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Quantitative Biochemical Profile of Leaves and Seeds of *Cajanus Cajan* L. (Fabaceae)

***Corresponding Author(s): Anjali Rawani**

Assistant Professor, Department of Zoology, Laboratory of Parasitology, Vector biology and Nanotechnology, The University of Gour Banga, Mokdumpur, Malda, 732103, West Bengal, India.

Email: micanjali@gmail.com

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Abstract

The objectives of this study were quantitative and qualitative analysis of leaves and seeds of *Cajanus cajan* L. for secondary metabolite and also to find out the amount of some primary biochemicals in the leaves of *C. cajan*. The methanol extract of leaves and seeds were prepared for the quantitative and qualitative analysis of secondary metabolite, which reveals the presence of alkaloids, steroid, flavonoids, saponins, phenolic compound, tannin and in the meantime their total amount, will also be calculated. The quantitative estimation of leaves of *C. cajan* for primary biomolecules showed the highest concentration of protein (19.93 mg/g) followed by total starch (9.18 mg/g), carbohydrate (5.82 mg/g), crude fibre (9.18 mg/g) and total lipid (1.56 mg/g). The quantitative estimation also revealed the ash (8.74 mg/g) and moisture (3.96) content in the leaves of *C. cajan*. So, it can be concluded that the leaves of *C. cajan* were represents a good source of protein, flavonoid and phenolic compound which may be used for medicinal purpose and in the food industry.

Keywords: *Cajanus cajan*; Phytochemical analysis; Quantitative analysis; Primary biomolecules.

Introduction

From the ancient times plants have been used by human beings for medicinal purpose and it is the basic of traditional medicine. The component of plant that possess the health regulatory and disease preventive quality is the source of the herbal drug and it termed as phytochemicals. These herbal drugs are not only used as a medicinal entity but it can also be consumed as the supplementary diet. Now a day's plant based products are used in various pharmaceutical companies in cosmetic products, as nutraceuticals, as functional food as well source of important trace element. About 80 % population of whole world believe in these traditional medicine for their health care [1]. Around 74% of the most important drugs contain active ingredients from plants used in traditional medicine [2]. The vast array complex secondary metabolites produced by plants and stored in their various part for their defence purpose, save themselves from pest attack and to combat with environmental changes. So bring about the maximum use of traditional medicine there is a need of exploration of medicinal plants in context of modern medicine. Before application, the quality control as well as to know the various ecological factor such as nutritional status, geographic variation and climatic condition is necessary to land them from laboratory to market.

Fabaceae (or Leguminosae) to which the *Cajanus cajan* and other leguminous plants belongs, is a cosmopolitan family and are notable for their ability to fix atmospheric nitrogen. This happens due to a mutualistic symbiotic relationship with bacteria (rhizobia) found in the root nodules of these plants. The family is broadly distributed to whole world with about 765 genera and nearly 20,000 known species, and is the third-largest land

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plant family in number of species [3,4]. Generally, legumes such as alfalfa, clover, peas, beans, lentils, lupins, menquite, carob, soy and peanuts are belonging to this family. *Cajanus cajan* (L.) Millsp is a protein-rich legume species of this family and it is commonly known as Arhar in hindi and Pigeon pea in English. This legume plant is distributed mainly in tropical areas such as Asian countries and India. Due to its high protein, content and presence of flavonoid and phenolic compounds as secondary metabolite make this plant in limelight for the investigation of medicinal as well as pharmacological properties.

Cajanus cajan is a perennial shrub which is erect branched and 1-2-meter long. Leaves are ovate to lanceolate with three leaflets. Flowers are yellow, in sparse peduncled racemes, about 1.5-cm long [5]. Flowering season is in between June and November. Pod or legume is oblong or linear oblong, hairy, 4-7 cm long, 1 cm wide, containing two to seven seeds. Seeds are sub spherical in shape and 3–6 mm in diameter [6,7]. Approximately 90% of the total world production of Arhar plant grown by India. Currently, it occupies an area of 3.85 million hectares with an annual production of 2.68 million tonnes [8].

In the past, it is mainly used for the food and in traditional medicines. The seeds are very high in protein and for the poor one this the solely a source of protein. The seeds are mainly used as a dal in all over the India. Amongst its many medicinal uses, C. cajan is indicated in the relief of pain in traditional Chinese medicine and as a sedative [9]. Ancient people used the extract of the leaf for the treatment of skin problems as well as in gingivitis. In recently *C. Cajan* plant is also discovered for the treatment of ischemic necrosis of the caput femoris, aphtha, bedsore and wound healing [5]. Chemical investigations have revealed the presence of two globulins, cajanin and concajanin [10]. From the ancient times, the plant has been used broadly for the treatment of diabetes, sores, skin irritations, hepatitis, measles, jaundice, dysentery and many other illnesses [5]. It is also reported for expelling bladder stones and stabilizing menstrual period [11].

