

## Research Article



## Evaluation of Phytochemicals and Fluorescent Analysis of Seed and Leaf Extracts of *Cajanus cajan* L.

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### ABSTRACT

*Cajanus cajan* L. is one of the major grain legume crops grown in semi-arid tropics. It is valued both as food and fodder. The present investigation is aimed to screen the various bioactive compounds present in seeds, both fresh and dry leaves and to study fluorescent characteristics of seed and leaf powder of *C.cajan* which are responsible for the medicinal properties of the plant using various standard tests. Qualitative analysis for the presence of phytochemicals was performed using aqueous, acetone, methanol, chloroform, diethyl ether and benzene extracts of seed and both fresh and dry leaves of *C.cajan* by standard procedures.<sup>1-4</sup> These extracts were further preceded for TLC for identification of bio constituents. The solvent system selected for TLC was Benzene: Acetic acid (9:1). The fluorescent characteristics of seed and leaf powder with various chemical reagents were noted under visible light and UV light. Phytochemical analysis revealed the presence of alkaloids, glycosides, tannins, flavonoids, phenols, saponins and steroids in all the fresh and dry leaf extracts. Seed extracts showed the presence of alkaloids, glycosides, resins, phenols, steroids, lignins, saponins, fats and oils in all the extracts in varying quantities. TLC analysis of fresh leaf and seed extracts resulted in the presence of various types of phytochemicals based on the number of spots. In fluorescent analysis characteristic colour changes were observed with different chemical reagents in leaf and seed powder of *C.cajan* under visible and UV light, it showed different colours of the powder in the presence or absence of chemical constituents. The plant is reliable to possess large number of medicinal values as it contains high amounts of these bioactive compounds. The leaves of plant are known to possess antihelmenthic, antiparasitic, antibacterial, antifungal, antitumor, antioxidant and antidiabetic activities.

**Keywords:** *Cajanus cajan*, Fluorescent analysis, Phytochemical analysis, Thin Layer Chromatography.

### INTRODUCTION

Pigeon pea [*Cajanus cajan* (L.) Millsp] (Fabaceae) commonly known as "Red gram" is an erect woody and annual perennial shrub, it is widely spread dietary legume crop predominantly grown throughout tropical and subtropical regions. Its leaves are used for rearing silkworms and it has many important medicinal properties. Decoction of leaves is used for cough, diarrhoea and abdominal pain. Its application in anti-ulcer, wound healing, hepatoprotective, anti-asthmatic ailments is practiced by the villagers. The seeds are nutritious and popular food in developing countries. The hypoglycaemic activity of seeds has been reported. Different parts of the plant are used in the management of disorders such as diarrhoea, diabetes, cough, sores and ulcer in South America, Asia and Africa. The extracts of Pigeon pea are often used for the treatment of hepatitis, measles, dysentery, as a febrifuge all over the world.<sup>5-8</sup> Leaves are used for treatment of malaria, bedsores, wounds, sore gums, mouthwash, toothache, child delivery as well as diet induced hypercholesterolemia. *Cajanus lactone* a natural coumarin has been isolated from leaves of *C.cajan* is an antibacterial agent against gram-positive microorganisms.<sup>9-13</sup> In Chinese medicine traditionally, leaves are widely used to relieve pain, arrest blood and kill worms.<sup>14</sup> The leaf decoctions cure genital and skin irritations in Argentina and Indochinese claim that powdered leaves help to expel bladder stones. In India and Java the young leaves are applied to sores.<sup>15</sup>

The leaf extracts show protective effects against alcohol induced liver damage and hypotoxic-ischemic brain damage.<sup>16,17</sup> The investigations on chemical constituents indicated that the pigeon pea leaves are rich in flavonoids, stilbenes which are considered to be responsible for the beneficiaries of leaves on human health.<sup>18,19</sup> The crop species *C.cajan* has ethano pharmacological importance; it is an important folk medicine in eastern Rajasthan as boiled juice of leaves is given orally to nullify effect of intoxication and as laxative. The leaf paste is applied for oral ulcers and inflammations.<sup>20</sup> In view of the importance of pigeon pea in ethanobotany as health remedy, the present study was designed to evaluate various phytochemicals and fluorescent characteristics of *C.cajan* leaf and seed extracts.

