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Physicochemical, phytochemicals and antioxidant activity of leaves of *Sesbania grandiflora* (L.)

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Abstract
The antioxidant activity of aqueous leaf extracts of *Sesbania grandiflora* was investigated using the DPPH radical scavenging assay, Hydrogen peroxide assay and Total antioxidant activity. In all testing a positive correlation existed between concentration of the extract and percentage inhibition of free radicals. Phytochemical screening, proximate analysis, mineral analysis by XRF were also determined. Qualitative screening for phytochemical showed the presence of alkaloids, flavonoids, phenols, tannins, terpenoids and cardiac glycosides. Proximate and mineral analysis showed moisture content (75.4%), ash content (2.24%), crude fibre (15.77%), crude lipid (1.81%), protein (7.14%), carbohydrate (12.8%) and minerals namely sodium, potassium and calcium which highlighted its good nutritional value. The results concluded that the presence of phytochemicals and some minerals proves that it is really an antioxidative.

Keywords: *Sesbania grandiflora*, free radical, aqueous, proximate, mineral analysis

Introduction

Free radicals in the body play a major driving factor for the initiation and progression of more than one hundred disorders in humans including cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases (Kumpulainen and Salonen, 1999; Pourmorad *et al*., 2006) [26, 44]. When it produced in excess, it causes deleterious process that can seriously alter the cell membranes and other structures such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA) (Droge, 2002; Pacher *et al*., 2007; Genestra, 2007; Halliwell, 2007) [9, 43, 13, 16]. The use of antioxidants is effective in delaying the oxidation of biomolecules. So, recently there has been an interest in the therapeutic activity of medicinal plants as antioxidants in reducing oxidative tissue damages. Besides, well known and traditionally used natural antioxidants from tea, wine fruits, vegetables, spices and many other plant species have been analyzed in the search for novel antioxidants (Koleva *et al*., 2002; Oke and Hamburger, 2002) [23, 38]. There is still the demand to find information concerning the antioxidant potential of more plant species.

The role of traditional medicine the solution of health problems is invaluable on a global level. Medical plants contribute valuable therapeutic agents, both in modern and in traditional medicine (Krentz and Bailey, 2005) [24]. With the associated side effects of the modern medicine, traditional medicines are gaining important and are now being to find the scientific basis of their therapeutic actions (Gupta and Briyal, 2004) [15]. It is also gaining greater acceptance from the public and the medical profession due to greater advances in understanding the mechanism of action by which herbs can positively influence health and quality of life (Fugh-Berman, 2000) [11].

Sesbania grandiflora (L.) Pers. is a small, erect, fast-growing, and sparsely branched tree belonging to the family Leguminosae. *Sesbania grandiflora* is native to Asia and is now widespread in most humid tropical regions of the world. It is often planted between rice fields (Gohl, 1982) or in association with Guinea grass (Cook *et al*., 2005) [8] which are distributed in warm and wet regions. The leaf is reported to contain protein, fat, fibre, calcium, magnesium, iron, vitamin A &C. It is believed to have Plants are the ridiculous sources of all the elements important for human beings. Like all living things, plants requisite obtains certain elements from their environment in order to endure their biological functions necessary for survival. Some elements are essential for growth for structure formation, as components of biologically active compounds while others have some other beneficial effects. Nutrients and minerals present in biological system play a significant role in the metabolism of plants and humans (Galan *et al*., 2005) [12]. Various parts of *Sesbania grandiflora* used for various ailments by the herbalists like migraine, sinusitis, rheumatism, arthritis, gout and wound healing, oral and throat infections, night blinds, headache, smallpox (Hari *et al*., 2014; Heghedűş-Mîndru *et al*., 2014) [17, 18]. Recent Scientific studies has proved potent hepatoprotective, cardioprotective, antiurolithiatic and anxiolytic activities of *Sesbania grandiflora* (Nafisa Binte *et al*., 2016; Shrivastav and Janin, 1996; Tamboli, 2000) [35, 50, 55]. The present study was undertaken to screen phytochemicals, nutritional value and evaluate *invitro* antioxidant activity of *Sesbania grandiflora* (L.).

Materials and Methods

Plant Collection

The leaves of *Sesbania grandiflora* were collected from Mannargudi, Thiruvarur District, Tamil nadu. The leaves were air dried under natural conditions for few days and were powdered by using home blender and aqueous extract was prepared and stored in refrigerator under 4^0C for further analysis.

