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RESEARCH ARTICLE

Proximate and Phytochemical Analysis of *Cajanus Cajan (*Pigeon Pea*)* Leaves

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Abstract: *Cajanus cajan* (Pigeon pea) leaves from 'ita osu' market of Ijebu Ode, Ogun state, Nigeria, were collected and qualitatively analysed for identification of phytochemical constituents. The results showed the presence of bioactive constituents of carbohydrates, alkaloids, flavonoids, tannins, saponins, and terpenes. Phlobatannins, anthraquinones and sterols were not detected. The proximate analysis of the leaves revealed a composition of 11.20% moisture, 8.22% ash, 22.4% crude protein, 2.74% crude fat, 7.25% crude fibre, 63.39% NFE, 9.8% alcohol soluble extractive value, 4.32% water soluble extractive value and 0.65% acid insoluble ash value. The significance of this plant was discussed in relation to the presence of these metabolites as well as the proximate values. The presence of some phytochemicals like saponins and flavonoids explained the medicinal action of the plant encountered in its therapeutic uses.

Keywords: *Cajanus cajan,* Phytochemical screening, Proximate analysis

Introduction

Leguminous plants belong to the family Fabaceae (or Leguminosae). The fruits of this plant are called legumes. Well-known examples of legumes include alfalfa, clover, peas, beans, lentils, lupins, menquite, carob, soy and peanuts. This ability in them is due to a mutualistic symbiotic relationship with bacteria (rhizobia) found in the root nodules of these plants.

Legume plants are notable for their ability to fix atmospheric nitrogen, thanks to a symbiotic relationship with bacteria (rhizobia) found in the root nodules of these plants. The ability to form this mutualism reduces fertilizer costs for farmers and gardeners who grow legumes, and allows it to be used in a crop rotation to replenish soil that has been depleted of nitrogen. The nitrogen fixation ability of legumes is enhanced by the availability of calcium in the soil and reduced by the presence of ample nitrogen.

Legume seed and foliage have comparatively higher protein content than non-legume material, probably due to the additional nitrogen that legumes receive through nitrogen - fixation symbiosis. The high protein content makes them desirable crops in agriculture. Legume seed coats, commonly referred to as hulls, are rich sources of polyphenolics and natural antioxidants[1] and have been extensively investigated, both from their beneficial physiological effects in humans and deleterious effects in animal nutrition.

Leguminous flower of pea flower family are characterized by noodles on their roots. These contain bacteria that are able to fix nitrogen from the atmosphere into the soil and the plants themselves. The process of crop rotation involves switching between crops that take nitrogen compounds out of the soil and leguminous plants that put them back in.

Cajanus cajan commonly called Pigeon pea in English is a leguminous plant belonging to the family Leguminosae-Fabaceae. Originating from Asia to East Africa and America around 3000 years ago. In Nigeria, pigeon pea is known as 'wake masa' by the Hausas; 'fiofio' by the Igbos; 'otili' by the Yorubas; and 'agwugwu' by the Igalas[2].

Pigeon peas are both a food crop (dried peas, flour, or green vegetable peas) and a forage/cover crop. They contain high levels of protein and the important amino acids methionine, lysine and tryptophan, (Nutrition Facts and Analysis). In combination with cereals, pigeon peas make a well-balanced human food. The dried peas may be sprouted briefly, and then cooked, for a flavor different from the green or dried peas. Sprouting also enhances the digestibility of dried pigeon peas via the reduction of indigestible sugars that would otherwise remain in the cooked dried peas.

Various work has been done on this plant as reported in the literatures but the bulk of the work were on the seed despite the fact that the leaves do almost all that the seed can be used for. This necessitated this work particularly for the fact that the leaves have also been described as useful medicinally. The plant has been locally used for the treatment of small pox, chicken pox, diuretic, haemostatic, astringent, measles and mouth wash[3].

The extracts or components of pigeon pea are commonly used all over the world for the treatment of diabetes, dysentery and hepatitis[4]. Now days, these leaves are used for the treatment of wounds, aphtha, bedsores and malaria as well as diet-induced hypercholesterolemia[5-6]. Chemical constituent's investigations have indicated that pigeon pea leaves are rich in flavonoids, stilbenes which are considered responsible for the beneficiaries of the leaves on human health[7-8].

