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Determination of Total Phenolic, Flavonoid and Saponin Contents of *Sesbania Cannabina* Leaves.

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ABSTRACT

Plant phenolics, saponins and flavonoids have a powerful biological activity, which outlines the necessity of their determination. The present work was aimed to determine the total phenolic, flavonoid and saponin contents in ethyl acetate and methanol extracts of *Sesbania cannabina* leaves. The total phenolic content was determined by folin-ciocatechu reagent using gallic acid as standard and total flavonoid content by aluminium chloride assay using rutin as a standard. The total saponin content was determined by vanillin reagent using diosgenin as a standard. Methanol extract of leaves showed highest amount of total phenolic content (231 ± 0.86 mg/g equivalent of Gallic acid), total flavonoid content (87.10 ± 0.38 mg/g equivalent of rutin) and total saponin content (50.66 ± 0.57 mg/g equivalent of diosgenin). The results obtained from the present study provides evidence that extracts of *Sesbania cannabina* leaves contains secondary metabolites and this justifies the use of plant species as traditional medicine for treatment of various diseases.

Keywords: *Sesbania cannabina*, leaves, phenolic, flavonoid, saponin, extract.

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INTRODUCTION

Medicinal plants are used to maintain and promote healthy life, prevent disease and cure ailments.¹ The World Health Organization supports the use of traditional medicine provided they are proven to be efficacious and safe.² The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body.³ Phytochemicals are primary and secondary metabolites, which are naturally occurring in the leaves, vegetables, and roots that have defense mechanism and protect from various diseases. Primary metabolites are proteins, carbohydrates, chlorophyll, lipids and common sugars, which are synthesized during photosynthesis, and these organic compounds are essential for plant life, growth and development.⁴ Secondary metabolites are tannins, flavonoids, phenolics, saponins and alkaloids, which are synthesized by the plant during development and are time, tissue and organ specific.⁵ Phenolic compounds are secondary metabolites in plants that are involved in a number of metabolic pathways and are essential for plant growth and reproduction, and as protecting agents against pathogens. Phenolic compounds may play an important role in preventing chronic illnesses such as cardiovascular disease, certain type of cancers, neurodegenerative disease, and diabetes.⁶ Flavonoids are essential in human diet and are present in plant extracts that have been used for medicinal purpose.⁷ The Flavonoids and phenolic compounds in plants have reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic.⁸ Saponins are a diverse group of compounds widely distributed in the plant kingdoms.⁹ The research has established saponins as the active components in many herbal medicines.¹⁰ Saponins have been reported to possess a wide range of biological activities.¹¹ In developing countries, a huge number of people lives in extreme poverty and some are suffering and dying for want of safe water and medicine, they have no alternative for primary health care.¹² There is therefore the need to look inwards to search for herbal medicinal plants with the aim of validating the ethno-medicinal use and subsequently the isolation and characterization of compounds which will be added to the potential list of drugs.¹³



Figure 1: *Sesbania cannabina* Poir. Plant

Sesbania cannabina is a multipurpose leguminous crop and is widely adaptable to various adverse climatic conditions. It belongs to family Fabaceae and is commonly used as a green manure crop. *Sesbania cannabina* is an annual shrub and can fix atmospheric nitrogen due to presence of root nodules.¹⁴ *Sesbania cannabina* is commonly called canicha, danchi, dunchi fibre, prickly sesban, prickysisham, sesbania pea (English), sesbane (French), canicha, danchi, dhaincha (Hindi), sanô (Tibetan), mrindazia, msalia-Nyuma (Swahili), sano-khangkhok (Thai).¹⁵ The leaves of *Sesbania cannabina* have aperient, diuretic, emetic, emmenagogue, febrifuge, laxative, and tonic properties and can be used to cure dysentery, eyes, fevers, headaches, small pox, sores, sore throat, and stomatitis.¹⁶ The astringent bark was used in treating small pox and other eruptive fevers. The juice of *Sesbania cannabina* flowers is also effective for treatment of headache, head congestion, or stuffy nose. Leaves are chewed to disinfect the mouth and throat.¹⁷ Leaves are also considered good for eyes and it is a common believe that regular use of it as a vegetable improves eyesight and prevent eye diseases. The natives also use it for anaemic patients. According to the healers, as medicine, this vegetable is good for patients suffering from dysmenorrhoea.¹⁴ Determining the total phenolic, saponin and flavonoid in plants is attracting attention for human and animal nutritionists worldwide due to their beneficial effects on human and animal health. The aim of the present study was to quantitatively estimate the total phenolic, flavonoid and saponin contents in ethyl acetate and methanol extracts of *Sesbania cannabina* leaves.