Preparation of Aqueous Extract

50g of the powdered leaves were weighed and poured into 500ml conical flask in which 200ml of distilled water was added. The mixture was kept for 12 hours with constant agitation at 30 minutes intervals. The extract was filtered using Whatman No. 1 filter paper.

Qualitative analysis of phytochemicals

The powdered samples were analysed for the presence of various phytochemicals using the following standard methods (Trease and Evans, 2002; Sofowora, 1993 and Roopashree *et al*., 2008) [56, 52, 47].

Quantitative Analysis

Determination of total Alkaloids

5 g of aqueous extract was taken to this 200 ml of 20% acetic acid was added and allowed to stand for 4h by covering the container. It was filtered and placed in a water bath to reduce the volume to one quarter. To this sample, concentrated ammonium hydroxide was added drop by drop until the precipitation process was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed (Obadoni and Ochuko, 2001) [36]. Alkaloid content was expressed as mg/ g of extract.

Total Flavonoids Content

The total flavonoids content was estimated using the procedure described by Chang *et al.,* (2002) [6]. 0.05ml of aqueous extract (1mg/ml) was taken and was made up to 1.0 ml with methanol. 4.0 ml of distilled water was added and mixed. It is then followed by the addition of 0.3 ml of 5% sodium nitrite solution; it was incubated for 5 minutes and 0.3 ml of 10% aluminium chloride solution was added. The mixture was then allowed to stand for 6 minutes. 2 ml of 1 mol/L sodium hydroxide solution was added. The total volume of the mixture was made up to 10ml with distilled water. After 15 minutes the colour developed was measured at 510 nm. A calibration curve was constructed using standard rutin and the total flavonoid content was calculated and the result was expressed as mg ascorbic acid equivalent per 100g extract.

Total Phenolic Content

The phenolic content of aqueous extract was estimated using Folin- Ciocalteau method of Kaur and Kapoor (2002) [22]. About 0.2ml of extract (1mg/ml) was taken and made up to 3 ml with distilled water. It was mixed well and 0.5ml of Folins-Ciocalteau reagent was added and mixed for 3 minutes, followed by the addition of 2ml of 20% sodium carbonate solution. The mixture was shaken well and incubated in dark for 60 minutes and the colour developed was measured at 650 nm.Total phenolic content was expressed as mg/ g of extract

Estimation of total Tannins Content

Tannins content of aqueous extract of leaves of *S.grandiflora* was estimated by the method of Siddhuraju and Manian (2007).To 1.0 ml of extract, 100mg of polyvinyl poly pyrrolidine and 1.0ml of distilled water was added. The mixture was vortexed and left for 15 minutes at 4°C. Then it was centrifuged at 5000rpm for 10 minutes and the supernatant was collected, the supernatant was free of tannin and contains only phenolic content, since tannins have been precipitated with polyvinyl pyrrolidine. The phenolic content in the supernatant was measured at 725nm and expressed as the non tannin phenolic content on a dry matter basis. From the results, tannin content was calculated as follows.Tannins (mg /g extract) = Total phenolics (mg /g extract) – Free phenolics (mg /g extract)

Estimation of Total Saponins Content

Estimation of total saponins was determined by the method described by Makkar *et al*. (2007) [31]. 50mg of extract was dissolved in 10ml of 50% aqueous methanol solution. 1.0 ml of aliquot was taken, to this 0.25 ml of 8% vanillin reagent and 2.5ml of 72% sulphuric acid was added, mixed well and the mixture was incubated at 60°C for 10 minutes in a water bath. Then cooled in ice and read at 544nm in a spectrophotometer against a blank. Ascorbic acid at a concentration of 0.5mg/ml in 50% methanol was used as a standard. The total saponin concentration was expressed as mg per g dry weight (DW).

