Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Research Article

Received on: 11-01-2017
Accepted on: 21-02-2017
Published on: 15-04-2017

Corresponding Author:

*** T. Vidhya Ananthi,**
PG and Research Department of Botany, Jamal Mohamed College, Affiliated to Bharathidasan University, Tiruchirappalli – 620024, Tamil Nadu, India.

*Email Id- vidhyarivoli@gmail.com

Preliminary Phytochemical and Trace metal Screening from *Cajanus cajan* (L.) Millsp.

Vidhya Ananthi*, Ahmed John

ABSTRACT

Cajanus cajan (L.) Millsp is a perennial legume shrub belong to the family Fabaceae. Its application in respiratory infection, vermifuge properties, anti – ulcer, wound healing, dermatosis, convulsions, hepatoprotective is practised by the villagers. The aim of the present study focussed on the phytochemical constituents and Heavy metal concentration of Ethanolic extract of leaves from *Cajanus cajan*. The leaves were collected from Tiruchirappalli District, Southern India. The primary metabolites like Chlorophyll content, Carbohydrate, Protein and Lipids were present in the ethanolic extract of leaves. Likewise the secondary metabolites revealed the presence of the bioactive compounds such as Steroids, Sugar, Alkaloids, Phenols, Flavonoids, Tannins, Glycosides and Amino acids. The Trace metal concentrations were analysed from the powdered plant leaves by 797 VA Computrace Voltammetry, Mertrohm. This study will provide referential information of anti-microbial and anti-fouling efficacy of the plant *Cajanus cajan.*

Key-words: Cajanus cajan, Fabaceae, Ethanol extract, Phytochemicals, Trace metals.

Cite this article as:
Vidhya Ananthi, Ahmed John, Preliminary Phytochemical and Trace metal Screening from Cajanus cajan (L.) Millsp., Asian Journal of Pharmaceutical Technology & Innovation, 05 (23); 01-06, 2017. www.asianpharmtech.com

1.0 Introduction

Higher plants are warehouses of phytochemicals which are useful in the pharmaceutical industry. Phytochemicals are chemical compounds formed during the normal metabolic process in plants. Some beneficial pharmaceutical actions of plant materials result from the combination of secondary metabolic products that are present in the plant[1]. Although secondary metabolic products may have a variety of functions in plants, it is likely that their ecological function may have some bearing on potential medicinal effects in humans. The 20th century brought further understanding of human health the development of synthetic or semi synthetic analogs of plant compounds that led to drugs with higher level of potency over the past decade there has been an increased interest in phytochemicals for the purpose of human health and other benefits in the food industry[2]. It is imperative to initiate an urgent step for screening of plants for secondary metabolites. The present work is an attempt to assess the status of phytochemical properties of *Cajanus cajan* to improve the health status of people and also to use in pharmaceutical products of commercial importance. This work therefore is designed to phytochemically screen *Cajanus cajan* leaf, with the objective of observing and analysing their respective chemical constituents and trace metal concentration. Phytochemicals are chemical compounds formed during the normal metabolic process in plants. They usually occur in complex mixtures that differ among plant organs and stages of development.

2.0 MATERIALS AND METHODS

2.1 Collection of Plant Material and Ethanol Extraction

Fresh leaves of *Cajanus cajan* was collected from Tiruchirappalli district of Tamil Nadu during the period of October – November 2015. 100 gram shade dried plant powder was loaded in the thimble of Soxhlet apparatus fitted with appropriate size of round bottom flask with 250 ml absolute ethanol. Constant heat was provided by Mantox heater for recycling of the solvent. After complete extraction, the extract in round bottom flask was transferred into clean and pre-weighed universal tubes. Universal tubes containing extracts were weighted and noted down and finally, the percentage yield was calculated. Percentage yield was calculated as dividing initial weight of raw material taken by final weight of extract. Then, the extracted solution was dried by hot air oven at 50 °C for 48 h.

2.2 Quantitative estimation of primary metabolites

The dried and powdered plant material of *Cajanus cajan* leaf, was used for quantitative estimation of primary metabolites such as proteins, carbohydrates and lipids by using different methods are as follows

Chlorophylls

The chlorophyll pigments in the leaves were estimated by the method of Arnon. After pre-cleaning, weighed fresh leaf material was homogenized and extracted thrice in chilled 80% acetone (v/v). The volume of the acetone extract was made up to a known one and the optical density was read at 645 nm and 663 nm wavelengths on a spectrophotometer. The concentration of the chlorophyll pigments was calculated and is expressed in mg/g fresh weight.

