

## PHYTOCHEMICAL ANALYSIS AND EVALUATION ON LEAF OF THE MEDICINAL HERB, SESBANIA GRANDIFLORA FOR IN VITRO ANTIOXIDANT ACTIVITIES

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### ABSTRACT

The aim of this study was to evaluate the antimicrobial activity of *Sesbania grandiflora* used in Ayurveda and traditional medicinal system for treatment of manifestations caused by microorganisms. Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action are also very likely to differ. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Hence, an attempt has been made to check the potency of *S. grandiflora* towards its range of phytochemicals and antioxidant activities. Methanol extract of *S. grandiflora* (leaf) contained relatively higher levels of total dietary phenolics than the other extracts. The antioxidant potential of the

extracts were assessed by employing different *in vitro* assays such as DPPH, FRAP and metal chelating ability.

**KEYWORDS:** Phytochemicals, antioxidant, *in vitro*, *Sesbania grandiflora*.

### INTRODUCTION

Recent reports indicate that there is an increased trend of antibiotic resistance for many of the broad spectrum antibiotics. In such a scenario, development of new antibiotics with a different mechanism of action than the presently used antibiotics is very crucial for the treatment of various infectious diseases. Plants have been used since time unknown as

primary healthcare need by the various civilizations around the globe. They have been used as antidiabetic, anticancer, antiseptic, antiproliferative and boosting immune system. Plants are integral part of human civilization. Medicinal plants are also been relied upon by over 80% of the world population for their basic health care needs. *Sesbania grandiflora* is a small erect, fast-growing, and sparsely branched tree that reaches 10 m in height. The bark of this species is light gray, corky and deeply furrowed and the wood is soft and white. All *Sesbania* sp. have pinnately compound leaves where each leaf is divided into multiple leaflets. It is commonly called as “agase” plant. In Ayurvedic medicine, the leaves are utilized for the treatment of epileptic fits and clinical research supports the anticonvulsive activity of agase leaves. The pharmacological potential of the plant is due to the type of chemical substance they produce and store.

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Antioxidants can prevent undesirable oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as O<sub>2</sub> scavengers.

The increase in prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs and has necessitated the search for new antimicrobials from alternative sources. Natural compounds are a source of numerous therapeutic agents. Recent progress to discover drugs from natural sources has resulted in compounds that are being developed to treat cancer, resistant bacteria and viruses and immunosuppressive disorders.

Therefore, there is need to search new infection-fighting strategies to control microbial infections (Sieradzki *et al.*, 1999; Nogueira *et al.*, 2008). Screening the active compounds from plants has lead to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases.

## MATERIAL AND METHODS

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Neethu and Dhanyaraj, 2016). The experimental procedure

employed in the present study to analyze the antioxidant and antimicrobial properties from the leaves of *S. grandiflora* is presented below.

**Plant material preparation:** Fresh leaves of *Sesbania grandiflora* were collected from Manasagangothri, Mysore. They were shade dried at room temperature ( $26 \pm 2^\circ\text{C}$ ) for 5-6 days. Then the dried samples were fine powdered and stored in screw cap bottles until further analysis.

### **Phytochemical analysis**

#### **Test for Alkaloids**

The extract was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

#### **Test for tannins (Ferric chloride test)**

To the extract add few drops of 1% ferric chloride solution and note the color of reaction. Formation of Green color precipitate indicates presence of tannins.

#### **Test for saponins**

About 5 ml of diluted extracts were taken in a test tube and shaken vigorously and kept for 5 min. Formation of foamy layer indicates the presence of saponins.

#### **Test for glycosides**

Extracts were treated with Ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of Benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniac layer indicates the presence of glycosides.

#### **Test for flavonoids**

Extracts were treated with few drops of sodium hydroxide solution (0.1N) solution. Formation of intense yellow colour, which becomes colourless on addition of dil. Hcl, indicates the presence of flavonoids.

#### **Test for protein**

The extract was treated with few drops of Con. Nitric acid. Formation of yellow colour indicates the presence of proteins.