Proximate Analysis

Determination of moisture content (AOAC, 2000) [4]

An empty china dish and lid were dried in an oven at 105°C for 3min and transferred to desiccator to cool. The empty dish and lid was weighed. About 3 g of fresh leaves was weighed and added to the dish. The sample was spread uniformly in the china dish. The dish with sample was placed in the oven and dried for 3 h at 105°C. After drying, the dish with partially covered lid was transferred to the desiccator to cool. The dish and its dried sample were re weighed. Moisture content % = (w1-w2)x10/W1. Where: W1 = weight (g) of sample before drying; W2 = weight (g) of sample after drying

Determination of Ash Content (AOAC, 2000) [4]

5 g of fresh leaves was weighed accurately in a previously weighed silica crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5 hours at 600°C. This was done to make the sample carbon free. A drop of concentrated nitric acid was added and heated in a furnace for 5 – 6 hours. The ash is white or grayish white in colour. The crucible was then cooled in a desiccator and weighed accurately. Percentage of Ash content = Weight of ash x 100/ weight of sample.

Determination of Crude Fibre

5 grams of the powdery form of plant material and 200 ml of 1.25 % H_2SO_4 were heated for 30 min and filtered with a buchner funnel. The residue was washed with distilled water until it was acid free. 200 ml of 1.25% NaOH was used to boil the residue 30 minutes; it was filtered and washed several times with distilled water until it was alkaline free. It was then rinsed once with 10% HCl and twice with ethanol. Finally, it was rinsed with petroleum ether three times. The residue was put in a crucible and dried at 105° C in an oven overnight. After cooling in a desiccator, it was ignited in a muffle furnace at 550° C for 90 minutes to obtain the weight of the ash (AOAC, 1990) [3].

Determination of Crude Lipid

This estimation was performed using the Soxhlet extraction method. 10 g of the powdery form of each plant sample

were weighed and wrapped with a filter paper and placed in a thimble. The thimble was covered with cotton wool and placed in the extraction column that was connected to a condenser. 200 ml of *n* – hexane was used to extract the lipid (AOAC, 1990) [3].

Estimation of Protein

The amount of protein present in the powdered leaves was estimated using Lowry's method (1951) [29].

Estimation of Total Carbohydrates

The total carbohydrate content of the powdered leaves was estimated using phenol sulphuric acid method of (Dubois *et al.*, 1956) [10].

XRF Mineral Analysis

One gram of ground samples was packed into a polyethylene cup of 20 mm internal diameter and covered with 6-µm-thick polypropylene film. The samples were irradiated in triplicate for 300 s under vacuum using an energy dispersive X-ray fluorescence spectrometer Shimadzu EDX-720. The samples were irradiated using an Rh X-ray tube operated at 15 kV (Na to Sc) and 50 kV (Ti to U). The current was automatically adjusted (maximum of 1 mA). A 10 mm collimator was chosen. The detection was carried out using the Si (Li) detector cooled with liquid nitrogen. Certified reference materials (CRMs) were analyzed using the same method as described above in order to verify trueness and precision. The intensity of element Kα counts per second (cps/µA) was obtained from the sample X-ray spectrum deconvolution using the EDX Shimadzu software package (Margul *et al*. 2009) [32].

Antioxidant assay

DPPH radical scavenging activity (Mensor *et al*., 2001) [34]

To 2.5ml of different of concentrations of aqueous leaves extract, 1.0ml of 0.3mM DPPH ethanol solution was added and the reaction was allowed to take place for 30 minutes at room temperature. After 30 minutes, the absorbance was measured at 518nm. The percentage of antioxidant activity was as follows. % Of scavenging activity = $Ab{-}As/AbX100$; Ab=Absorbance of blank As=Absorbance of sample

Hydrogen Peroxide Scavenging Activity (Ruch *et al.*, 1989) [48]

40mM solution of hydrogen peroxide in phosphate buffer (pH 7.4) was prepared. To 0.6ml of hydrogen peroxide solution varied concentrations of extract (100-400µg/ml) in distilled water was added. Absorbance of hydrogen peroxide was measured after 10 minutes at 230nm. A blank was prepared with phosphate buffer having no hydrogen peroxide. Ascorbic acid was used as a standard.

% of hydrogen peroxide scavenging activity was calculated as follows. % of hydrogen peroxide activity = $Ac{-}As/AcX100$.

