

Comparative Phytochemical Composition of *Cajanus cajan* Leaf and Seed

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Abstract: The quantitative phytochemical analysis of *Cajanus cajan* leaves and seeds were carried out in dry samples. Quantitative phytochemical analyses were carried spectrophotometrically and revealed the presence of the bioactive compounds such as flavonoids (423.75 ± 57.81 and 31.08 ± 8.20 mg/100g), tannins (31.55 ± 2.67 and 17.30 ± 0.47 mg/100g), alkaloids (3118.86 ± 79.35 and 385.54 ± 75.15 mg/100g), saponins (51.21 ± 4.66 and 1.82 ± 0.29 mg/100g), cyanogenic glycosides (43.91 ± 5.99 and 12.42 ± 1.84 mg/100g), glycosides (3.55 ± 1.98 and 3.80 ± 1.01 mg/100g) and anthocyanins (8.35 ± 0.172 and 4.75 ± 0.174 mg/100g) in the leaf and seed samples respectively. The results also indicated that the leaves contain more of the bioactive compounds than the seeds. This explains the use of this plant parts in ethnomedicine for the management of various diseases.

Key words: Phytochemical • *Cajanus cajan* • Quantitative • Leaf And Seed

INTRODUCTION

Throughout history plants have been used by human beings for medicinal purposes and even in modern times have formed the basis of many pharmaceuticals in use [1]. Plants produce a vast array of secondary metabolites as defense against environmental stress or other factors like pest attacks, wounds and injuries [1]. The complex secondary metabolites produced by plants have found various therapeutic uses in medicine from time immemorial [1]. Many of the modern medicine in the early history contains descriptions of phytochemicals derived from plants that have beneficial effects on long-term health when consumed by humans and can be used to effectively treat human disease [2].

At least 12,000 such compounds have been isolated so far, a number estimated to be less than 10% of the total chemical compounds used in conventional drugs [2]. Thus herbal medicines do not differ greatly from conventional drugs in terms of how they work [2]. Medicinal plants are becoming more main stream as improvement in analysis and quality control along with advances in clinical researches have shown the value of folk medicine in the treatment and prevention of diseases [3]. The use of herbs to treat disease is almost universal among non-industrialized societies and is often more affordable than purchasing expensive modern

pharmaceuticals, this enables herbal medicine to be as effective as conventional medicines but also gives them the same potential to cause harmful side effects [4].

Cajanus cajan is a tropical woody herb with yellow flower and flat pods much cultivated in tropics, it is commonly known as Pigeon pea in English, “Fiofio” in Igbo and “Otili” in Yoruba and it is a highly nutritious seed of the tropical Pigeon pea plant [5]. Its leaves are used for rearing silkworms; green leaves and the top are used as fodder and also as green manure [6]. *Cajanus cajan* has been cultivated in ancient Egypt, Africa and Asia, This plant was introduced also in America and in other several tropical countries and it is mainly produced in India [6]. It is a multipurpose plant and it is extensively eaten as a spice, it is rich in proteins [6].

Cajanus cajan has been used in the management of pains in traditional Chinese medicine and as a sedative [7]. It has been used widely for many years for treating diabetes, sores, skin, bedsores, measles, jaundice, dysentery and other illness, for expelling bladder stones and stabilizing menstrual period [8]. The leaf and seed are applied as poultice over the breast to induce lactation [9].

MATERIALS AND METHODS

The leaves and seeds of *Canjanus cajan* were collected from Agara-Oza Village in Abakaliki, Ebonyi

State, Nigeria and classified by a taxonomist Prof. J.C. Okafor of Department of Applied Biology University of Nigeria, Nsukka, Nigeria. The seeds and leaves were dried at room temperature and ground into fine powder with electrical grinding machine and stored in an air tight container in a refrigerator.

Quantitative Phytochemical Analysis of *Cajanus cajan*

Leaf and Seed: The phytochemical constituents of the samples were carried out by the methods modified by the following

Determination of Flavonoids: This was determined by the method of Harborne [10].

Principle: Flavonoids reacts with dilute ammonia (NH₃) to produce a colored complex which can be measured spectrophotometrically at 470nm.

Procedure: 1g of the each sample was macerated with 20ml of ethyl acetate for 5min, 5ml from each sample were transferred into a triplicate tubes and 5ml of dilute ammonia (NH₃) each were added and stirred for 5min and allowed to stand for some time. The lower layers were collected and the absorbance was read at 470nm against dilute ammonia.

Determination of Alkaloids: This was determined using the method of Harborne [10].

Principle: H₂SO₄ reacts with alkaloids in the presence of formaldehyde to form a colored complex which is read spectrophotometrically at 565nm.