**Test for triterpenoids**

The extract were treated with chloroform and filtered. The filtrates were treated with few drops of conc. H<sub>2</sub>SO<sub>4</sub>, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenoids.

**Test for polyphenols**

Add 3-4 drops of 5% ferric chloride solution to the extract and observe the formation of dark blue or blackish color which may indicates the presence of phenol in the extracts.

**Test for steroids**

To the leaf extract add few drops of acetic anhydride, warmed and cooled under tap water and add few drops of concentrated sulfuric acid and observe the color change violet to green color indicates the presence of steroids.

**Test for terpenoids**

About 5 ml of each leaf extract was taken and add 2 ml of chloroform and 3 ml of concentrated sulfuric acid notice the formation of layer and color. A reddish brown coloration of the interface confirms the presence of terpenoids.

**Determination of total phenolic content**

The amount of total phenolic content in *S. grandiflora* leaf was determined according to Folin-Ciocalteu method with slight modifications. Briefly, 10 µL of extract solution from the stock solution was mixed with 100 µL of Folin-Ciocalteu reagent. After 10 mins of incubation, 300 µL of 20% Na<sub>2</sub>CO<sub>3</sub> solution was added and the volume was adjusted to 1 mL using distilled water. The mixture was incubated in dark for 2 hrs and the absorbance was measured at 765 nm. The total phenolic content was measured as gallic acid equivalents (mg GAE)/gram of dry weight (dw) and the values were presented as means of triplicate analysis.

**Determination of total flavonoids content**

The content of flavonoids was determined by a pharmacopeia method using rutin as a reference compound. For brief, One ml of aqueous extract in methanol (mg/ml) was mixed with 1 ml aluminium trichloride in methanol (20 g/l) and diluted with methanol to 25 ml. The absorption at 415 nm was read after 40 min at 20 C. Blank samples were prepared from 1 ml plant extract and 1 drop acetic acid, and diluted to 25 ml. A standard graph was constructed using rurtin as the reference standard using the above method.

**Metal chelating assay**

The chelation of ferrous ions by extracts was estimated by method of Dinis *et al.* (1994). Briefly, 50µl of 2 mM FeCl<sub>2</sub> 1.6 ml of deionised water were added to 0.5 ml of the extract. The reaction was initiated by the addition of 0.1 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min and then it absorbed under 562 nm. The metal chelating activity % was calculated by using the formula.3.

$$\text{Inhibition (\%)} = \left[ \frac{A_0 - A_s}{A_s} \right] \times 100$$

**Ferric reducing antioxidant power (FRAP) activity**

The FRAP assay was carried out according to Benzie and Strain (1996). Briefly the working FRAP reagent produced by combination of 300 mM acetate buffer (pH 3.6). 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCL and 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O in 10:1:1 ratio prior to use and heated to 37°C in water bath for 10 min. *Sesbania grandiflora* leaf extract of various concentration were allowed to react with 0.5 ml of the FRAP reagent. The final volume of the reaction mixture was made up to 3 ml with DW. The reaction mixture was kept in dark for 30 min. The readings of the colored product (ferrous tripyridyltriazinecomplex) were taken at 593 nm. The FRAP values were determined as optical density readings. Higher optical density indicated the higher ferrous reducing power.

**Radical scavenging activity using DPPH method**

The ability of the plant extracts to scavenge the stable free radical DPPH was assayed by the method of Mensor *et al.* (2001). DPPH (2,2-diphenyl-2-picryl hydrazyl), a stable free radical, when acted upon by an antioxidant, is converted into diphenyl-picryl hydrazine with a colour change from deep violet to light yellow colour. This can be quantified spectrophotometrically at 518 nm to indicate the extent of DPPH scavenging activity by the plant extracts.