MATERIALS AND METHOD

Collection of plant material

The leaves of plant were collected from Bhopal, Madhya Pradesh, India. The plant was identified and authenticated by Dr. Zia Ul Hasan, Professor & Head -Department of Botany, Safia college of Science, Bhopal.

Preparation of plant extracts

The leaves of *Sesbania cannabina* were washed, dried in shade, coarsely powdered in a grinder, weighed and then kept in a closed jar. The successive extraction of the samples from non polar to polar solvents was done by using three different types of solvents such as petroleum ether, ethyl acetate, methanol using standard technique of maceration. The leaves were kept in Petroleum ether for 2 days, and 7 days each in ethyl acetate and methanol, with occasional shaking/ stirring. The extracts thus obtained were evaporated to dryness at room temperature and stored in a sterile air tight container. The concentrated mass obtained, i.e. the crude extract for the three solvents was weighed and kept in a refrigerator for further experimental procedure.

Determination of Total Phenolic Content^{18, 19}

The total phenolic contents were determined by using Folin-Ciocalteu reagent. Gallic acid was used as a reference standard for plotting calibration curve. A volume of 0.5 ml of the plant extract (100 µg/ml) was mixed with 2 ml of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765 nm using UV-VIS spectrophotometer. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The total phenolic contents were expressed as mg/g gallic acid equivalent.

Determination of Total Flavonoid Content²⁰

The total flavonoid content of *Sesbania cannabina* leaves was determined using the aluminium chloride assay. An aliquot (0.5 ml) of extracts were taken in different test tubes then 2ml of distilled water was added followed by the addition of 0.15 ml of sodium nitrite (5% NaNO₂, w/v) and allowed to stand for 6 min. Later 0.15 ml of aluminium trichloride (10% AlCl₃) was added and incubated for 6 min, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4% w/v) and volume was made upto the 5ml with distilled water. After 15 min of incubation the mixture turns to pink whose absorbance was measured at 510 nm using a spectrophotometer. Distilled water was used as blank. The total flavonoid content was expressed in mg of rutin equivalents per gram of extract.

Determination of Total Saponin Content²¹

Standard saponin solution was prepared by dissolving 10 mg of diosgenin and add (16 ml) methanol and distilled water (4 ml). To the aliquots for each tube, vanillin reagent (8%, 0.25 ml) was added and sulphuric acid (72% v/v, 2.5 ml) added slowly on the inner side of the wall. The solutions were mixed well and the tubes were transferred to a 60 °C water bath. After 10 mins incubation, the tubes were cooled in ice cold water bath for 3 – 4 min. The absorbance was measured at 544 nm against the reagent blank. 0.1 g of freeze dried sample was dissolved in aqueous methanol (80%, 0.1 ml). 0.25 ml of aliquot was taken for spectrophotometric determination for total saponins at 544 nm.

RESULTS AND DISCUSSION

Table 1: Total phenolic, flavonoid and saponin contents of *Sesbania Cannabina* leaves.