Procedure: 1g of the sample was macerated with 20ml of methanol and 20% sulfuric acid at the ratio of 1:1 (i.e. 10ml of each) for 5min and centrifuged for 5min. Then, 0.5ml of the supernatant was transferred into triplicate tubes. In the tubes 2.5ml of 60% sulfuric acid was added and stirred. After 5min, 2.5 ml of 0.5% formaldehyde in 60% sulfuric acid was added and allowed to stand for 3hrs. The absorbance was taken at 565nm against the blank. The same procedure was repeated with the second sample.

Determination of Tannins: This was determined by the method of Harborne [10].

Principle: Tannins reduce phosphotungstomolybdic acid in alkaline solution to produce highly colored blue

solution, the intensity of which is proportional to amount of tannins. The intensity is measured by the spectrophotometer at 720nm.

Procedure: 1g of the sample was macerated with 20ml of methanol for 10mins and centrifuged for 5min. Then, 5ml of the supernatant was transferred into triplicate tubes. In the tubes, 0.3ml of 0.1molar ferric chloride in 0.1molar HCl was added and stirred. Then 0.3ml of 8/10000 molar potassium ferricyanide was added and mixed and stood for 5mins. The absorbance was taken at 720 nm against the blank. The same procedure was repeated with the second sample.

Determination of Cyanogenic Glycosides: The method modified by Trease and Evans [11] was used to extract and estimate cyanogenic glycosides.

Principle: Cyanogenic glycosides react to alkaline picrate under boiling temperature to produce a color that is read spectrophotometrically at 490 nm.

Procedure: 1g of sample was macerated in 20ml of distilled water for 5min and was allowed to stand overnight. It was centrifuged for 10minutes. 1ml of the supernatant was transferred into triplicate tube and 4ml of alkaline picrate solution was added into each tube. They were boiled in a water bath for 5min and allowed to cool at room temperature. The absorbance was taken at 490nm against the blank. The same procedure was repeated with the second sample.

Determination of Anthocyanin: This was determined using method of Trease and Evans [11].

Principle: Anthocyanin reacts with citrate buffer at pH of 3.4 to give a colored complex in which the absorbance is read spectrophotometrically at 500nm.

Procedure: 1g of the sample was macerated with 20ml citrate buffer of pH of 3.5 for 5min and centrifuged for 5min. Then, 1ml of the supernatant was taken into two set of triplicate tubes, to one set of the triplicate tubes 4ml of citrate buffer at pH 3.4 of 1:1 HCl were added and mixed. They were allowed to stand for 1hr and the absorbance was taken at 500nm against distilled water. The same procedure was repeated with the second sample.

Determination of Saponin: This was determined by the method of Harborne [10].

Principle: Saponin reacts with anisaldehyde and ethyl acetate to give a colored complex which is read spectrophotometrically at 430nm.

Procedure: 0.5g of the sample was macerated with 10ml of methanol for 10min and centrifuged for 5min; 2ml of the supernatant was transferred into triplicate tubes. The tubes were placed in water bath to evaporate the methanol and allowed to cool. Then, 2ml of ethyl acetate and 1ml of 0.5% anisaldehyde in ethyl acetate and 1ml of 5% H₂SO₄ in ethyl acetate were added and placed in a hot water bath at 60°C for 20min and allowed cool in cold water for 10min. The absorbance was taken at 430nm. The same procedure was repeated with the second sample.

RESULTS

The results of phytochemical analyses of *Cajanus cajan* Leaf and seed revealed that alkaloids (3118.86±79.35 and 385.54 ±75.15 mg/100g) and flavonoids (423.75±57.81 and 31.08± 8.20 mg/100g) were found to be the highest in leaf and seed samples respectively (Table 1). The results also showed high levels of the phytochemicals in the leaf sample than the seed sample as shown in Table 1.

DISCUSSION

High levels of photochemicals obtained in the results showed that *Cajanus cajan* Leaf and seed are good source of phytochemicals as shown in Table 1. Aja *et al.* [12], reported the presence of high phytochemicals in dry sample of *Talinum triangulare* than the wet samples of *Talinum triangulare*. Aja *et al.* [12] had revealed that *Moringa oleifera* leaf is a good source of phytochemicals. Nwali *et al.* [13] showed that *Bryophyllum pinnatum* leaf contained low levels of photochemicals. High level of alkaloids in *Cajanus cajan* leaf and seed obtained in the results showed that *Cajanus cajan* is a good source of alkaloids as shown in Table 1. Alkaloids have been implicated for inducing a stress response and apoptosis in human breast cancer cell [14]. Alkaloids which are nitrogen – containing naturally occurring compounds commonly found to have anti-microbial properties [14]. The alkaloids can be used as a central nervous system stimulant as well as powerful pain relievers [15].