In this DPPH assay, 1 ml of DPPH (10µg/ml) was added with various concentrations of the extract and incubated for 30 minutes at room temperature. It is considered as standard; of which for clean seven test tubes DPPH of 1ul, 8ul, 16ul, 24ul, 32ul and 40ul, respectively, then the concentration will 40-200, respectively, then incubate for 30 min then OD at 517 nm in spectrometer. Before using the test sample in the spectrophotometer first we should take methanol as a blank and made it to auto zero after auto zero one side we kept methanol and another side the DDPH is kept and taken a reading. In that 2 test tubes were considered as blank and control for the blank only the 1 ml of methanol was added and for the control only

the DPPH is added. From the control what we get the reading is subtracted by the value which we get in other tubes. The radical scavenging activity was calculated as follows.

$$\text{Scavenging activity (\%)} = \frac{-100 - A518 [\text{sample}] - A518 [\text{blank}] \times 100}{A518[\text{blank}]}$$

## RESULTS AND DISCUSSION

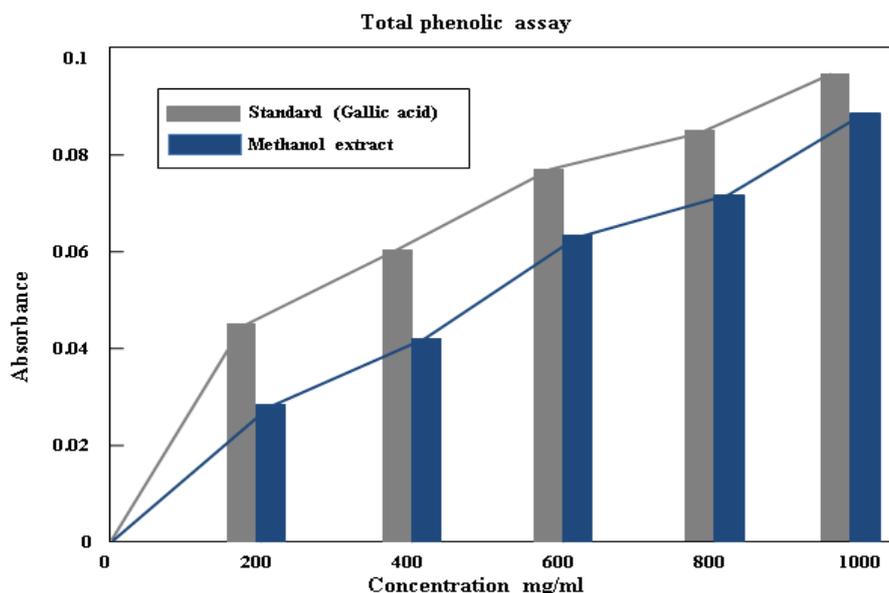
Various tests have been performed to find out the phytochemical constituents (Kartikeyan *et al.*, 2014). Natural phenolics exert their beneficial health effects mainly through their antioxidant activity (Fang *et al.*, 2002). These compounds are capable of decreasing oxygen concentration, intercepting singlet oxygen, preventing 1st—chain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to non radical species and breaking chains to prevent continued hydrogen attraction from substances (Shahidi and Naczki, 2003). Phenolic compounds contribute to the overall antioxidant activities of the plant foods (Table 1).

**Table 1: Phytochemical analysis of *Sesbania grandiflora* leaf extract**

Sl no	Phytochemicals	Observation	Result
01	Alkaloids	Formation of Green colour	Presence of Alkaloids
02	Tannins	Formation of Green colour	Presence of Tannins
03	Saponins	Formation of foamy layer	Presence of Saponins
04	Glycosides	Formation of rose-pink colour in the amminical layer	Presence of Glycosides
05	Flavonoids	Formation of intense yellow colour	Presence of Flavonoids
06	Proteins	Formation of yellow colour	Presence of Proteins
07	Triterpenoids	Formation of golden yellow colour	Absence of Triterpenoids
08	Phenols	Formation of dark blackish color	Presence of Phenols
09	Steroids	Formation of violetto green colour	Presence of Steroids
10	Terpenoids	Formation of reddish brown	Presence of Terpenoids