Proteins

Plant material of 0.5 g was homogenized in 5 ml of 0.1 M phosphate buffer (pH 7.0) by grinding with a mortar and pestle. The homogenate was centrifuged at 15,000 g at 40º C for 15 min. The obtained supernatant was used for protein estimation. Protein estimation was made according to Lowry's method (Lowry et al., 1951). Plant extract (0.1 ml) was taken in test tubes and the volume made upto 1 ml using distilled water. A 5 ml of the alkaline copper solution was added and incubated for 10 min then 0.5 ml of Folin-Ciocalteu's reagent was added to the mixture and allowed to stand for 30 min and measure the absorbance at 660 nm against the reagent blank. Protein content was calculated using the calibration curve of bovine albumin.

Carbohydrates

Total carbohydrate was estimated by Anthrone reagent (Yemm and Willis, 1954). Plant liquid (0.05 ml) was taken in test tubes and the volume was made up to 1 ml and 4 ml of Anthrone reagent was added to this solution and the mixture was heated in boiling water bath for 8 min followed by cooling. Optical density of green colour to dark colour was read at 630 nm.

Lipids

Total lipid was estimated by the method of Barned and Blackstock (1973). The plant tissue was homogenized in 5 ml of chloroform and methanol mixture (2:1). The homogenate is filtered using Whatmann filter paper No. 1. Then, 0.1 ml of the filtrate is taken in a test tube and left aside for evaporation. After complete evaporation, 1 ml of H2SO4 was added and boiled for 10 min. Then 0.2 ml of this solution was added with 5 ml of vanillin reagent

and shaken vigorously. The colour thus obtained is read at 520 nm, after 10 min against a reagent blank. The content of lipids was calculated using the calibration curve of cholesterol.

2.3 Identification tests for secondary metabolites

The phytochemical constituents were analysed from the plant *Cajanus cajan* by the standard procedures[4].

Steroids

A 3 ml of test solution and minimum quantity of chloroform was added with 3-4 drops of acetic anhydride and one drop of concentrated H2SO4. Purple colour thus formed changes into blue or green colour indicating the presence of Steroids.

Tritrepenoids

A 3 ml of test solution was added with a piece of tin and 2 drops of thionyl chloride.
Formation of violet or purple colour indicates the presence of Tritrepenoids.

Reducing Sugars

A 3 ml of test solution was added with 2 ml of Fehling's reagent and 2 ml of water.
Formation of reddish orange colour indicates the presence of Reducing Sugar.

Sugars

A 3 ml of the test solution was added with very small quantity of Anthrone reagent and a few drops of concentrated H2SO4 and heated. Formation of green or purple colour indicates the presence of Sugars.

Alkaloids

A 3 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity indicates the presence of Alkaloids.

Phenols

A 3 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue colour indicates the presence of Phenols.

Flavonoids

A 3 ml of test solution in alcohol was added with a bit of magnesium and one (or) two drops of concentrated HCl and heated. Formation of red or orange colour indicates the presence of Flavonoids.

Saponins

A 3 ml of test solution was added with water and shacked. Formation of foamy lather indicates the presence of Saponins.

Tannins

A 3 ml of test solution was added with water and lead acetate. Formation of white precipitate indicates the presence of tannins.

Anthroquinones

A 3 ml of test solution was added with magnesium acetate. Formation of pink colour indicates the presence of Anthroquinones.

Glycoside

To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added and observed for a reddish brown coloration at the junction of two layers and the bluish green colour in the upper layer.

Amino Acids

A 3 ml of test solution was added with 1% ninhydrin in alcohol. Formation of blue or violet colour indicates the presence of amino acids.

Catechins

A 3 ml of test solution in alcohol was added with Ehrlich reagent and a few drops of concentrated HCl. Formation of pink colour indicate the presence of Catechins.

