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Pharmacognostic Evaluation and Phytochemical Analysis of Leaves of *Cajanus cajan* L.

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Abstract

Pharmacognostic evaluation of *Cajanus cajan* L. leaf powder for ash analysis, organoleptic characters, fluorescent analysis, elements and phytochemical analysis. The total ash value, acid insoluble ash value and water soluble ash value were 14.50, 8.35 and 3.39% respectively. The highest extractive values are found with alcohol and lowest in chloroform. These are sticky to powder in nature and bitter to pungent in taste. The fluorescence characters of leaf powder with various reagents were noted under UV and under normal ordinary light. Elements like Fe>Cu>Zn>Mg>Pb were found in leaf at concentration in decreasing order. Preliminary phytochemical analysis revealed the presence of phenol, tannin, lignins, glycosides and steroids are positive to all extracts, where as flavonoids, alkaloids and terpenoids were positive to ethanol and aqueous extracts. These studies provide referential information for correct identification and selection of the drug from various adulterations.

Key words: Identification of drug, drug standardization, leaf powder.

Introduction

A pharmacognostic study is the preliminary step in the standardization of crude drugs. Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential, the first step towards ensuring quality of starting material authentication. Thus in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance^{1, 2}. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. Pharmacognostic studies have been done on many important drugs and the resulting observation has been incorporated in various pharmacopeias³. There are a number of crude drugs where the plant source has not yet been scientifically identified. Hence pharmacognostic

study gives the scientific information regarding the purity and quality of the plant drugs⁴. In recent years growing demand for herbal products has been increased and plant materials traded within and across the countries activity. Previously the phytochemicals with unknown pharmacological activities have been extensively investigated as source of medicinal agents⁵.

Cajanus cajan L is a perennial member of the family Leguminosae, commonly known as pigeonpea or red gram. The extracts or components of pigeonpea are commonly used all over the world for the treatment of diabetes, dysentery and hepatitis⁶. Now days, these leaves are used for the treatment of wounds, aphtha, bedsores and malaria as well as diet-induced hypercholesterolemia etc^{7, 8}. Chemical constituent's investigations have indicated that pigeonpea leaves are rich in flavonoids, stilbenes which are considered responsible for the beneficiaries of the leaves on human health^{9, 10}. The *Cajanus cajan* L. leaves'

proper and detailed pharmacognostical studies have not been reported so far. So an attempt was made to standardize the drug on the basis of botanical and phytochemical parameters. The objective of the present study was to evaluate various pharmacognostical standards like ash and extractive values, organoleptic, fluorescence analysis, mineral contents and phytochemical analysis of *Cajanus cajan* L. leaf.

Experimental

Plant collection and extraction

The leaves of *Cajanus Cajan* L. variety Maruti (ICP-8863) were collected from the field of Gulbarga District in Karnataka. The leaves were dried under shade and made into fine powder using pestle and mortar. These fine powder are analysed for following pharmacognostic parameters.

Pharmacognostic evaluation of the plant *Organoleptic studies of crude extract*

For the determination of organoleptic characters such as color, nature, taste and yield of the extracts, 100g of dried and powdered plant material was successively extracted in the soxhlet extractor using petroleum ether, chloroform, ethanol (95%, v/v) and distilled water solvents in the increasing order of polarity for 18h. The resulting liquid extracts were evaporated to dryness under reduced pressure. The yield of the extracts were calculated using the following formula.^{11,12}

$$\text{Extractive value (\%)} = \frac{\text{Weight of the residue obtained} \times 100}{\text{Weight of the plant material taken}}$$

Determination of total ash and acid insoluble ash content

Two grams of dried and powdered plant material was taken in the pre-weighed clean sintered silica crucibles. Then, they were incarnated by gradual increasing of the temperature (400-500°C) in the muffle furnace for 6h. The crucible was cooled to room temperature in a desecrator and the weight of ash content was weighed in electronic digital balance (Anamed make). The total ash content of the plant material thus obtained was boiled for 15min, after adding 25ml of 25% (v/v) HCl and was allowed to cool. It was filtered through a Whatman filter paper No. 44 (ash less). The insoluble ash thus retained on filter paper along with paper was ignited in a pre-weighed sintered crucible (100°C). Then the crucible along with the residue was weighed and calculated

the total ash and acid insoluble ash content using the following formula¹¹.

$$\text{Total ash content (\%)} = \frac{Z-X}{Y} \times 100$$

Where, Z= Weight of the crucible; X = Weight of the crucible with ash; Y = Weight of the plant material taken (g).