However, although there are many reported folkloric claims on the medicinal usefulness of this plant and some research reports on the seed, there is no report on the phytochemical screening and proximate composition of the leaves of this plant especially with regards to the one obtained in Nigeria. The purpose of this work therefore was to establish and thus report the phytochemical and proximate composition of the leaves in Nigeria.

Experimental

Cajanus cajan leaves (sun dried) were purchased from ita Osu market at Ijebu Ode in Ogun State and were identified at the University herbarium of the Department of Botany, Faculty of Science of the University of Lagos, Nigeria. The sun dried leaves were further oven dried at 105 ^{o}C for 5 h using a Genlab Oven after which it was pulverized using pestle and mortar to powder and further dried at the same oven for another 5 h at 105 ^{o}C and finally stored in a dry container.

Phytochemical analysis

The pulverized sample was used for phytochemical analysis to determine the secondary metabolites present using standard methods[9].

Preparation of extract

50 g of the crude powder was extracted twice with ethanol using Soxhlet extraction method. The solid residue obtained was kept in a capped container ready for the phytochemical screening.

Proximate analysis

The proximate analysis was carried out according to the procedure of Association of Official Analytical Chemist[10]. This constitutes the class of food present in sample such as carbohydrate, protein, fat, fiber, ash content and moisture content.

Methodology of proximate analysis

Nitrogen

It was determined as total Kjeldahl nitrogen. 2.0 g of powdered plant and 20 mL of 1 M sulphuric acid were transferred into a clean 100 mL Kjeldahl flask and heated until there was complete decomposition. After about 20 h of digestion, the solution was not colourless as expected but brownish with no trace of any leave in it.

100 mL of concentrated (2 M) sodium hydroxide solution was added slowly down the neck of the flask. Being heavier, it forms a layer underneath the diluted acid digestion mixture. The flask is connected to the condenser and mixed before heating and distillation begins. The mixture in the flask was distillated into a 5 mL solution of 1 M boric acid containing methyl orange indicator.

Crude protein

This was obtained by multiplying Total Kjeldahl Nitrogen (TKN) value by a conversion factor[14] of 6.25.

$$\%\text{Crude Protein} = \%\text{N} \times 6.25$$

To calculate crude lipid, 2.0 g of powdered sample was weighed into a sample thimble, petroleum ether was poured into a clean extraction beaker and the sample was inserted into the sample chamber. The beaker and the solvent were weighed before the start of extraction; extraction was carried out at condensation rate of 8-9 drops per seconds for 2 h. after the extraction, the beaker and its content (solvent and extract) were weighed again to record the gain in mass, the percent lipid was calculated as:

$$\%\text{ Crude lipid} = \frac{\text{Mass of extract} \times 100}{\text{Mass of sample}}$$

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 ^oC for 4 h. The percentage crude was calculated as:

$$\%\text{ Crude fibre} = \frac{\text{Mass of crude fibre} \times 100}{\text{Mass of sample}}$$

Ash content

It was determined by subjecting 2.0 g of the powdered plant to ignition in a pre heated muffle furnace for 6 h, cooled in desiccator and weighed. The percentage ash was calculated using:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash} \times 100}{\text{Original weight of sample}}$$

Acid-insoluble ash value

The ash was transferred into a beaker with 15 mL 10 v/v hydrochloric acid. The crucible was rinsed with 5 mL of the same acid twice. The beaker was then boiled for 5 min and filtered hot through an ash less filter paper. The beaker was washed with water and passed through

the filter paper. The washing was repeated thrice and filtered in a manner that allowed the residue collect at the tip of the cone of the filter paper. The funnel along the paper was dried in the oven at 105 ^{0}C. The crucible was also dried to a constant weight. The crucible was gently heated to 600 ^{0}C for 2 h for complete washing of its content. The crucible and content was cooled to room temperature in a desiccator and weighed to constant weight.