Total Antioxidant Activity

The total antioxidant capacity of the extracts was evaluated according to the method described by Prieto *et al*. (1999).An aliquot of 0.5 Ml of samples solution was combined with 4.5 Ml of reagent solution (0.6 M sulfuric acid, 28 Mm sodium phosphate, and 4 Mm ammonium molybdate). In case of blank, 0.5 Ml of 45% ethanol was used in place of sample. The tubes were incubated in a boiling water bath at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each sample was measured at 695 nm against blank in UV-2450 spectrophotometer. The total antioxidant activity was expressed as the absorbance of the sample at 695 nm.The higher absorbance value indicated higher antioxidant activity. % of Total antioxidant activity = $Ac{-}As/AcX100$

Statistical Analysis

Results were given as Mean ± Standard Deviation of 3 replicates. Experimental results were analyzed by SPSS version 16.0. Differences between means were determined using one-way ANOVA and Duncan's test.

Results and Discussion

Medicinal plants are in the food obtained from the vegetation. It is useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents that produce a definite physiological action on the human body. Therefore medicinal plants come into preparation of various drugs singly or in combination or even are used as the source of raw material for the other medicines.

Phytochemicals constituents are non-nutrient, bioactive, secondary metabolites, naturally occurring plant compounds found in seeds, fruits, spices and vegetables. In the present study, plant extract revealed the presence of alkaloids, flavonoids, phenols, terpenoids, cardiac glycosides and tannins and absence of steroid, saponin,sugar and aminoacids which are considered very important components from medicinal point of view (Table 1). Plants have an almost limitless ability to synthesize aromatic substances and derivatives which form the phytochemical constituents (Olukoya *et al*., 1992; Okarter *et al*., 2009) [42, 37]. Phytochemical constituents and derivatives are commonly used for medicinal purposes against number of disease such as analgesic, antimalarial, bactericidal and antiseptic (Stray, 1998) [53]. It is well documented that the presence of these chemicals is responsible for various medicinal properties and there are many reports available to support the role of phytochemical constituents and their activity against specific disease (Suresh and Nagarajan, 2009) [54].

Table 1: Preliminary phytochemical screening aqueous extract of *Sesbania grandiflora*

S. No	Constituents	Aqueous extract
1	Alkaloids	+
2	Flavonoids	+
3	Phenols	+
4	Steroids	-
5	Tannins	+
6	Saponins	-
7	Terpenoids	+
8	Cardiac glycosides	+
9	Sugar	-
10	Aminoacids	-

+ indicates presence whereas – indicates absence

Phytochemical constituents and derivatives are commonly used for medicinal purposes against number of disease such as analgesic, antimalarial, bactericidal and antiseptic (Stray, 1998) [53]. Table 2 represented the data of quantitative

determination of secondary metabolites as showed that phytochemicals determined from the leaves of *S.grandiflora*, phenols were found to be the most abundant one followed by flavonoids and saponins while tannins and alkoloids were least in concentration. Phenols and tannins have anti-oxidant properties. Saponins are produced by plants as a defense mechanism to stop attacks by foreign pathogens, which makes them natural antibiotics (Okwu and Emenike, 2006) [39]. Saponins were also demonstrated to have potential to kill or inhibit cancer cells (Okwu and Nnamdi, 2008) [40]. Flavonoids are known to protect inflammation, platelet aggregation, allergies and microbial infection (Okwu and Omodimiro, 2005) [41].Various reports indicate that regular flavonoids may trim down the risk of several chronic diseases including neurodegenerative diseases, atherosclerosis, and cancer (Maher, 2019) [30]. The flavonoids also reported to have antiviral, anti-allergic, anti-platelet and anti-inflammatory properties.

Table 2: Quantitative Phytochemical composition of *Sesbania grandiflora* leaves

S. No.	Parameters	Value (mg/100g)
1	Alkaloids	3.19 ± 0.87
2	Flavonoids	12.9 ± 0.05
3	Phenols	25.4 ± 0.09
4	Tannin	4.2 ±0.07
5	Saponin	4.5 ±0.08

Values are expressed as Mean ± S.D

The proximate compositions determined in the leaves are summarized in Table 3. It shows that the plant has a high moisture content (75.4%), crude fibre (15.77%) and carbohydrate (12.8%), moderate concentration of protein (7.14%) and ash (2.24%), and low concentration of fat (1.81 %).