2.4 Analysis of trace metals in plant

The *Cajanus cajan* plant sample was collected from the Tiruchirappalli district, Tamil Nadu. The plant leaves were carefully removed and washed with sterile distilled water, separately. The cleaned leaves were dried in shadow area and were grinned with agate mortar and pestle. The powered plant samples were stored in sterile plastic container. The 1 g of powdered plant sample was treated with aqua-regia mixture (hydrochloric acid + nitric acid) in Teflon bomb and was incubated at 140 °C for 2-3 days [3, 4]. After incubation, the reaction mixture was filtered with nitrocellulose (0.45 µm) filter paper by Millipore vacuum filtration unit. Then the extraction

was test for trace metals (Fe, Cu, Zn, Pd, Cd, Cr and Ni) analysis. Trace metal analysis were determined by the 797 VA Computrace voltammetry, Metrohm.

3.0 RESULTS AND DISCUSSION

The results thus obtained are discussed in the light of the available literature. The Ethanolic leaf extract of *Cajanus cajan* screened for the presence of various biologically active primary and secondary metabolites. The quantitative results revealed of primary metabolites of chlorophylls A, chlorophylls B, Total chlorophylls, carbohydrates, proteins and lipids presented in 0.419, 0.852, 1.271, 22.2, 40, 25 mg/g respectively (Table.1). Likewise, the presence of secondary metabolites are steroids, sugars, alkaloids, phenol, flavonoids, tannins, glycoside and amino acids (Table.2). A common role of secondary metabolites in plants is defence mechanisms. They are used to fight off herbivores, pests and pathogens. Although Researchers know that this trait is common in many plants it is still difficult to determine the precise role of each secondary metabolite. Secondary metabolites are used in anti-feeding activity, toxicity or acting as precursors to physical defence systems[6]. The preliminary test such as phenol and Ellagic acid tests showed positive to all extracts of leaf, seed coat and cotyledon. Whereas, aqueous extract of cotyledon shows negative to these test. Formation of muddy yellow colour by the Ellagic acid test shows the presence of trihydroxy phenolics like ellagic acid and gallic acid which are known to be the nuclear compounds of tannins. Similarly, the test solutions have also shown positive response to phenol test. It is known that ellagic acid is a potent inhibitor of mutagenicity and cytogenecity [4]. The ethanol and aqueous extracts of leaf shows presence of tannins by showing positive to the tests of tannins namely FeCl3 and tannin tests. Similarly, ethanol extract of seed coat and cotyledon shows positive to above tests, remaining extracts shows negative to these tests. The pet ether, chloroform, ethanol and aqueous extracts of leaf, seed coat and cotyledon showed positive to the tests of flavonoids i.e., Shinoda, Pew's and NaOH tests. The Shinoda and Pew's tests showed developing deep cherry red colour indicating the occurrence of dihydrokaempferol flavonoids and deep red or magenta colour indicating the presence of flavones respectively. The positive response to different flavonoid tests indicates the occurrence of more than one type of flavonoids. The earlier report of Duke (1981) supports the present results. The ethanol and aqueous extracts of all parts of Cajanus cajan shown positive response to steroids by the development of wine red colour precipitation and bluish green colour in the tests of Salkowski and Liebermann and Burchard tests indicates the presence of steroids has reported by Duke (1981). The ethanol and aqueous extracts were positive to the preliminary alkaloid tests i.e., iodine, Dragendroffs and Wagner's reagents indicating the presence of alkaloids. The positive responses to iodine, Wagners and Dragendroffs to all tests confirms the occurrence of more than two types of alkaloids in the test solutions by the formation of yellow, white and orange precipitation respectively. Sinthiya and Koperuncholan (2015) has also shown the occurrence of alkaloids in Cajanus cajan. The chloroform, ethanol and aqueous extracts of all parts of Cajanus cajan indicated the presence of glycosides by giving characteristic reaction with the tests of glycosides namely Conc. H2SO4 and Kellar Killiani test by formation of reddish brown colour ring at the junction of two liquids and formation of the reddish colour respectively, indicating more than one type of glycosides may be present. Presences of glycosides are seen in Cajanus cajan by Duke (1981). The leaf, seed coat and cotyledon extracts were completely devoid of saponins. The foam and haemolysis tests showed the absence of saponin. According to tannin is present in the plant extracts the haemolysis test gives negative result. The preliminary screening tests have revealed that the presence of the various groups of secondary metabolites such as phenols, tannin, flavonoids, alkaloids, glycosides, lignins and steroids in the leaf, seed coat and cotyledon[6]. Some of the trace metals are essential for plant growth whereas many of them affect the plant physiology. Especially, the role of trace metal pollutants causing injury to plants either by direct toxic effect or modifying the host physiology rendering it more susceptible to infection which leads to affects the photosynthesis process, growth and their efficiency[7]. The mean concentrations of metals such as Cd, Cr, Cu, Fe, Ni, Pb and Zn were below detectable limit (BDL), BDL, 0.06, 0.42, BDL, BDL and 0.33 mg kg-1, respectively (Table 3). Through the natural process of bio-magnifications, minute quantities of metals become part of the various food chains and concentrations become elevated to levels which can prove to be toxic to human, animal, plant and other living organisms[5].In particularly, higher trace metal in the plants caused progressive reduction in the photosynthetic ability of leaves, closure of leaf stomata, and productivity of plants, ascorbic acid content and chlorophyll content.