### MATERIALS AND METHODS

#### Plant collection and extraction

The leaves and pods of *C.cajan* variety ICP-26 were collected from the field of Department of Biotechnology, Kakatiya University, Warangal. The leaves and pods were collected, cleaned and air dried under shade for about 14 weeks. After drying, the seeds were separated from pods. The dried seeds and leaves were then blended using a house hold electric blender. This fine powder was analysed for phytochemicals and fluorescent characters in various plant extracts.



**Preparation of extracts**

For phytochemical analysis, the extracts were prepared by taking 2gms of each dried powder into separate 100 ml conical flask and 50ml of each solvent ( Aqueous, Benzene, Chloroform, Diethyl ether, Methanol, Acetone ) was added. The conical flasks were plugged with cotton plugs, labelled and allowed to stand for 1-2 hrs and then filtered using Whatman No.1 filter paper. Thus, the filtrates obtained were used as test solutions.

**Screening of phytochemicals**

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures.<sup>1-4</sup> After the addition of specific reagents to the test solution, the tests were detected by visual observation of colour change or by precipitate formation.

**A. Test for Alkaloids****Dragandroffs test**

To 1 ml of test solution 2 ml of Dragandroffs reagent was added and mixed. To this 2 ml of dilute HCL was added. Orange precipitate indicates the presence of alkaloids.

**Mayer's test**

To 1 ml of test solution add few drops of Mayer's reagent (Potassium Mercuric Iodide Solution). Cream precipitate indicates the presence of alkaloids.

**Wagner's test**

To 1 ml of test solution add equal volumes of Wagner's reagent (Iodine in Potassium Iodide). Reddish precipitate indicates the presence of alkaloids.

**Hager's test**

To 2 ml of test solution add few drops of Hager's reagent (Saturated Picric Acid Solution). Bright yellow precipitate indicates the presence of alkaloids.

**Tannic acid test**

The extracts were treated with 10% tannic acid. A pale yellow precipitate indicates the presence of alkaloids.

**FeCl<sub>3</sub>**

To 1 ml of test solution add few drops neutral ferric chloride solution. Yellow precipitate indicates the presence of alkaloids.

**B. Tests for Glycosides****Raymond's test**

When the extracts were treated with dinitrobenzene in hot alkali, Violet or pink colour indicates the presence of glycosides.

**Legal's test**

When the extracts were treated with pyridine and alkaline sodium nitroprusside solution, cherry colour indicates the presence of glycosides.

**Bromine water test**

The test solution treated with bromine water yield a pale yellow colour which indicates the presence of glycosides.

**Keller Kiliani test**

To 1ml of test solution 1ml of glacial acetic acid was added, dissolved and then cooled. After cooling 2-3 drops of ferric chloride was added. Then carefully, 2 ml of conc. H<sub>2</sub>SO<sub>4</sub> was added along the walls of test tube. Reddish brown ring at the junction of two layers indicates the presence of Glycosides.

**Molisch's test**

To 1ml of test solution 2-3 drops of molisch reagent was added and mixed well. Then conc. H<sub>2</sub>SO<sub>4</sub> was added along the walls of test tube. Reddish purple ring at the junction of two layers indicates the presence of glycosides.

**Conc. H<sub>2</sub>SO<sub>4</sub> test**

To 1ml of test solution 1ml of conc. H<sub>2</sub>SO<sub>4</sub> was added and allowed to stand for 2 min. Red precipitate indicates the presence of glycosides.

**C. Tests for Tannins****Ferric chloride test**

Few drops of FeCl<sub>3</sub> solution was added to the test solution. Blackish precipitate indicates the presence of tannins.

**Gelatine test**

To 1ml of test solution few drops of gelatine solution was added. White precipitate indicates the presence of tannins.

**Lead acetate test**

Basic lead acetate was added separately to 1ml of test solution. Bulky red precipitate indicates the presence of tannins.

**Alkaline reagent test**

The test solution was treated with sodium hydroxide solution. Yellow to red precipitate indicates the presence of tannins.

