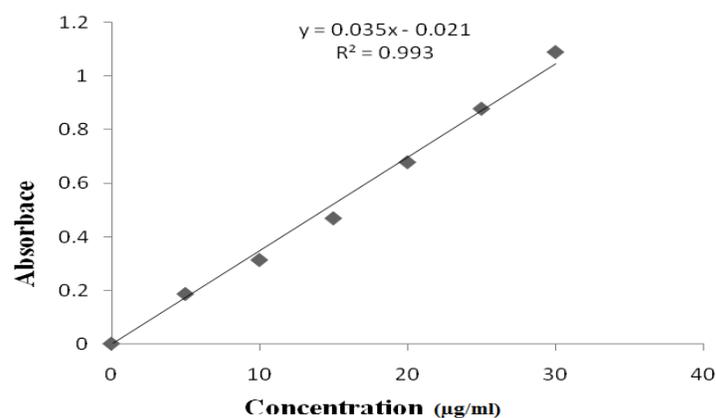


Figure II: Catechol Calibration Curve at 650 nm

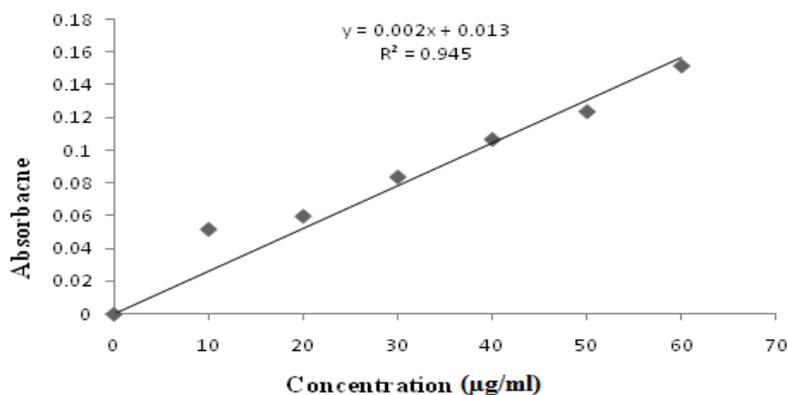


Figure III: Quercetin Calibration Curve at 510 nm

Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, involved in ageing and ementia¹³. Experiment evidence suggests that free radicals (FR) and reactive oxygen species (ROS) can be

involved in a high number of diseases. Numerous physiological and biochemical processes in the human body may produce oxygen-centered free radicals and other reactive oxygen species as byproducts. Overproduction of such free radicals can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA) eventually leading to many chronic diseases¹⁴. Recently, enormous growth is evidenced in the field of herbal medicines as synthetic drugs pose severe toxicity with increased financial burden. Many natural antioxidants like carotenoids, ascorbic acid, tocopherols, poly phenols and flavonoids protect the human body against free radicals induced organ/cell injury. The antioxidative properties of poly phenol and flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free-radical generation. Depending on their structure, flavonoids are able to scavenge practically all known ROS including superoxide anions, hydrogen peroxide, hydroxyl radicals, singlet oxygen, alkoxy, aroxy, and peroxy radicals, as well as alkyl-, aryl-, and nitrogen-derived radicals¹⁵. In our current investigation, the plant of the present study possess rich concentration of Polyphenolics and flavonoids and further these constituents are involved in countering the generated free radicals and it may be suggested here that the plant may be one of the potential source for antioxidants. The plant possessing antioxidants can be employed in the treatment of many human ailments including organ protective properties.

CONCLUSION

The 70% alcoholic extract of flowers of *Sesbania grandiflora* (Linn) showed significant amount of total phenol and flavonoid content. Phenolic compounds (flavonoid and tannins) can influence the enzyme involved in the activation and detoxification. Phenolic components are known to antioxidants and antioxidants are reported to have organ protective role. Hence it may be concluded that the plant may be potential source of antioxidant principles, may be employed in the treatment of many human ailments.

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