### Determination of total phenol

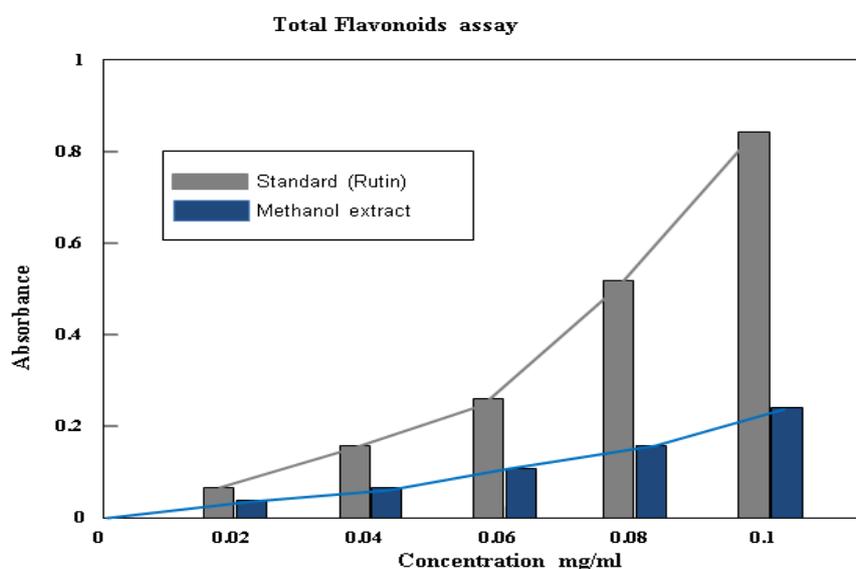
Total phenolic content in the methonolic extract of *Sesbania grandiflora* was (mg of Gallic acid equivalent per gram) estimated by gallic acid. Major role of phenols in scavenging the free radicals is due to the presence of hydroxyl groups. Antioxidant activity of the extract is proportional to the amount of phenol content present in the extract (Figure 1).



**Fig 1: Total phenolic assay for Standard (Gallic acid) and Methanol extract (*Sesbania grandiflora*)**

#### Determination of total flavonoids

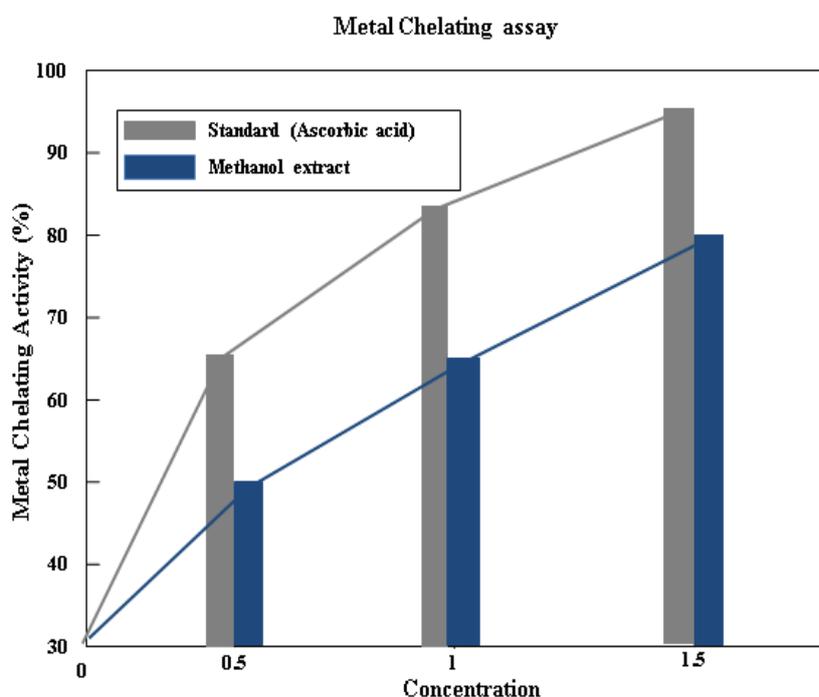
Total flavonoid content was determined using  $\text{NaNO}_2$  and  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ , and result was expressed as mg quercetin equivalents/g methanol extract content was determined using rutin reagent, and absorbance was recorded at 490 nm (Figure 2). The total methanol extract content was then determined from a rutin standard curve, and result was expressed as mg methanol extract equivalent/g DM.