Alcohol extractive value

5 g of powdered material was measuring into 250 mL stopper conical flask containing 100 mL of 90% ethanol and the stopper replaced. The flask and content was placed in a mechanical shaker for 6 h and then allowed to stand for 18 h. The mixture was filtered and 200 mL of the filtrate was measured into an evaporating dish with a known weight and evaporated to dryness. The constant weight of the residue was gotten after drying in the oven at 105 ^{0}C for about 3 min. The extractive value was then determined by extrapolation.

Water extractive value

The above procedure was used except that 0.25% chloroform in water was prepared and used in place of 90% ethanol.

Results and Discussion

Table 1 and 2 show the result of both the proximate and phytochemical analysis done on the leaves of *Cajanus cajan.*

Table 1. Results of the proximate analysis of the leaves of *Cajanus cajan*

Parameter	Moisture	Ash	Nitrogen	Crude protein	Crude fat	Crude fibre	Alcohol extractive	Water extractive	Acid insoluble ash
Values %	11.20	8.22	3.58	22.40	2.74	7.25	9.80	4.32	0.65

Table 2. Results of the phytochemical analysis of the leaves of *Cajanus cajan*

Phytochemicals	Alkaloids	Flavonoids	Terpenes	Steroids	Saponins	Tannins	Anthraquinones	Phlobatannin
Results	+	+	+	_	+	+	_	_

Key: + = Present; - = Absent

Proximate results

Table 1 show that the plant's leaves in its dried form may have a good shelf-life with reduced chance of microbial growth due to its relatively low moisture content of 11.20%. The value is comparable to 11.02%, 11.91% and 10.83% of the three *Acalypha hispida*, *Acalypha racemosa* and *Acalypha manginata* respectively[11].

The crude protein was 22.4% is high and compare favourably with *Amaranthus caudatus* (20.59%)[12,13], cassava leaves (*Manihot utilisima*), 24.88%, *Piper Guineeses* 29.78% and *Talinum triangulare* 31.00%[13].

Total ash value of 8.22% is low compared to 14.5% obtained on the same leaves from India[14]. The ash content of the leaves are however higher than some other vegetables such as *Occimum graticimum* (8.00%) and *Hibiscus esculentus* (8.00%)[13] ash content is a reflection of the mineral contents preserved in the food materials. The result therefore suggests a high deposit of mineral elements in the leaves[15]. The total ash is particularly important in the evaluation of purity of drug *i.e*. the presence or absence of foreign organic matter such as metallic salts or silica[16].

The value of the crude fat for the leaves was 2.72% which is low compared to those of *Talinum triangulare* (5.90%), *Baseila alba* (8.71%), *Amaranthus hybridus* (4.80%), *Calchorus africanum* (4.20%)[17,13] Dietary fats function is the increase of palatability of food by absorbing and retaining flavours[15]. A diet providing 1-2% of its caloric of energy as fat is said to be sufficient to human beings as excess fat consumption is implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging[15].

The crude fibre content of 7.25% is relatively high compared with *Talinum triangulare* (6.20%), *Piper guineeses* (6.40%), *Corchorus olitorius* (7.0%), bitter leaves (*Vernonia amygdalina),* 6.5%[13].

Mean proximate composition of whole pigeon pea seeds showed that the ash content was within the range of 4.5 to 5.0% reported by Khalil *et al.,*[18]. This value is higher than the 3.8% reported by Salunkhe *et al.,*[19] for whole pigeon pea seeds from Pakistan. This specie has higher ash content than the 3.8% reported by Oshodi *et al.,*[21], 3.6% reported by Hardallon *et al.,*[22] and 3.1 to 4.0% reported by Jambunathan and Singh[24]. The moisture content did not differ much from the 6.1% reported by Hardallon *et al.,*[22].