**Mitchell's test**

The test solution was treated with iron and sodium tartarate. Water soluble iron-tannin complex, which is insoluble in solution of ammonium acetate, indicates the presence of tannins.

**D. Test for Flavonoids****Shinoda test (Magnesium Hydrochloride reduction test)**

To 1ml of the test solution, add few fragments of Magnesium ribbon. Then conc. HCl was added carefully along the walls of the test tube drop wise. Crimson red colour indicates the presence of flavonoids.

**Zinc Hydrochloride reduction test**

To 1ml of test solution a mixture of zinc dust and conc. HCl was added. Appearance of red colour after few min. indicates the presence of flavonoids.

**Lead acetate test**

Basic lead acetate was added separately to 1ml of test solution. Bulky reddish brown precipitate indicates the presence of flavonoids.

**Alkaline reagent test**

The test solution was treated with sodium hydroxide solution. Yellow to red colour indicates the presence of flavonoids.

**Ferric chloride test**

Few drops of  $\text{FeCl}_3$  solution was added to the test solution. Blackish precipitate indicates the presence of flavonoids.

**E. Test for Fats and Fixed Oils****Stain test**

Small quantity of extract was taken and pressed between the two Whatman No. 1 filter papers; the stain on the filter paper indicates the presence of fixed oil.

**Saponification test**

To small quantities of various test solutions add few drops of 0.5N alcoholic potassium hydroxide and a drop of Phenolphthalein and heat on water bath for 1-2 hrs. Presence of fixed oils and fats is indicated by the formation of soap.

**F. Test for Steroids****Salkowski test**

To 1ml of test solution 5ml of chloroform was added and then few drops of Conc.  $\text{H}_2\text{SO}_4$  was added to the above mixture and mix well. Allow the mixture to stand for some time, reddish precipitate in the lower layer indicates the presence of steroids.

**Liebermann- Buchard test**

To 1ml of test solution few drops of acetic anhydride was added and then few drops of Conc.  $\text{H}_2\text{SO}_4$  was added to the above mixture. Steroids were indicated by reddish brown coloured ring at the junction of two layers.

**G. Test for Phenols****Phenol test**

To 2ml of test solution 1ml of  $\text{FeCl}_3$  solution was added. Phenol is indicated by an intense colour.

**Ellagic acid test**

To 2ml of test solution add few drops of 5% (w/v) glacial acetic acid and 5% (w/v) sodium nitrate solution. Phenol is indicated by Niger brown precipitate.

**H. Test for Quinones**

To 1ml of test solution Alcoholic KOH solution was added separately. Quinones were indicated by colour ranging from red to blue.

**I. Test for Lignin's**

To 1ml of the test solution 2% (w/v) furfuraldehyde was added. Red colour indicates the presence of lignins.

**J. Test for Resins**

To 1ml of the test solution 2-3ml of copper sulphate solution was added the contents were mixed well for 2min, and then the solution was allowed to separate. Resins were indicated green coloured precipitate.

**K. Test for Saponins**

3ml of test solution was taken in a test tube and vigorously shaken. Formation of foam indicates the presence of saponins.

**L. Test for Coumarins**

Take few drops of distilled water in a test tube and add 1gm of plant powder. The paper soaked in NaOH was covered over the test tube is diluted and boiled. Coumarins is indicated by yellow fluorescence and examined under UV light.<sup>21</sup>

**Thin layer chromatography (TLC)**

TLC is a chromatographic technique which is used for the separation of mixture of compounds. TLC is performed on a sheet of aluminium foil which is coated with a thin layer of adsorbent silica gel, which are commercially available 60 F<sub>254</sub> (Merck). Samples of all the fresh leaf extracts and seed extracts prepared with different solvents were spotted onto the TLC plate as a single spot with capillary tubes.<sup>22</sup> After trying different solvent systems a suitable solvent system was selected. After applying different samples on the silica gel plate, it is placed in a TLC chamber containing the solvent system (known as mobile phase) and covered with a lid on top of it. By the capillary action the solvent is drawn up by the plate and it is developed. The developed plate was taken out from the chamber and the solvent front was marked and allowed to air dry for few minutes. Iodine vapours are used as visualizing agent for the detection of separated compounds. Qualitative evaluation of separated substance was carried out by calculating  $R_f$  values.