**Fig 2: Total flavonoids assay for standard (Rutin) and methanol extract (*Sesbania grandiflora*)**

### Metal chelating activities

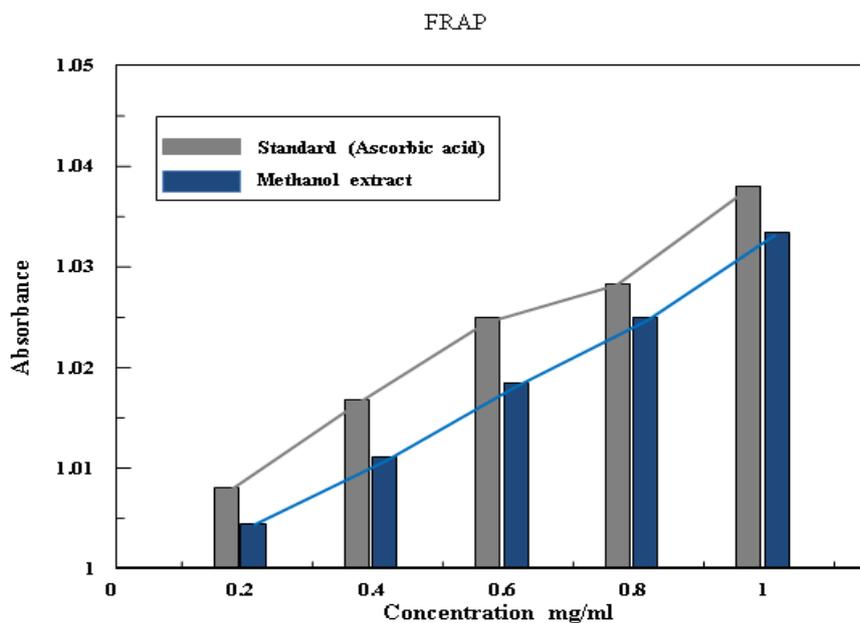
As excess free irons have been implicated in the induction and formation of free radicals in biological systems, the plant extract was tested in a metal chelating assay. It was tested in the concentration range of 0.5 to 1.5 mg/mL, which showed strong chelating activities in concentration-dependent manners (Figure 3). Here the concentration of methanol extract (*S. grandiflora*) increased the percentage of metal chelating assay up to 50-75 but not as much as standard ferrozine.



**Fig 3: Metal Chelating assay for standard (Ascorbic acid) and methanol extract ((*Sesbania grandiflora*))**

### Ferric reducing antioxidant power (FRAP) activity

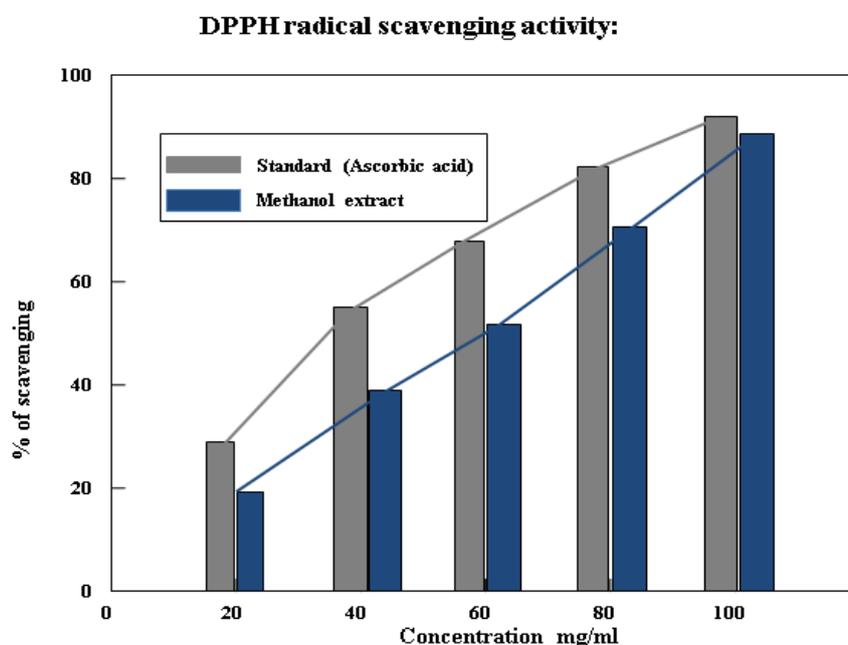
The FRAP assay gives fast, reproducible results with methanol extract, with single antioxidants in pure solution and with mixtures of antioxidants in aqueous solution and added to methanol extract. The dose response characteristics of different antioxidants showed different activities, but the dose response of each individual antioxidant tested was linear, showing that activity is not concentration-dependent, at least over the concentration ranges that was tested in this study (Figure 4).