Some literature reported the pharmacological and medicinal properties but there is little information about chemical composition of leaves of *Cajanus cajan* L. Present study was carried out to examine the biochemicals present in the matured leaves and seeds of *C. cajan* and to obtain a preliminary data on some primary and secondary biochemicals of this plant for optimum utilization of bioactive principle.

Materials and methods

Study area

The present study was conducted at Malda (district), West Bengal, India during January to October 2017.

Collection of leaves

Random collections of mature leaves growing in different fields situated at the outskirt of Malda district, West Bengal, were done thrice a month for twelve months during the study period. Leaves were initially cleaned in the laboratory with distilled water and soaked with paper towels before each biochemical assay.

Preparation of leaf extracts

One gram of the mature leaves of *C. cajan* were cut into pieces (0.5-1 cm) and plunged in boiling methanol and ground for 10 min. Leaf extract was centrifuged at 2000 rpm for 20 min, and the supernatant and pellet was separated. To remove leaf pigment, supernatant was passed through charcoal powder and filtered through Whatman No-41 (Maidstone, IIK) filter paper. The supernatant was treated as sample for estimation of total soluble carbohydrate and pellet was treated as sample for estimation of total protein and starch.

Quantitative estimation of primary biochemicals

Estimation of total soluble carbohydrate

Sum of soluble carbohydrate was measured by modified Anthrone method provided by Dubios et al., [12] with some modifications. To estimate the total soluble carbohydrates 1 ml of the supernatant clear plant extract was collected and treated with 4 ml of Anthrone reagent. The carbohydrate content was measured by the preparation of standard curve against a reagent blank of D- glucose in the UV-visible spectrophotometer read at 630 nm.

Estimation of crude protein

Total protein was measured following the method of Lowry et al., [13]. To estimate the sum of soluble proteins, pellet separated earlier was suspended in 5 ml of 5% TCA solution at 0-5 ºC in an ice bath for 10 min. Aliquotes (1 ml each) were taken in a centrifuge tube, 1 ml of 10% TCA was added and centrifuged at 5000 rpm for 45 min. The supernatant was discarded and the pellet was re-extracted once with absolute ethanol and twice with hot ethanol-ether mixture, every time discarding the supernatant. The pellet was treated with alkaline copper reagent [alkaline sodium carbonate : Copper sulphate - sodium potassium tartarate solution (50:1 in v/v)] and the level of total protein was measured against a reagent blank of bovine serum albumin by the addition of Folin - Ciocaltu reagent in the UV-Visible spectrophotometer read at 750 nm.

Estimation of crude lipid

The lipid content of the leaves of *C. cajan* was estimated by the procedure of Folch et al., [14]. To determine lipids, 1 g of fresh mature leaves were separately homogenized in 20 ml chloroform: methanol ($CHCl_3$: MeOH) (1:2v/v) mixture for 10 min in a cell homogenizer. The extract was filtered after vigorous shaking and residue was mixed with 25 ml $CHCl_3$: MeOH mixture and stirred for 30 min. To remove any chlorophyll, the mixture was filtered through activated charcoal. The filtrate was shaken with a 0.9% aqueous sodium chloride solution, and non–lipid contaminants were eliminated. The lipids thus extracted were dried in a desiccator and weighed in an electro balance.

Estimation of starch

The amount of starch was measured by modified Anthrone method with necessary modification. To estimate the starch components, 0.5 g of the sample was homogenized in hot 80% ethanol to remove the soluble sugars. The residue was homogenized and centrifuged after washing with hot 80% ethanol (repeated five times) till the washing did not give color with Anthrone reagent. The residue was dried in a water bath and 5.0 ml of distilled water and 6.5 ml of 52% perchloric acid was added to it. The extraction procedure was continued using fresh perchloric acid until 100 ml of the supernatant was collected. To 1 ml of the supernatant (0.2 ml supernatant + 0.8 g ml distilled water solution) 4 ml of the Anthrone reagent was added and the amount of starch was measured in the UV-visible spectrophotometer read at 630 nm.

Estimation of crude fibre

This was determined as loss of ignition of dried lipid-free residues after digestion with 200 mL of 1.25 N H_2SO_4 and 200 mL of 1.25 N NaOH and rinsed at 600 °C for 4 h. The percentage crude was calculated as:

Crude fibre = Mass of crude fibre / Mass of sample

Estimation of Ash content and moisture content

1 g of mature leaves were placed separately in a hot-air oven at 50 ± 0.5 ºC for 72 h, and, materials that showed constant dry weights was removed from the oven and weighed. The difference between fresh and dry weights gave moisture contents. A portion (140-150 mg/replicate) of each kind of oven-dried leaves was separately ashed in a muffle furnace at 450 ± 10 ºC for 20 min. The ash thus produced in crucible was soaked with 2ml distilled water and placed in a watch glass for drying at 110 ºC for 2 h. The respective ash sample was weighed.