Variation in moisture content could be attributed to a number of variations in sample treatment such as storage factors before the laboratory analysis. Salunkhe *et al.,*[19] reported that mature, whole pigeon pea seed contained 10.13 (moisture), 19.2 (protein), 57.3 (carbohydrate), 1.5 (fat), 8.1 (crude fibre) and 3.8% (ash). These values were similar to those of Khalil *et al.,*[18] who reported 10.0 (moisture), 21.3 (crude protein), 1.2 (fat), 54.8% (NFE), but higher percent ash (4.5) and crude fibre (8.2). From the report of Hardallon *et al.,*[22], pigeon pea from Sudan contained 6.1 (moisture), 19.3 (crude protein), 2.0 (fat), 3.6 (ash), 6.4 (crude fibre) and 62.7% (carbohydrate). Oshodi *et al..*[21] reported that the pigeon pea from parts of western Nigeria contained 10.1 (moisture), 23.11 (crude protein), 1.5 (fat), 57.3 (carbohydrates), 8.1 (crude fibre) and 3.8% (ash). Other reports revealed that percent crude protein varied from 20.4[25], 20.6[26] to 21.2[27].

For instance, 23.11% value was reported by Oshodi *et al.,*[21]; 21.2% by Ene-Obong and Carnovale[27], 20.6% by Oyenuga[26] and 20.4% by Nnenna[25]. Some of the foreign species from India and Pakistan reportedly had higher protein contents of 21.3%[18] and 19.2%[19]. Percent crude fat, crude fibre and NFE respectively, obtained in these species did not differ much from those reported in literature. Oshodi *et al.,*[21] reported 1.5% (fat), 8.1% (fibre) and 57.3% (carbohydrates).

Consequently, these Nigerian species appeared to be richer in crude fat and fibre compared to those from India and Pakistan. Again, these variations in proximate composition would imply that the seed variety, stage of maturity, soil type and weather conditions might have affected the physiochemical parameters on this crop.

Alcohol soluble extractive value of 9.80% and the water soluble extractive value of 4.32% showed that alcohol rather than water would be a better solvent of extraction of the leaves of the plant.

Phytochemical results

Table 2 reveals the presence of alkaloids, tannins, flavonoids, terpenes, and saponnins. The leaves did not show the presence of steroids, anthraquinones, resins and phlobatannins. The presence of these secondary metabolites suggests that the plant might be of industrial and medicinal importance.

Terpenes are very important group of organic compounds that have been reported as potent drugs used in treatment of wide range of ailments. They can be simple essential oils to the more complex triterpenes and teraterpenes. The most rapidly acting anti-malarial, artemisinin and its derivatives are terpenes[28]. The presence of terpenes will encourage further research for possible new drugs leads.

Saponins from plants have long been employed for their detergent properties. It is used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hypercholesterolaemia, hyperglycaemia, antioxidant, anti-cancer, anti-inflammatory and weight loss *etc*.[29]. This compound has also been reported to have antihyper-cholesterol, anti-inflamatory, cardiac depressant properties[20] and appear to kill or inhibit cancer cells without killing the normal cells in the process[23]. Seigler[30] reported that saponnins have anti-carcinogens' properties, immune modulatory activity and cholesterol lowering activity. It is also been reported to have anti-fungal properties[31]. Some saponins glycosides are cardiotonics while others are contraceptives and precursors for other sex hormones[28].

Tannins sacs are known to be common in Caesalpinoideae and known to exhibit antiviral, antibacterial and anti-tumor activities. It was also reported that certain tannins are able to inhibit HIV replication selectively and is also used as diuretic. Plant tannins are also source of commercial tannic acids and tanning agents[28].

Flavonoid has been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities. Some flavonoids have also been reported to behave like the some coumarins in the inhibition of giant cell formation in HIV-infected cell cultures[28].

Conclusion

Plants have contributed immensely to the medical field. It has been the source of most drugs used for combating infections. The plant used in this study was found to contain the important constituents needed to combat various kinds of infections in human beings.

The leaves of *Cajanus cajan* has been shown to possess secondary metabolites, some of which has also been reported in its seeds and therefore may be a very important source of phytochemical for new drug leads. It is our recommendation that bioassay guided work be carried out on the leaves with a view to isolating a useful pharmacologically active component as a drug.

It is recommended that more work be done on the plant leaves for isolation and characterization of bioactive compounds that may be active against malaria parasites and other diseases.

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