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# Phytochemical Screening for Secondary Metabolites and Nutraceutical Value of *Sesbania grandiflora* (L) Pers leaf extract

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**ABSTRACT:** *Sesbania grandiflora* (L) Pers, commonly known as Agasthya is an Indian medicinal plant which belongs to family Fabaceae. The present study intended with various phytochemical screening, estimation of secondary metabolites and Nutraceutical value of leaf extract. The phytochemical test undertaken revealed the presence of Phenols, Flavonoids, Alkaloids, Steroids, Saponins, Glycosides and Terpenoids. Sterols content (37%) was found maximum followed by Flavonoids(18%), Cardiac Glycosides(16%), Phenols(13%), Terpenoids(11%), Tannins(4%) and Alkaloids(1%). The Nutraceutical value with respect to estimation of Primary metabolites (Carbohydrates and Proteins) and Elemental analysis was done. The total carbohydrate content was found to be maximum (162mg/100g), proteins (144mg/100g), Fructose(1.4mg/100g), and the Starch (1.08mg/100g). The Elemental analysis was carried out by AAS method, calcium was found to be the highest (12.4497ppm) and the least was Cadmium (0.0073ppm). 

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## INTRODUCTION

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases [1]. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts [2]. In India, almost 95% of the prescriptions were plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha[3].

*Sesbania grandiflora* (L) Pers. is a plant belongs to family Fabaceae. It is commonly known as Agati, Agasthya, widely available plant, it is an open branching tree tall up to 15cm and 39cm in diameter [4]. It is native to India, Australia, Indonesia, Malaysia, Myanmar, Philippines. In Ayurvedic system, whole plant of Agasthya is medicinally valued. It goes as a principal ingredient in preparation of Ayurvedic medicinal formulations. Classical texts of Ayurveda have attributed wide ranging therapeutic indications to the herb. Agasthya leaves contain a non-poisonous saponin and are known to possess anthehelmintic, alixeteric, aperients, tonic, diuretic, and laxative properties. Further they have been documented as therapeutically useful in Kaphaja disorders, pruritis, skin disorders, night blindness, epilepsy, gout, ophthalmia, nasal catarrh and headache [5]. The bark of the plant is used as astringent, febrifuge and tonic and its infusion in small-pox. Besides the root juice along with honey is used as expectorant [6].The flowers, young leaves and tender pods of the white flowered Agati are edible and are sold in local ethnic markets [7]. They are excellent source of vitamin C and calcium. Seeds are rich in protein [8].

## MATERIALS AND METHODS

### COLLECTION OF PLANT MATERIAL

Fresh plants were collected from a place named Mantralayam, Andhra Pradesh, India. The leaves were separated, washed

under running tap water and shade dried. The dried leaves were ground in to fine powder using a blender. The powder was preserved in an air tight bottle for further use.

**PREPARATION OF PLANT EXTRACT**

Ten gram of the leaf powder was mixed with 100ml of solvent and kept shaking on a shaker overnight. Different solvents used for extraction were petroleum ether, chloroform, ethyl acetate, and methanol and distil water. The extracts kept overnight were centrifuged at 8000rpm for 6minutes and the supernatant was transferred to fresh vials. The collected supernatants were used for phytochemical screening.

**PHYTOCHEMICAL SCREENING**

**Test for Alkaloids [9]**

A. **Dragendroff's test**: To 2 mg of the extract 5 ml of distilled water was added, 2M Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendroff's reagent was added. Formation of orange or orange red precipitate indicated the presence of alkaloids.
B. **Wagner's test:** 2 mg of the extract was acidified with 1.5 % v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids.
C. **Mayer's test:** To 2mg of extract few drops of Mayer's reagent was added. Formation of pale yellow white precipitate indicated presence of Alkaloids.