S.No.	Phytochemicals	Ethyl acetate extract	Methanol extract
1.	Total phenolic content in mg/g equivalent of Gallic acid	171.16±0.28	231±0.86
2.	Total flavonoid content in mg/g equivalent of Rutin	69.88±0.19	87.10±0.38
3.	Total saponin content in mg/g equivalent of Diosgenin	25.33±0.57	50.66±0.57

The values are means of three replicates with standard deviations (mean ± S.D)

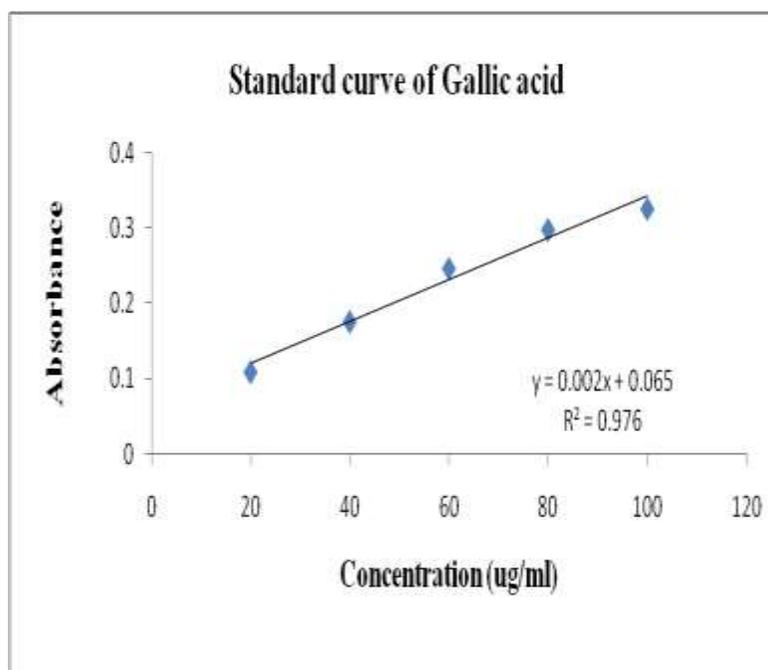
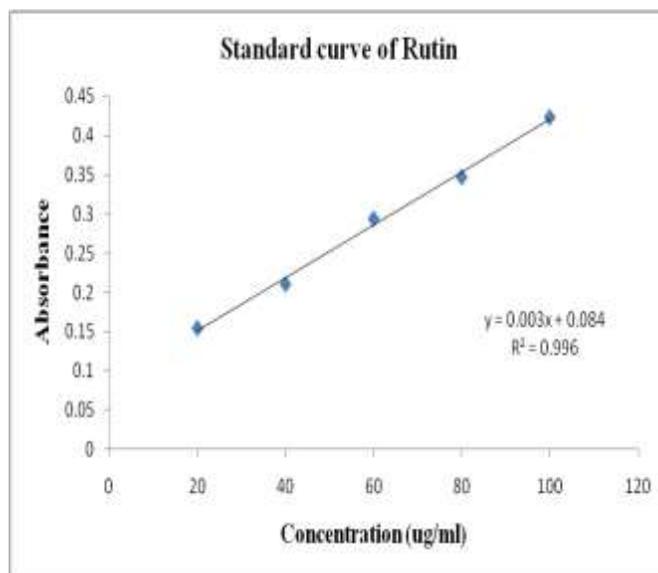


Figure 2: Standard curve of Gallic acid for determination of Total Phenolic Content.

Table 2: Absorbance of standard compound (Gallic acid) at λ_{\max} =765nm

S.No	Gallic acid Concentration (ug/ml)	Absorbance at λ_{\max} =765nm
1.	20	0.1098
2.	40	0.1763
3.	60	0.2468
4.	80	0.2981
5.	100	0.3258

**Figure 3: Standard curve of Rutin for determination of Total Flavonoid Content.****Table 3: Absorbance of standard compound (Rutin) at λ_{\max} = 510nm**

S.No	Rutin Concentration (ug/ml)	Absorbance at λ_{\max} = 510nm
1.	20	0.155
2.	40	0.211
3.	60	0.294
4.	80	0.347
5.	100	0.423