Estimation of minerals

Estimation of mineral elements from leaf ash of *Cajanus cajan* L. was done by AAS (Atomic Absorption Spectrometer) method¹³. Five gram of leaf ash was dissolved in 25ml of dilute HCl (1:1v/v) in the china dish and covered with a watch glass and incubated for 20-25 minute on hot water bath, gradually cooled and filtered through a whatman filter paper No.44 (ashless). 10ml of filtrate was taken in 100ml volumetric flask and made its final volume to 100ml by adding distilled water. This solution was diluted to 10 times using distilled water, this stock ash solutions were used for the detection and estimation of elements using Atomic Absorption Spectrometer. The mineral content of the sample was calculated using the following formula

$$\text{Mineral content (ppm)} = \frac{Z \times S \times A}{Y \times W \times 100}$$

Where, Z= Mineral content of the standard solution, S=Reading of the test sample of a mineral, Y= Reading of the standard solution of the mineral, A= Dilution factor, W= weight of plant material (g), 100= volume of the stock solution.

Flourescent studies of powder drugs

A pinch of dried and powdered plant material was taken in a clean test tube with about 10ml of solvent like acetone, benzene, petrpleum ether chloroform, ethanol, glacial acetic acid, HCl, HNO₃, methanol and distilled water. All the tubes were shaken well and incubated for about 30min. The colors of the drug solutions thus obtained were observed for their characteristic color reaction under the visible light (fluorescent tube) and ultra violet light (UV366nm) and were recorded by comparing with a standard color chart¹⁴.

Phytochemical screening:

The extracts of the plant were screened for alkaloids, flavonoids, glycosids, saponins, tannins, phenols, lignins and steroids as described by¹⁵.

Results and Discussion

The physico- chemical constant evaluation of the drug is an important parameter in detecting

adulteration or improper handling of drugs. 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 silica¹⁶. The total ash content of the *Cjanus cajan* leaf is 14.50%, whereas the water insoluble ash is less than that of acid insoluble ash at 3.39% and 8.35% respectively (Table-1). Similar results are reported in *Elephantopus scaber* leaf¹⁷. In the present investigation considerable amount of total ash was noticed in leaf. Findings can be employed as quality parameter to evaluate *Cajanus cajan* L. biomass for any adulteration.

Table 1. Ash value of *Cajanus cajan* L.

| S. No. | Physical constants | Value (%) |
|--------|---------------------|-----------|
| 1 | Total ash value | 14.5 |
| 2 | Acid insoluble ash | 8.35 |
| 3 | Water insoluble ash | 3.39 |

Results of organoleptic study such as extraction yield, colour, odor and nature of leaves extract of *Cajanus cajan* L. are shown in Table-2. The extraction yield of different solvents varied from 6.2% to 12.29 and could be ranked from high to low i.e. ethanol > methanol > petroleum ether > chloroform > aqueous extracts¹⁸. The percentage of extraction yield will increase with the ratio of solvents, temperature and sample extraction. The crude extracts of *Cajanus cajan* have exhibited a

wide range of colour. The petroleum ether and chloroform extracts are greenish brown and greenish black colour whereas ethanol and aqueous extract are dark brown and brown colour. Further the taste of pet ether and chloroform are pungent bitter while the remaining are bitter in taste. Similarly the nature of these extracts varies from sticky (petroleum ether), waxy (chloroform), resinous (ethanol) and powdery (aqueous) respectively.