**Test for Phenols [9]**

A. **Ellagic Acid Test**: The test solution was treated with a few drops of 5% (w/v) glacial acetic acid and 5% (w/v) NaNO2 solution. The solution turned muddy or Niger brown precipitate occurred in the extract. It indicates the presence of phenol solution.
B. **Ferric chloride test**: 0.5 ml of FeCl3 (w/v) solution was added in 2 ml of test solution, formation of an intense color indicates the presence of phenols.

**Test for Flavonoids [10]**

A. **Shinoda's test:** In a test tube containing 0.5 ml of the extract 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicated the presence of flavonoids.
B. **Ferric chloride test:** Test solution with a few drops of ferric chloride solution shows intense green colour.
C. **Zinc-Hydrochloric acid reduction test:** Test solution with zinc dust and a few drops of hydrochloric acid shows magenta red colour.
D. **Alkaline reagent test:** Test solution when treated with sodium hydroxide solution, shows an increase in the intensity of yellow colour which becomes colourless on addition of a few drops of dilute acid.
E. **Lead acetate solution test:** Test solution with a few drops of lead acetate (10%) solution gives a yellow precipitate.

**Test for Triterpenoids**

A. **Libermann - Burchard's test (LB test):** 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a violet coloured ring indicated the presence of triterpenoids.
B. **Salkowaski test:** When a few drops of concentrated sulphuric acid were added to the test solution, shaken and allowed to stand, lower layer turns yellow indicating the presence of triterpenoids.

**Test for Saponins** [9]

**Foam test:** In a test tube containing about 5 ml of extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicated the presence of saponins.

**Test for Steroids**

A. **Libermann-Burchard's test:** 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicated the presence of steroids.
B. **Salkowaski reaction:** 2 mg of dry extract was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of the test tube. Formation of red colour indicated the presence of steroids.
C. **Sulphur test:** pinch of sulphur powder added to test solution it sinks to the bottom it indicates the presence of steroids.

**Test for Tannins [11]**

A. **Ferric chloride test:** To 1-2 ml of the extract, few drops of 5% w/v FeCl3 solution were added. A green colour indicated the presence of gallotannins, while brown colour indicated the presence of pseudo tannins.
B. **Gelatin test:** Test solution when treated with a gelatin solution gives white precipitate. This confirmed the presence of a naphthoquinone.

**Test for glycosides**

A. **Keller-Killiani test:** The test solution was treated with a few drops of ferric chloride solution and mixed. When concentrated sulphuric acid containing ferric chloride solution was added, it forms two layers, lower layer reddish brown and upper acetic acid layer turns bluish green.
B. **Raymond test:** The test solution was treated with dinitrobenzene. Formation of alkal violet indicates presence of glycosides.
C. **Bromine water test:** when the test solution was dissolved in Bromine water yellow precipitate was formed, this indicates presence of glycosides.
D. **Legal's test:** Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gives pink to red colour.

**Test for Resins**

1 ml of extract was dissolved in acetone and the solution was poured in distilled water. Turbidity indicated the presence of resins.

**QUANTITATIVE ESTIMATION OF SECONDARY METABOLITES**

Alkaloids [12], Flavonoids [13], phenols [14], terpenoid [15], Tannin, cardiac glycosides, sterols were estimated by using the standard methods. Alkaloid was found to be in negligible amount whereas sterols were found to be maximum. (**Fig 1**)

**QUANTITATIVE ESTIMATION OF PRIMARY METABOLITES**

The nutritive value of powdered plant material (leaves) was carried out according to standard Spectrophotometric method for the quantitative estimation of Carbohydrates and Proteins. In Carbohydrates, the Total Carbohydrate, Fructose and Starch content was estimated.

**ELEMENTAL ANALYSIS BY AAS (Atomic Absorption Spectroscopy)**

From leaf extracts the content of minerals were analyzed using AAS which revealed the presence of calcium, potassium, magnesium, silicon, titanium, aluminium, iron, copper, vanadium, molybdenum, manganese, zinc, chromium and cadmium. Calcium was found to be maximum whereas Chromium and Cadmium were found to be in negligible amount.