Estimation of secondary metabolite

Qualitative estimation of secondary metabolite

Chemical tests were carried out with the methanolic extract for the qualitative determination of phytochemical constituents as described by Harborne [15], Trease and Evans [16] and Sofowora [17].

Alkaloids

0.5 g of extract was diluted with 10 ml of acid alcohol, then boiled and filtered. To 5 ml of the filtrate, 2 ml of dilute ammonia was added. 5 ml chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent reagent) was regarded as positive for the presence of alkaloids.

Anthraquinones

The Borntrager reaction was used to observe the alkaline red colour phase indicating positive reaction otherwise its negative.

Phenolic compounds

The presence of phenolic compounds was tested by adding 5 % $FeCl_3$ drops in the sample extract solution. The formation of a dark precipitate indicated positive reaction.

Saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. An appearance of creamy mass of small bubbles indicated the presence of saponins.

Tannins

About 0.5 g of the dried powdered samples (dried mature leaves of *C. cajan*) was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue–black coloration, indicating the presence of Tannins.

Flavonoids

About 0.5 g of plant extract was dissolved in diluted NaOH and then HCl was added to the solution. A yellow solution that turns colorless indicated the presence of Flavonoids.

Steroids

2 ml acetic anhydride was added to 0.5 g of ethanol extract of each sample with 2 ml H_2SO_4. The Colour changed from violet to blue or green in some samples indicating the presence of steroids.

Terpenoids (salkowski method)

About 0.5 g of the extract was added 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.

Cardiac glycosides (keller-killiani test)

About 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardioids. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Quantitative estimation of secondary metabolites estimation of phenolics

Estimation of phenolics

To determine the level of phenolics the process of Bray and Thrope was adopted [18]. The level of phenols (hydrolysable and condensed Tannin) in the leaves was determined from each of the leftovers in the carbohydrate estimations. The extract was taken in a test tube, and to that 1 ml Folin Ciocalteu reagent and 2 ml of 20% sodium–carbonate solution were added. The solution was diluted up to 25 ml with water and phenol levels were estimated following standard method against a known level of pyrocatechol.

Estimation of tannins

The follins-Dennis spectrophotometric method [19] was used to determine the amount of tannin in both types of leaves. 1g each of the test samples was dispensed in 50 ml of distilled water. The mixture was filtered and filtrate was used for the experiment. 5 ml of the filtrate was diluted with 35 ml of distilled water. 1 ml of follins–Dennis reagent was added to it followed by 2.5 ml of saturated sodium carbonate solution. The absorbance of the developed Colour was measured at 760 nm wavelength with the standard tannic acid reagent.

Estimation of flavonoids

This was determined by gravimetry using the method of Harborne [15]. 2 g of the sample was dispensed into a conical flask and to 100 ml of distilled water, 2 ml HCL acid was added. After boiling and reduction in volume to half of its original, the solution was treated with ethyl acetate. The precipitate was recovered and weighed using a preweighed filter paper (Whatman No. 42).

Estimation of saponins

Saponin content of the sample was determined by double solvent extraction gravimetric method [15]. 2 g of each sample was boiled in 50 ml of 20% ethanol solution for 90 min with periodic agitation in a water bath. Diethyl ether was added after cooling and it was shaken to mix well. The aqueous layer was collected and treated with equal volume of n-butanol. The precipitate was recovered and weighed with a pre–weighed filter paper.

Estimation of alkaloids

The quantitative determination of alkaloids was carried out by the alkaline precipitation through gravimetric method described by Harborne [15]. 2 g of the sample was dispensed in ethanolic acetic acid. The mixture was allowed to stand and alkaloids, concentrated ammonium hydroxide (NH_4OH) solution was added. The resulting alkaloid precipitate was recovered and weighed by filtration using a weighed filter paper.

Estimation of steroids

2 ml of ethanol extract of dried mature leaves of *C. cajan* was dispensed into a test tube and 1 ml of acetic anhydride was added to the mixture. 1 ml of concentrated H_2SO_4 was added carefully by the side of the test tube. A brown coloration at the interface of the two layers was indicative of the presence of sterols. The brown coloured area was recovered by separating funnel and weighed by filtration using a weighed filter paper.