Table 3: Proximate composition of *Sesbania grandiflora* leaves

S. No.	Parameters	Value (%)
1	Moisture content	75.4
2	Total Ash	2.24
3	Crude Fibre	15.77
4	Crude lipid	1.81
5	Protein	7.14
6	Carbohydrates	12.8

Water determines the energy value in terms of moisture (Rahimi and Rabani, 2010) [46]. The proximal parameters studied would be used to characterize and standardize the experimental plant. A Moisture content of 75% in the leaves primarily explains the higher degree of food spoilage and microbial contamination. The percentage of ash content defines the quality of a food material which gives an identity to a substance of its carbon free nature and also denotes the organic, inorganic matter and impurities present in the sample. The total ash content predicts the soluble and insoluble minerals in the sample (Llodibia *et al*., 2016) [28].

The crude fiber is the organic residual content remaining after digesting with enzymes, acid and base. It is an important constituent of balance diet that decreases blood cholesterol level, heart risks, colon cancer and diabetes (Ishida *et al*., 2000) [21]. The RDA values of fibers for children are 19-25% and for lactating mother is 29 % (Belewu and Babalola, 2009) [5]. Stated that crude fibers can be used for useful purposes if treated with microorganisms. Crude fats and oils is the part of a complex organic material that is soluble in ether consists chiefly of fats and fatty acids. It is a measure of the fat or oil (lipid) of plant which is considered as medicinal or nutritious feed and extremely rich sources of energy. Oils impede microbial fermentation, ruminant diets should be limited to about 4% fat. Protein contents in the plant vary according to climatic and habitat conditions. Proteins are considered to be the building block of cells and carbohydrate is a group of organic compounds that includes sugars, starches, cellulose, and gums. It serves as a major source of energy in the diet of humans. Carbohydrates performs numerous important roles in human and animal bodies. Polysaccharides serve for the storage of energy (e.g. starch and glycogen) and as structural components (e.g. cellulose in plants and chitin in animals).

Mineral Analysis

Sesbania grandiflora leaves were found to have quantities of Calcium (77.23%). But Potassium (18.38%) and Sodium (4.38%) were present but not in very high concentration (Table 4 and Figure 1). Qualitative determination of mineral elements present in plants is important because the concentration and type of minerals present must often be stipulated on the label of a food. Mineral elements also are needed in minute quantities for the proper functioning of the human system, health growth and development (Igwenyi *et al*., 2014) [19]. The content of mineral elements in plants depends to a high degree on the soils abundance, including the intensity of fertility (Kruczek, 2005) [25]. Calcium is one of the mineral believed to be an important factor governing fruit storage quality (Lechaudel *et al*., 2005) [27]. Ca is the main constituent of the skeleton and is important for regulating many vital cellular activities such as nerve and muscle function, hormonal actions, blood clotting and cellular mortality (Sakina *et al*., 2013) [49]. Calcium is essential for healthy bones, teeth and blood (Charles, 1992) [7]. The health of the muscles and nerves depends on calcium. It is required for the absorption of dietary vitamin B, for the synthesis of the neurotransmitter.Potassium is an intracellular cation and with sodium it controls the electric potential of the body's nerve pressure (Adeyeye and Aye, 2005) [1].

Sodium plays a major role in regulating the amount of water in the body; also, the passage of sodium in and out of cells is necessary for many body functions, like transmitting electrical signals in the brain and in the muscles. Distorted enzymatic activity and poor electrolyte balance of blood plasma are related to inadequate Na+, K+ and Mg2+, as they are the most required elements in living cells (Alli, 2009) [2].