# RESULTS AND DISCUSSION

**Fig I: Quantitative estimation of Secondary metabolites**

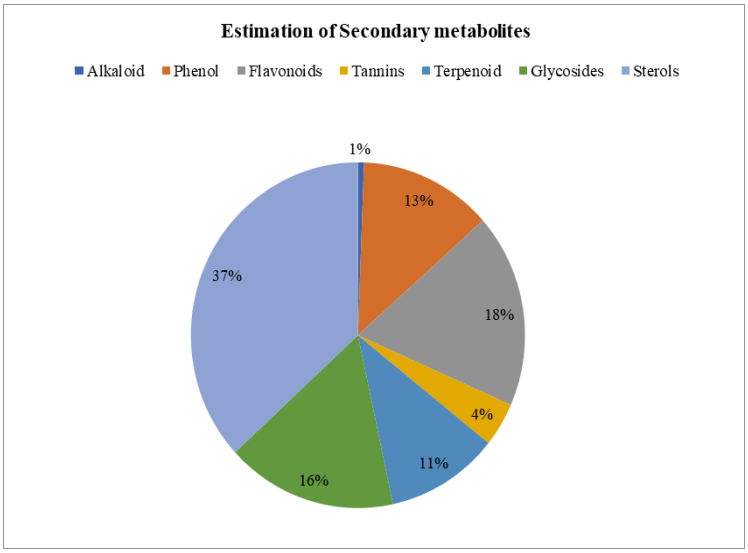


**Table 1: Phytochemical result of secondary metabolites obtained from leaf in different solvent extracts**

| Phytochemical test | Petroleum ether | Chloroform | Ethyl acetate | Methanol | Aqueous |
|---|---|---|---|---|---|
| **ALKALOIDS** | | | | | |
| Dragendroff's test | + | + | + | + | + |
| Wagner's test | - | - | + | - | + |
| Mayer's test | - | - | - | - | - |
| **PHENOLS** | | | | | |
| $FeCl_3$ test | + | + | + | - | - |
| Ellagic test | + | + | + | + | + |
| **FLAVONOIDS** | | | | | |
| **Shinoda test** | - | - | - | - | - |
| $FeCl_3$ test | + | + | + | - | - |
| $ZnCl_2$ test | - | - | - | - | - |
| Alkaline reagent test | - | - | - | - | + |
| Lead acetate test | - | - | - | - | - |
| **TERPENOIDS** | | | | | |
| Libermann Burchard (LB) | + | + | + | + | + |
| Salkowaski test | - | - | - | - | - |
| **SAPONINS** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Foam test | + | + | + | + | - |
| **STEROIDS** | | | | | |
| LB test | + | + | + | + | - |
| Salkowaski test | - | - | - | - | + |
| Sulphur test | + | + | + | + | + |
| **TANNINS** | | | | | |
| $FeCl_3$ test | + | + | + | + | + |
| Gelatin test | - | - | - | - | - |
| **GLYCOSIDES** | | | | | |
| Keller-killiani test | - | - | - | - | - |
| Raymond test | - | - | - | - | - |
| Bromine water test | - | - | - | - | - |
| Legal's test | - | - | - | - | + |
| **RESINS** | + | + | + | + | + |

**Table 2: Quantitative estimation of Primary metabolites**

| Primary metabolites | OD (nm) | Result (mg/100g sample) |
|---|---|---|
| Total carbohydrates | 630 | 162 |
| Fructose | 520 | 1.4 |
| Starch | 630 | 1.08 |
| Protein | 660 | 144 |

**Table 3: Elemental analysis by AAS**

| ELEMENTS | CONCENTRATION |
|---|---|
| Calcium | 12.4497 |
| Potassium | 6.8466 |
| Magnesium | 5.9854 |
| Silicon | 3.0638 |
| Titanium | 2.402 |
| Aluminium | 2.0537 |
| Iron | 0.8976 |
| Copper | 0.5599 |
| Vanadium | 0.5113 |
| Molybdenum | 0.3998 |
| Manganese | 0.2544 |
| Zinc | 0.1592 |
| Chromium | 0.023 |
| Cadmium | 0.0073 |

The phytochemical analysis of leaf extract of *S.gradiflora* was analyzed for the compounds such as alkaloids, phenols, cardiac glycosides, flavonoids, saponins, steroids and tannins.