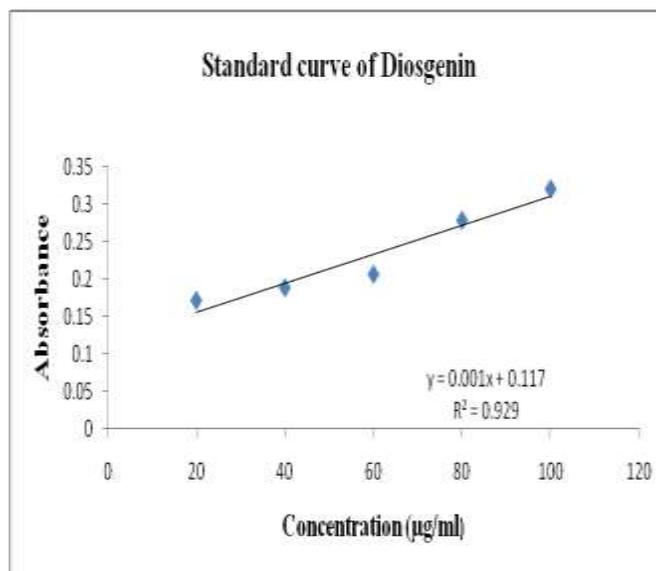


Figure 4: Standard curve of Diosgenin for determination of Total Saponin Content.

Table 4: Absorbance of standard compound (Diosgenin) at λ_{max} =544nm

S.No	Diosgenin Concentration (µg/ml)	Absorbance at λ_{max} =544nm
1.	20	0.172
2.	40	0.189
3.	60	0.207
4.	80	0.279
5.	100	0.321

Phytochemical screening of the medicinal plants is very helpful for the development of new drugs.²² Phytochemicals of plant extracts are known to exhibit medicinal as well as physiological activities.²³ For centuries, extracts from plants have been used as folk remedies against various health problems²⁴ and with many natural products are leading to the development of clinically beneficial drugs.²⁵ In the present study quantitative estimation of total phenolic, flavonoid and saponin contents in ethyl acetate and methanol extracts of *Sesbania cannabina* leaves was done.

The total phenolic content was determined using Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenolic contents were expressed as mg/g gallic acid equivalent using the standard curve equation: $y = 0.002x + 0.065$, $R^2 = 0.976$ (Figure 2); where y is absorbance at 765 nm and x is total phenolic content in the extracts of *Sesbania cannabina* leaves expressed in mg/g equivalent of Gallic acid. The total phenolic content in the ethyl acetate and methanol extract of *Sesbania cannabina* leaves was found to be 171.16 ± 0.28 and 231 ± 0.86 in mg/g equivalent of Gallic acid (Table 1). The total flavonoid content was determined using aluminum tri chloride reagent. Rutin was used as a standard compound and the total flavonoid content was expressed as mg/g rutin equivalent using the standard curve equation: $y = 0.003x +$

0.084, $R^2 = 0.996$ (Figure 3); where y is absorbance at 510 nm and x is total flavonoid content in the extracts of *Sesbania cannabina* leaves expressed in mg/g. The total flavonoid content was 69.88 ± 0.19 and 87.10 ± 0.38 in mg/g equivalent of rutin in the ethyl acetate and methanol extracts, respectively (Table 1). The total saponin content was determined using vanillin reagent. Diosgenin was used as a standard compound and the total saponin content were expressed as mg/g diosgenin equivalent using the standard curve equation: $y = 0.001x + 0.117$, $R^2 = 0.929$ (Figure 4); where y is absorbance at 544 nm and x is total saponin content in the extracts of *Sesbania cannabina* leaves expressed in mg/g. The total saponin content in the ethyl acetate and methanol extract of *Sesbania cannabina* leaves was found to be 25.33 ± 0.57 and 50.66 ± 0.57 in mg/g equivalent of diosgenin (Table 1). Methanolic extract of leaves showed highest amount of total phenolic content (231 ± 0.86 mg/g equivalent of Gallic acid), total flavonoid content (87.10 ± 0.38 mg/g equivalent of rutin) and total saponin content (50.66 ± 0.57 mg/g equivalent of diosgenin). The results obtained from the present study provides evidence that extracts of *Sesbania cannabina* leaves contains secondary metabolites and this justifies the use of plant species as traditional medicine for treatment of various diseases.

CONCLUSION

Phenolics, Saponins and flavonoids are important phytochemicals present in the plant leaves. These compounds could be responsible for the biological activity of the leaves. Therefore, further research is on going to isolate, characterize and determine some biological parameters of the plant leaves.

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