The cyanogenic glycosides levels in leaf and seed of *Cajanus cajan* are shown in Table 1. Cyanogenic glycosides in plant-based food can improve glucose metabolism and can enhance the overall health of diabetic

Table 1: Phytochemical Composition of *Cajanus cajan* Leaf and Seed

Name of the Photochemical	Leaf	Seed
Flavonoids (mg/100g)	423.75±57.81	31.08± 8.20
Tannins (mg/100g)	31.55±2.67	17.30± 0.47
Alkaloids (mg/100g)	3118.86±79.35	385.54±75.15
Saponins (mg/100g)	51.21± 4.66	1.82± 0.29
Cyanogenic glycoside (mg/100g)	43.91± 5.99	12.42± 1.84
Glycosides (mg/100g)	3.55± 1.98	3.80± 1.01
Anthocyanin (mg/100g)	8.35± 0.17	4.75± 0.17

patients by improving the lipid metabolism, antioxidants status, also in improving capillary function and lowering of cholesterol level [16, 17]. The glycosides levels in the leaf and seed of *Cajanus cajans* are shown in Table 1 which revealed that the plant is not a good source of glycosides. Glycosides contribute in the modification of tumourgenesis and are also inhibiting carbohydrate mediated in tumor growth [18]. Glycosides (Glycyrrhetic acid) inhibit the enzyme 15-hydrox prostaglandin dehydrogenase which metabolizes the ether soluble prostaglandins (PGE₂) to active 15 keto –13 metabolite. This causes an increased level of prostaglandins in the digestive system. The prostaglandin inhibits gastric secretion and stimulates pancreatic and mucous secretion in the intestines and then may markedly increase the intestinal motility. Phosphate soluble prostaglandins (PGF₂ alpha) stimulate the contraction of the uterus and can cause abortion in a pregnant woman. Glycoside (Glacyrrhizin) is used in the treatment of chronic hepatitis and cirrhosis in Japan. It also inhibits the growth of several DNA and RNA viruses and inactivates herpes simplex virus particles [19].

The valuable pharmaceutical properties of *Cajanus cajan* may be attributed to the high values of flavonoids in the leaf and seed (Table 1). This shows that the plant is good for the management of cardiovascular diseases and oxidative stress, since flavonoids are biological antioxidants, flavonoids may help to provide protection against these diseases by contributing along with antioxidants, vitamins and enzymes to the total antioxidant defense system to human body [20]. Flavonoids possess substantial anti-mutagenic and anti carcinogenic activities due to their antioxidant and anti-inflammation properties [21].

The results of the phytochemical analyses obtained showed that *Cajanus cajan* contained appreciable amount of tannins as shown in Table 1. This indicates that *Cajanus cajan* leaf and seed have antimicrobial properties [22]. According to Carson and Riley [22], it is believed that tannic acid has anti-bacterial and astringent properties which have action upon mucous tissue such as

tongue and inside the mouth; the indigestion of tannic acid cause constipation and can be used in the treatment of diarrhea. Tannins are polyphenols that are obtained from various parts of different plant belonging to multiple species. Tannins can also be effective in curbing hemorrhages and as well restrict bare swellings [22].

High level saponins showed that the leaf of *Cajanus cajan* is a good source of saponins as shown in Table 1. Saponins have been implicated for the control of high cholesterol level and they bind to the bile salts. The bile salts form small micelles with cholesterol and facilitate its absorption. Saponins cause the reduction of blood cholesterol by preventing its reabsorption [15]. Schneider and Woliling [23] reported that saponins inhibits sodium ion (Na⁺) efflux by the blockage of the entrance of Na⁺ out of the cell. This leads to higher Na⁺ concentration in the cells, by activating the Na²+Ca²⁺ an anti-porter in cardiac muscle which strengthens the contraction of heart muscle. Rausch *et al.* [24] reported that saponins have antioxidant, anti-inflammatory, anti apoptosis and immunostimulant properties, which raised speculation that these compounds could positively affect neurodegenerative effects and delay neural aging.

The result revealed that anthocyanins were found to be present in the leaf and seed of *Cajanus cajan* (Table 1). Anthocyanins have the strongest anti-oxidizing power that helps to protect human against oxidative stress from free radicals [25]. Anthocyanins have a positive influence in preventing inflammation and subsequent blood– vessel damage [26]. It also helps in stimulation of insulin in pancreatic cells [27] and helps in improving the eyesight [28].

CONCLUSIONS

The present study confirmed that the *Cajanus cajan* leaf and seed contain appreciable amount of phytochemicals. Phytochemicals have been linked to have many positive health effects in humans and animals. Phytochemical constituents have pharmacological actions that are vital for healthy living conditions, especially in rural areas where access to modern health facilities is limited. Plant/ herbs still remain the main stay of health care system.

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