Table 4: Mineral analysis of *Sesbania grandiflora* leaves

S. No	Element	Mass (%)	Atomic intensity (%)
1	Sodium	4.38	5.45
2	Potassium	18.38	18.55
3	Calcium	77.23	76.05

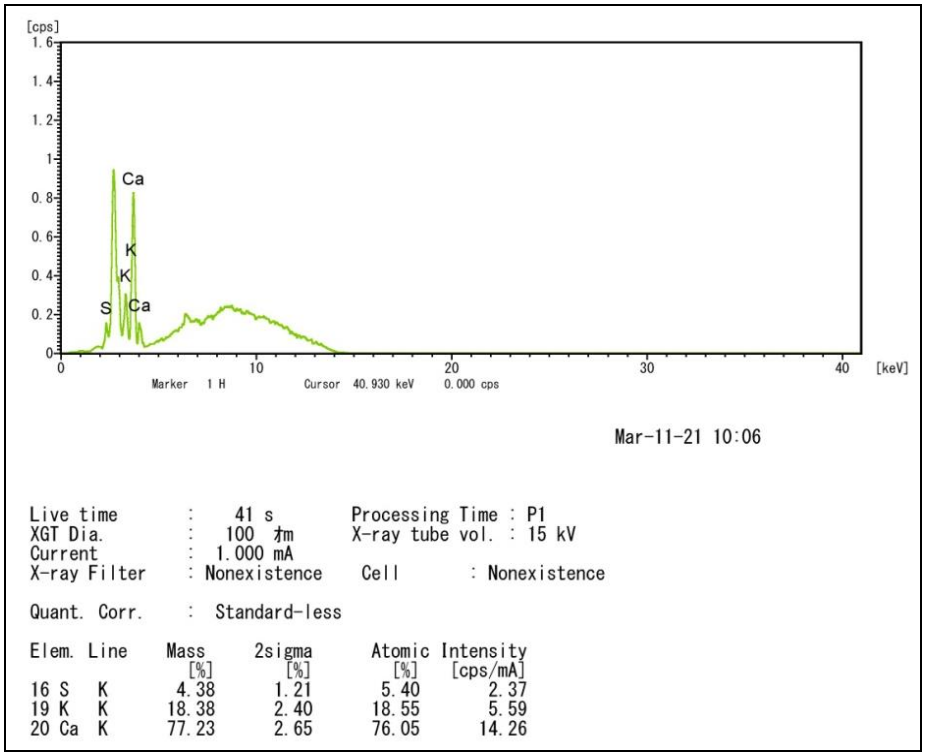

Fig 1: XRF spectra analysis of *Sesbania grandiflora* leaves

In vitro Antioxidant Activity

In the present study, aqueous leaf extract of *Sesbania grandiflora* were screened for *In vitro* antioxidant activity using DPPH assay, Hydrogen peroxide assay and total antioxidant activity. In all methods, various concentrations (100, 200, 300, 400µg/ml) of the extract showed inhibitory activity in concentration dependent manner and it compared with standard ascorbic acid (Table 5). Antioxidant capacity of leaves of *S.grandiflora* was checked by different assays because of the lack of a validated assay that can reliably measure the antioxidant capacity of foods, herbs and biological samples. This study put forwarded that aqueous extract of *S.grandiflora* possess antioxidant properties when compared with the ability of well exemplified and widely used antioxidant standards. The high potential of being good antioxidant and free radical scavengers of extract of *S.grandiflora* is due to the presence of phytoconstituents, which possess a number of therapeutic and pharmacological properties and are promising source of herbal medicinal cure of various ailments including cancers and neurogenerative disorders.

Table 5: Antioxidant activity of *Sesbania grandiflora* leaves

S. No.	Concentration of plant extract (µg/ml)	DPPH (%)	H2O2 %	TAC (%)	Ascorbic acid (%)
1.	100	17.9±1.4	19.55±0.1	15.8±0.5	22.8±0.25
2.	200	22.5±1.8	22.05±0.2	22.06±0.1	28.7±0.6
3.	300	28. 3±0.9	24.2±0.8	25.2±0.8	32.15±0.7
4.	400	32.5±0.5	29.6±0.7	28.6±0.4	38.6±0.8

Values are expressed as Mean ± S.D

The DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm and also for a visible deep purple color. The degree of color change is proportional to the concentration and potency of the antioxidants. Outcome of our study revealed that, changes in color (the more decolourisation is, the more reducing ability is; from deep violet to light yellow) followed by low absorbance of the reaction mixture indicated a high free radical scavenging activity (Meng *et al.*, 2016) [33]. *In vivo* condition Hydrogen peroxide is formed by many oxidising enzymes and it has oxidizing properties also. It can pass through membrane barriers and may oxidize many numbers of compounds slowly (Ilhami *et al.*, 2010) [20]. As a consequence there is a possibility of causing oxidative DNA damage. The aqueous leaves extract of *S.grandiflora* was found to have a good hydrogen peroxide radical scavenging activity. The total antioxidant capacity was measured by the phosphomolybdenum assay which is a quantitative method based on the reduction of Mo^{6+} to Mo^{5+} by the formation of a green phosphate/Mo (V) complex at acidic P^{H}.

Conclusion

From the results, a high positive correlation was observed between presence of phytochemicals and free radical scavenging activity. Our findings add one more attribute to spectrum of pharmacological properties supporting their use in traditional system of medicine.

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