Table: 1 Qualitative phytochemical constituent of *Cajanus cajan*

Phytochemical Constituents	Ethanol
Steroids	+
Triterpenes	-
Reducing sugars	-
Sugars	+
Alkaloids	+
Phenolics	+
Catechins	-
Flavonoids	+
Saponins	-
Tannins	+
Anthroquinones	-
Glycosides	+
Amino acids	+

+ = Present; - =Absent

Table 2. Quantitative phytochemical constituent of *Cajanus cajan*

Biochemical constituents	*Cajanus cajan* (mg/g)
Chlorophyll A	0.419
Chlorophyll B	0.852
Total Chlorophyll	1.271
Protein	22.2
Carbohydrate	40.0
Lipid	25.0

Table 3. Concentration of trace metals in *Cajanus cajan*

Sampling Site	Sample Name	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Tiruchirappalli Tamil Nadu	Cajanus cajan	0.07	BDL	0.26	0.72	BDL	BDL	0.43

BDL – Below detectable limit

4.0 CONCLUSION

This study shown that all the primary and secondary metabolites were present in the *Cajanus cajan*. The *Cajanus cajan* were absorbed the chemicals and metals which may affect the growth and biochemical properties of the plants. Hence, we recommended that *Cajanus cajan* will act as an alternative antibiotic in the near future for fungal diseases.

5.0 ACKNOWLEDGEMENT

The authors thank the Biospark Biotechnological Research Center (BBRC), Tiruchirappalli, Tamil Nadu, India for trace metal studies.

6.0 REFERENCES

1.Ahmed John S and Koperuncholan M. Direct root generation and Indirect Organogenesis in Silybum marianum and preliminary phytochemical, antibacterial studies of its callus, The International Journal of Pharmaceutics, 2012; 2:52-57.

2. Ahmed John S and Koperuncholan M. Antibacterial Activities of various solvent extracts from Impatiens balsamina. International Journal of pharma and bio sciences, 2012; 3: 401-406.

3. Duke, J. A.: Handbook of Legumes of World Economic Importance (Handbuch der Hülsenfrüchte von weltwirtschaftlicher Bedeutung). Plenum Press, New York and London, 1981.

4. Koperuncholan M and Ahmed John S. Antimicrobial and Phytochemical Screening in *Myristica dactyloides* Gaertn. Journal of Pharmacy Research, 2011; 4: 398-400.
5. Koperuncholan M and Ahmed John S. Biosynthesis of Silver and Gold Nanoparticles and Antimicrobial Studies of Some Ethno medicinal Plants in South-Eastern Slope of Western Ghats. IJPI'S
Journal of Pharmacognosy and Herbal Formulations, 2011a; 1(5): 10-15.
6. Koperuncholan M and Ahmed John S. Antimicrobial and Phytochemical Screening in *Myristica dactyloides* Gaertn. Journal of Pharmacy Research, 2011; 4: 398-400.
7. Ramesh V, Ahmed John S and Koperuncholan M. Impact of cement industries dust on selective green plants: A case study in Ariyalur industrial zone, International Journal of Pharmaceutical, Chemical and Biological Sciences, 2014; 4: 152-158.
8. Suresh K, Saravana Babu S and Harisaranraj R. Studies on In Vitro antimicrobial activity of ethanol extracts of *Rauvolfia tetraphylla*. Ethnobotanical leaflets, 2008; 12: 586590.
9. Vidhya Ananthi T and Ahmed John S.2016. Screening of Trace metals and secondary metabolites from *Cajanus cajan* (L.) Millsp. And study their Antimicrobial efficacy. World Journal of Pharmaceutical Research.5 :(4):1059-1071, impact factor 6.805.