**Fluorescent study of seed and leaf powder**

0.5gms of seed powder and dried leaf powder were taken into clean and dried test tubes. To each tube 5ml of different organic solvents like distilled water, acetone, ethanol, benzene, chloroform, diethyl ether, methanol, glacial acetic acid, sulphuric acid, nitric acid, hydrochloric acid, 5%  $\text{FeCl}_3$ , 5%  $\text{I}_2$ , picric acid, 1N NaOH and 1N NaOH + methanol were added separately. Then, all the tubes were shaken and they were allowed to stand for about 20-25 min. The solutions obtained were observed under the visible light and UV light for their characteristic colour



reaction and were compared with a standard colour chart and colours were recorded.<sup>23</sup>

## RESULTS

The results of phytochemical screening of seeds, fresh and dried leaves for the six solvents viz., Acetone, Aqueous, Benzene, Chloroform, Diethyl ether, Methanol extracts of *C.cajan* are given in the Tables 1-2. In seed extracts phytochemical analysis revealed the presence of alkaloids, glycosides, resins, phenols, steroids, lignins,

saponins, fats and oils in all the extracts in varying quantities. Tannins, flavonoids and quinones are completely absent in seed extracts. Sterols are absent in aqueous and acetone extracts Table 1. In fresh and dried leaves, phytochemical analysis revealed the presence of glycosides, resins, phenols, lignins in more amounts in fresh leaf and dry leaf extracts. Quinones are completely absent in all the leaf extracts. Steroids are absent in aqueous and methanol leaf extracts Table 2.

**Table 1:** Phytochemical analysis of seed extracts of *C.cajan*

Phytochemical tests	Aqueous Extract	Acetone Extract	Benzene Extract	Chlorofom Extract	Diethyl Ether Extract	Methanol Extract
<b>ALKALOIDS</b>						
Dragendorff's test	+	+	+	+	+	+
Mayer's test	+	+	+	+	+	+
Wagner's test	+	+	+	+	+	+
Hager's test	+	+	+	+	+	+
Tanicacid test	+	+	+	+	+	+
FeCl <sub>3</sub> test	+	+	+	+	+	+
<b>GLYCOSIDES</b>						
Raymond's test	-	+	+	+	+	+
Legal's test	+	+	+	+	+	+
Bromine water test	-	-	+	+	+	-
Kellar Kiliani test	+	+	+	+	+	+
Conc. H <sub>2</sub> SO <sub>4</sub> test	-	-	-	-	-	-
Molisch test	+	+	+	+	+	+
<b>TANNINS</b>						
FeCl <sub>3</sub> test	-	-	-	-	-	-
Gelatin test	-	-	-	-	-	-
Lead acetate test	-	-	-	-	-	-
Alkaline reagent test	-	-	-	-	-	-
Mitchell's test	-	-	-	-	-	-
<b>FATS &amp; OILS</b>						
Stain test	+	+	+	+	+	+
Saponification test	+	+	+	+	+	+
<b>FLAVONOIDS</b>						
Lead acetate test	-	-	-	-	-	-
FeCl <sub>3</sub> test	-	-	-	-	-	-
Shinoda's test	-	-	-	-	-	-
Alkaline reagent test	-	-	-	-	-	-
Zn-Hcl Reduction test	-	-	-	-	-	-
<b>STEROIDS</b>						
Liebermann Burchard test	-	-	+	+	+	+
Salkowski test	-	-	+	+	+	+
<b>RESINS</b>	-	-	+	+	+	-
<b>PHENOLS</b>	+	+	+	+	+	+
<b>LIGNINS</b>	+	+	+	+	+	+
<b>QUINONES</b>	-	-	-	-	-	-
<b>SAPONINS</b>	+	+	+	+	+	+

