



Analysis of total phenols, total tannins and screening of phytochemicals in *Indigofera aspalathoides* (Shivanar Vembu) Vahl EX DC

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ABSTRACT

The plant *Indigofera aspalathoides* is popularly known as Sivanar vembu in Tamil belongs to the family fabaceae. The stem is traditionally used for various skin disorders and cancer. In the traditional medicinal system, the leaves, flowers and tender shoot are said to be cooling and demulcent, they are used in the form of decoction for leprosy and cancer affections. Qualitative and quantitative Phytochemicals test were screened in *Indigofera aspalathoides*. The total content of some important phytochemical constituents like phenols, tannins of *Indigofera aspalathoides* by studied using spectrophotometric methods. The results showed that the plant is rich in phenols (47.38 ± 1.532) compare than Tannins (34.59 ± 1.788).

KeyWords: *Indigofera aspalathoides*, Total phenol, Total Tannin, Screening of phytochemicals

INTRODUCTION

Natural antioxidants such as phenols, flavonoids and tannins are increasingly attracting because they are natural disease preventing, health promoting and anti-ageing substances [1]. Antioxidants may serve the task of reducing oxidative damage in humans induced by free radicals and reactive oxygen species under oxidative stress conditions. These conditions can cause DNA and protein damage, lipid peroxidation, cancer, ageing and inflammatory activity [2]. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radical induced tissue injury [3].

Phenolic compounds are secondary metabolites which synthesize in plants. They possess biological properties such as: antioxidant, anti apoptosis, anti-aging, anti carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilities [4].

Plant foods have phenolic compounds, which affect their appearance, taste, odor and oxidative stability. In cereal grains, these compounds are located mainly in the pericarp [5]. The major phenolic acids in cereals are ferulic and p-coumaric acids [6,7,8,9]. Anthocyanins are water-soluble pigments mostly studied in cereals [10]. The tannins are widely distributed in almost all plant foods [11] species [12]. According to World Health Organization more than 21 000 plants are being in use for medicinal purpose all around the world [13]. Numbers of plants were screened for primary and secondary metabolites for their medicinal values [14,15,16].

The objective of this research was to determine the total phenol content and total tannins by using Folin-Ciocalteu method and also Phytochemicals test were screened

EXPERIMENTAL SECTION

Chemicals

Analytical grade chemicals collected from Loba, Hi-Media, S.D.Fine Chemicals, E.Merck, Qualigens and Sigma Chemicals (U.S.A.) were used.

Plant collection

Fresh plants of *Indigofera aspalathoides* were collected from the fields located in *Kelambakkam*, tamil nadu, India.

Preparation of the Plant extract

The plants were carefully washed with tap water, rinsed with distilled water, and air-dried for 1 hour. Then whole plants are separated & dried in room temperature for one week. Then they were ground into powder and stored in room temperature. The material (1 gm) was extracted with 10 ml methanol in conical flask in shaking condition. The extract was decanted in to pre-weighed glass vials. The process was repeated 3 times and the same plant material but using fresh solvent. The solvent was removed by placing the extracts in front of a steam of air in a fume hood at room temperature. The extracted residues were weighed and re-dissolved in different solvents to yield 10mg/ml solutions ready for phytochemical analysis.

Phytochemical screening of *Indigofera aspalathoides*

The different qualitative chemical tests were performed for establishing the profile of given extract for its chemical composition. The following tests were performed on the extracts to detect various phytoconstituents present in them.

Detection of alkaloids

Solvent free extract (50mg) was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents.

Mayer's test

To a few mL of filtrate, a drop or two of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate indicated the test as positive.

Detection of carbohydrates and glycosides

The extract (100mg) was dissolved in 50mL of water and filtered. The filtrate was subjected to the following test.

Benedict's test

To 0.5 mL of filtrate, 0.5 mL of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic colored precipitate indicated the presence of sugar.

Detection of glycosides

50 mg of extract was hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate was subjected to the following test.

Borntrager's test

To 2 mL of filtrate hydrolysate, 3 mL of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Pink color indicated the presence of glycosides.

Detection of saponins

Foam test

The extract (50mg) was diluted with distilled water and made up to 20 mL. The suspension was shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicated the presence of saponins.

Detection of proteins and amino acids

The extract (100mg) was dissolved in 10 mL of distilled water and filtered through Whatmann No.1 filter paper and the filtrate was subjected to tests of proteins and amino acids.

Biuret test

An aliquot of 2 mL of filtrate was treated with one drop of 2% copper sulphate solution. To this, 1 mL of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of proteins.

Detection of Phenol Compound**Ferric chloride test**

The extract (50 mg) was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of

Lead acetate test

The extract (50 mg) was dissolved in 5 mL of distilled water. To this, 3ml of 10% lead acetate were added. A bulky white precipitate indicated the presence of phenol compounds.

Determination of total phenol content

The amount of total phenol content, in various solvent extracts of flower was determined by Folin-Ciocalteu's reagent method¹³. 0.5ml of extract and 0.1 ml (0.5N) Folin-Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm. Gallic acid was used as a positive control. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of extracted compounds). The assay was carried out in triplicate and the mean values with \pm SEM is presented.

Determination of tannins

The tannins were determined by Folin and Ciocalteu method. 0.1 ml of the sample extract was added with 7.5 ml of distilled water and adds 0.5 ml of Folin Phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 725 nm. Blank was prepared with water instead of the sample. A set of standard solutions of gallic acid is treated in the same manner as described earlier and read against a blank. The results of tannins are expressed in terms of gallic acid mg/g of extract

RESULT AND DISCUSSION**Screening of Phytocomponents in *Indigofera aspalathoides***

Table1 contains the preliminary phytochemical screening of *I. aspalathoides* has revealed the presence of phenolics, carbohydrates, amino acid, and glycosides in high amounts.

Parameter	Test/Reagents	Result
Alkaloids	Mayer's	-
Carbohydrates/glycosides	Benidict's	+++
Glycoside	Borntrager's	+++
Saponins	Foam's	-
Protein/ Aminoacids	Biuret	+++
Phenolic compound	Ferric Chloride	+++

Total Phenol and Tannins

The results of total phenol content, tannins and are given table1. Total phenolic content was found to be more in methanol extract followed by tannin(Table2). Phenolic and polyphenols like flavonoid are secondary plant metabolites that are ubiquitously present in plants. The values presented as the mean \pm SD of three measurements.

Table -2 Bioactive compounds in Methanol extract of *Indigofera aspalathoides*

Bioactive compounds	<i>Indigofera aspalathoides</i> (Methanol extract)
Total phenol	47.38 \pm 1.532
Tannin	34.59 \pm 1.788

It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color,taste, aroma, and flavor and also in providing health beneficial effects. They also serve in plant defense

mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by micro organisms, insects and herbivores [18].

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