Result & discussion

Quantitative estimation pf primary biomolecules

Table 1 showed the quantitative value of some primary and secondary biochemicals in the leaves of *C. cajan*. Carbohydrate is the most important component for metabolism as it supplies the energy needed for respiration and other metabolic processes. The calculated value for carbohydrate is 5.82 mg/g. While the soluble starch quantitative value obtained was 9.18 mg/g which is slightly higher than carbohydrate. Starch is the most predominant reserve carbohydrate available for energy, growth and maintenance. So peak leaf starch concentration was observed in flowering season which is happened due to the completion of leaf expansion and maturation. Low starch reserve normally observed with lower flowering intensity, fruit set and yield i.e. during non- flowering season. Generally, starch represents the greatest fraction of Non-Structural Carbohydrate (NSC) than the soluble carbohydrate [20,21]. Crude protein value calculated was 19.93 mg/g, which is higher than any other primary biomolecules, and it can be treated as an indicator of the physiological state of the plant. Protein actually one of the most important building component of living organisms and it plays a major role in controlling tree growth rate and reproduction similar to other plant systems [22,23]. The value of the total lipid for the leaves was 1.56 mg/g which is low compared to other primary biomolecules. Lipids are mainly associated with storage of energy, maintenance of membrane structure and synthesis of plant hormone. Temperature has a typical influence on the lipid value as with the increase in temperature, the lipid level decreases. So the study is during the growing season of plant i.e. in summer and the calculated value was a smaller amount than the other primary biomolecules [24]. The crude fibre content value is 7.56 mg/g which represents moderate value. This crude fibre content represents the dietary fibres which increases the nutritional value of plant. Leaves can be consumed as diet by both human beings and animal.

Total ash content value obtained is 8.74 gm/g which is generally depiction of the mineral contents reserved in the food materials. Here the result reveals the moderate deposit of mineral elements in the leaves. However, the importance of ash content necessary for the estimation of purity of drug that means to examine the existence of any foreign organic matter such as metallic salts or silica [25]. Table 1 also showed low moisture content of the leaves which revealed that the leaves may have a good shelf-life with reduced chance of microbial growth due to its relatively low moisture content [26].

Qualitative and quantitative analysis of secondary metabolite

Table 2 revealed the presence of some secondary metabolites such as saponins, flavonoids, tannins, alkaloids and steroids and phenolic compound in both leaves and seeds. The leaves and seeds did not show the presence of terpens, Anthraquinones, and cardiac glycosides. The presence of this secondary metabolite responsible for medicinal properties as well as physiological activity [17]. *C. cajan* leaves were found to be a better source of total phenolic content and total flavonoids content than seeds (Table 3). Table 3 showed that the leaves contain 0.73 mg/g and 1.17 mg/g of flavonoid and phenolic compound respectively, which is higher than the 0.22 mg/g, and 0.44 mg/g of flavonoid and phenolic compound in seeds. Flavonoid has been reported to have inherent ability to modify the body's reaction to allergen and that's why they show anti-allergic, anti-inflammatory as well as anthelmintic activity [27]. Saponin is one of the active constituents from plants which have long been employed for their detergent properties. Besides that, from medicine point of view they are used in hypercholesterolaemia, hyperglycaemia condition and also helpful in weight loss etc., [28]. Saponin is also been reported for anti-fungal properties Tannins are a widely distributed polyphenolic allelochemicals that play important role in plant–plant and plant–litter–soil interactions. It also plays a major role in decomposition and nutrient cycling [29]. The presence of alkaloids and steroids might be associated with medicinal uses of *C. cajan* and thus the plant is reported as a potential source of some useful drugs. Steroids are cholesterol like molecules found in vegetable oils, both leaves and seeds showed very little amount of steroid. Steroids generally helps in immune modulation [30]. Major constituent of secondary metabolite in both leaves and seeds is phenolic compounds have been found to have antioxidant properties. This phenolic compound reported for decreased the activities of liver marker enzymes and thus increased antioxidant enzyme activities and showed a potential efficacy in therapeutic use in alcohol- induced liver dysfunction.

Conclusion

C. cajan is the most widely used and cultivated crop in the whole world. It is a rich source of protein and there are many reported medicinal usefulness of this plant. The plant has been used from the ancient time as a traditional medicine in many parts of the world for its innumerable medicinal properties. So, in the present study, there is report on the qualitative and quantitative estimation of secondary metabolite as well quantitative estimation of primary biomolecules. Secondary metabolite like flavonoids, phenolic compound, tannins and protein fractions have been isolated from its different parts. But still it does not belong to medicinal plant category. So this study brings about the scope towards the isolation of bioactive constituents and pure active compounds from the different parts of plant so that it can be used by phytochemists and pharmacologists in near

future for various therapeutic uses.

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