The preliminary phytochemical analysis revealed the presence of seven compounds i.e. alkaloids, phenols, cardiac glycosides, flavanoids, saponins, steroids and tannins (**Table 1**). Quantitative estimation of secondary metabolites was carried out using standard methods, sterol content was found maximum(37%), followed by Flavonoids(18%), Cardiac glycosides(16%), Phenols (13%), Terpenoids (11%), Tannins(4%) and the least was Alkaloid (1%). (**Fig I**)

The nutritive value of leaf extract was estimated by quantitative estimation of Primary metabolites i.e., Carbohydrates and Proteins. The Total carbohydrate content was found to be 162mg/100g of sample, Fructose 1.4mg/100g, Starch 1.08mg/100g, Protein 144mg/100g.(**Table 2**)

The Elemental analysis was also carried out by AAS method where the Calcium was found to be highest (12.4497ppm) and Cadmium was found to be present in least amount (0.0073ppm). (**Table 3**)

In this research, chemical studies such as preliminary phytochemical investigation, elemental analysis, and examination of nutritional values of *Sesbania grandiflora* L. leaves had been studied.

The preliminary phytochemical investigation revealed the high presence of sterols and least amount of alkaloids. The solubility in methanol and ethanol were found to be the greatest in the solubility tests [16]. The present study revealed more positive results in aqueous extracts.

Various tests have been performed to find out the phytochemical constituents [17]. The results have shown that each and every phytochemical has the ability to get extracted with different solvents. Methanol can be used as a active extracting solvent as the evaporation of methanol is soon thus suggest that methanol can be used as an active extracting solvent[18]. But according to the present study methanolic extract didn't give more positive results. This might differ according to the polarity of the solvent. The presence of various secondary metabolites such as glycosides, phytosterols, alkaloids, saponins, phenols and flavanoids were believed to exhibit the antibiotic properties of *S.grandiflora* (L) leaves. With aqueous extract has shown the presence of alkaloids, phenols and steroids.

According to the result of elemental analysis, it was found that the macronutrient elements in calcium, phosphorus, potassium, sulphur, chlorine and iron. The micronutrient elements in strontium, bromine, manganese and zinc were

found in *S. grandiflora* leaves [16]. According to the present study calcium, potassium, magnesium, silicon, titanium, aluminium, iron, copper were the macronutrient elements whereas vanadium, molybdenum, zinc, chromium and cadmium were the micronutrients. Calcium was found in high amounts and Cadmium was found to be negligible.

## CONCLUSION

The phytochemical study revealed the presence of almost all the secondary metabolites which indicates that the plant can be used to treat various diseases like Rheumatism, epilepsy, stomach disorders, skin disorders, night blindness, ophthalmia, nasal catarrh and headache. It has antibiotic, anti-helminthic, anti tumor and contraceptive properties. The results of Quantitative estimation of Phytochemicals showed high concentration of Sterols.

The Nutraceutical study revealed that the Total carbohydrate content is maximum, and also the presence of fructose, starch and proteins. The elemental analysis showed that Calcium which is found in high amount is important for bone development, teeth and shells whereas Cadmium was found to be in low quantity, which is a toxic metal and causes poisoning. Hence the plant is non-toxic and considering the above results the leaves can be used as vegetable for daily purposes. It is denoted elsewhere that the leaves and the pod are being consumed as food and also acts as a supplement meal and as a vegetable.

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## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

## DATA AVAILABILITY

Not declared.

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