**Fig 4: FRAP for standard (Ascorbic acid) and Methanol extract of (*Sesbania grandiflora*)**

#### DPPH radical scavenging activity

In DPPH radical scavenging assay, all plant extracts exhibited scavenging activities in a concentration-dependent manner, in the range of 20 to 100  $\mu\text{g/mL}$  (Figure 5). Here the concentration of methanol extract increased the percentage of scavenging up to 20 -90.



**Fig 5: DPPH radical scavenging activity for standard (Ascorbic acid) methanol extract (*Sesbania grandiflora*).**

Plants are blessed with various bioactive chemical with potential antioxidant. The potential is due to the ability of the phytochemical to donate or accept electrons from the free radicals which makes the free radicals unreactive. Polyphenols are the compounds which bear multiple phenolic rings and have the ability to either donate or accept a pair of electrons. The quantitative assays indicate that polyphenols are present in the leaf in a notable quantity. The potential radical scavenging activity, metal chelating ability of the plant extract may be attributed to the presence of flavanoids, polyphenols and alkaloids in the extract. Preliminary studies indicate the promising antioxidant potential of the leaf. Though *in vitro* assays indicate the potential *ex vivo* and *in vivo* validation of the antioxidant potential is needed for the development of the plant as an antioxidant for the treatment of disorders involving oxidative stress.

## CONCLUSION

The study suggests the great value of the species *S. grandiflora* in both pharmacy and phytotherapy. In conclusion, *S. grandiflora* is a natural source of antioxidant substances which are of high importance. The methanolic extract manifested greater power of extraction for phenolic compounds from *S. grandiflora*. Further studies of this plant species should be directed to carry out the *in vivo* studies because of the presence of medicinally active components in order to prepare a natural pharmaceutical products of high value.

## REFERENCES

1. Arun A, Karthikeyan P, Sagadevan P, Umamaheswari R, Rex PR. Phytochemical screening of *Sesbania grandiflora* (Linn). *Int J Biosci Nanosci*, 2014; 1(2): 33-6.
2. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power : the FRAP assay. *Anal Biochem*, 1996; 239(1): 6-70.
3. Dinis TCP, Madeira, VMC, Almeida LM. Action of phenolic derivatives (acetoaminophen, salicylate and 5- aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Arch Biochem Biophys*, 1994; 315: 161-9.
4. Fang YZ, Yang S, Wu G. Free Radicals Antioxidant and Nutrition. *Nutr J*, 2002; 18(10): 872-9.
5. Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, Leitão, SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother Res*, 2001; 15(2): 30-127.

6. Neethu SK, Dhanyaraj FS. Phytochemical analysis and antimicrobial activities of *Sesbania grandiflora* (L) leaf extracts. *Int J Pharmaceut Sci Rev Res*, 2016; 36(1): 144-8.
7. Nogueira JC, Diniz Mde F, Lima EO. In vitro antimicrobial activity of plants of acute Otitis externa. *Braz J Otorhinolaryngol*, 2008; 74: 118-24.
8. Shahidi F, Naczki M. "Antioxidant properties of food phenolics." In: Shahidi F, Naczki M, (eds.), *Phenolics in Food and Nutraceuticals*, Boca Raton; CRC Press, 2003; 1-403.
9. Sieradzki K, Wu SW, Tomasz A. Inactivation of the methicillin resistance gene *mecA* in vancomycin-resistant *Staphylococcus aureus*. *Micro Drug Resist*, 1999; 5(4): 253-7.