The result of fluorescence studies of leaf *Cajanus cajan* L powder using different reagent of are given in Table-3. Under visible light and UV light the powdered leaf sample treated with distilled water, HCL, nitric acid, chloroform and sulphuric acid different samples shows a characteristic colouration whereas, acetone, methanol, ethanol, glacial acetic acid and petroleum ether treated samples didn't showed fluorescence. Fluorescence is an important phenomenon exhibited by various chemical constituents show fluorescence in the visible range in day light, The UV light produces fluorescence in many natural products (eg. Alkaloids like berberine) which do not visibly fluorescence in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation¹⁹.

Table 2. The organoleptic study of the leaf powder of *Cajanus cajan* L.

| S. No. | Physical characteristic | Petroleum ether extract | Chloroform extract | Ethanol extract | Aqueous extract |
|--------|-------------------------|-------------------------|--------------------|-----------------|------------------|
| 1 | Percentage of yield | 10.25 | 6.2 | 12.29 | 8.6 |
| 2 | Taste | Pungent bitter | Pungent bitter | Bitter | Bitter |
| 3 | Nature | Sticky | Waxy | Resin | Amorphous powder |

Table 3. Fluoroscnet study of *Cajanus cajan* L. leaf powder

| S. No. | Organic solvents | Visible light | Fluorescence in UV light |
|--------|---------------------|-----------------|--------------------------|
| 1 | Distilled water | Yellowish | Yellowish |
| 2 | Ethanol | Colourless | Colourless |
| 3 | Methanol | Colourless | Colourless |
| 4 | Acetone | Colourless | Colourless |
| 5 | Chloroform | Light yellow | Yellow |
| 6 | Glacial acetic acid | Colourless | Colourless |
| 7 | Sulphuric acid | Black | Black |
| 8 | Nitric acid | Greenish yellow | Light green |
| 9 | Hydrochloric acid | Brownish | Dark purple |
| 10 | Petroleum ether | Colourless | Colourless |

Results of element analysis of leaf by AAS (Atomic absorption spectrophotometer) are recorded in Table-4. Elements like Fe>Cu>Zn>Mg>Pb were found in leaf at concentration in decreasing order as mentioned here. The mineral content is essential part of plant biomass like; iron, copper and sulphur were detected in different crops^{20, 21, 22}. Several (*Nelumbo nucifera*, *Emblica ribes* and *Eugenia jambolana*) plant contain elements like potassium, magnesium, iron, and copper with medicinal activity²³.

Table 4. Element analysis of leaf of *Cajanus cajan* L.

| S. No. | Name of elements | Content (ppm) |
|--------|------------------|---------------|
| 1 | Iron | 2.1927 |
| 2 | Magnesium | 0.1532 |
| 3 | Copper | 0.1457 |
| 4 | Zinc | 0.2657 |
| 5 | Lead | 0.0004 |

The phytochemicals detected in *Cajanus cajan* L. leaf extracts are listed in Table-5. Test for alkaloids, steroids, tannins, phenols, glycosides and lignins were positive in all extracts, where as flavonoids and terpenoids were detected only in ethanol and aqueous extracts. The saponine is absent in all extracts. Results show that *Cajanus cajan* L. are rich in phytochemicals. Biologically important specific compounds have been identified in extracts of the plant by many workers²⁴.

The present studies revealed that pharmacognostic and phytochemical screening can serve as a basis for preparation of the herbal monograph for its proper identification, authentication and standardization of drugs.

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Table 5. Phytochemical screening of various leaf extract of *Cajanus cajan* L.

| Photochemicals | Petroleum ether extract | Chloroform extract | Ethanol extract | Aqueous extract |
|----------------|-------------------------|--------------------|-----------------|-----------------|
| Alkaloids | - | - | + | + |
| Flavonoids | - | - | + | + |
| Terpenoids | - | - | + | + |
| Steroids | + | + | + | + |
| Tannins | + | + | + | + |
| Phenols | + | + | + | + |
| Glycosides | + | + | + | + |
| Lignin's | + | + | + | + |
| Saponins | - | - | - | - |

+ = Present, - = Absent

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