+ = Present; - = Absent

**Table 2:** Phytochemical analysis of fresh and dry leaf extracts of *C. cajan*

Phytochemical tests	Aqueous Extract		Acetone Extract		Benzene Extract		Chloroform Extract		Diethyl Ether Extract		Methanol Extract	
	F	D	F	D	F	D	F	D	F	D	F	D
<b>ALKALOIDS</b>												
Dragendorff's test	-	-	-	+	-	+	-	+	-	+	-	-
Mayer's test	-	-	-	+	-	+	-	+	-	+	-	-
Wagner's test	-	-	-	+	-	+	-	+	-	+	-	-
Hager's test	-	-	-	+	-	+	-	+	-	+	-	+
Tanicacid test	-	-	-	+	-	+	+	+	+	+	+	+
FeCl <sub>3</sub> test	-	-	+	+	+	+	+	+	+	+	+	+
<b>GLYCOSIDES</b>												
Raymond's test	-	-	+	-	-	-	+	-	-	-	-	-
Legal's test	+	-	+	-	+	+	+	+	+	+	+	-
Bromine water test	+	+	+	+	+	+	+	-	+	+	+	+
Kellar Kiliani test	-	-	+	+	+	+	+	+	+	+	+	-
Conc. H <sub>2</sub> SO <sub>4</sub> test	-	-	-	-	-	-	+	-	+	-	+	+
Molisch test	+	-	+	+	+	+	+	+	+	+	+	+
<b>TANNINS</b>												
FeCl <sub>3</sub> test	-	+	-	+	+	+	+	+	+	+	+	+
Gelatin test	-	+	-	+	+	+	+	+	+	+	-	-
Lead acetate test	-	-	-	-	-	-	-	-	-	-	-	-
Alkaline reagent test	-	+	-	-	-	+	+	+	+	-	-	+
Mitchell's test	+	+	-	+	-	+	-	+	-	+	-	+
<b>FLAVONOIDS</b>												
Lead acetate test	+	+	-	-	-	-	-	-	-	-	-	+
FeCl <sub>3</sub> test	+	+	+	+	+	+	+	+	+	+	+	+
Shinoda's test	+	+	-	+	-	+	-	+	-	-	-	-
Alkaline reagent test	+	+	-	-	-	+	+	+	-	-	-	-
Zn-Hcl Reduction test	-	+	-	+	+	+	+	+	+	+	+	+
<b>STEROIDS</b>												
Libermann Burchard test	-	-	+	-	+	+	+	+	-	+	-	-
Salkowski test	-	-	+	+	+	+	+	+	+	+	-	-
<b>COUMARINS</b>	+	+	+	+	+	+	+	+	+	+	+	+
<b>RESINS</b>	+	+	+	+	+	+	+	+	+	+	+	+
<b>PHENOLS</b>	+	+	-	+	+	+	+	+	+	+	+	-
<b>LIGNINS</b>	+	+	+	+	-	+	+	+	+	+	+	-
<b>QUINONES</b>	-	-	-	-	-	-	-	-	-	-	-	-
<b>SAPONINS</b>	+	+	+	+	+	+	+	+	+	+	+	+

F = Fresh leaf; D = Dry leaf, + = Present; - = absent

The solvent system selected for TLC was Benzene: Acetic acid (9:1). TLC analysis of fresh leaf extract resulted in identification of 6 spots in benzene and chloroform extract each, 5 spots in diethyl ether and aqueous extract each and 7 spots in methanol and acetone extract each with  $R_f$  values 0.25, 0.31, 0.55, 0.61, 0.82, 0.91 in benzene; 0.23, 0.31, 0.53, 0.61, 0.78, 0.89 in chloroform; 0.21, 0.29, 0.53, 0.85, 0.93 in diethyl ether; 0.21, 0.29, 0.55, 0.78, 0.85 in aqueous; 0.21, 0.29, 0.53, 0.68, 0.74, 0.85, 0.93 in methanol and 0.21, 0.29, 0.34, 0.57, 0.74, 0.80, 0.91 in acetone extracts of *C.cajan* Figure A. TLC analysis of seed extracts resulted in identification of 3 spots in aqueous, acetone, benzene and diethyl ether

extract each, 2 spots in chloroform extract and 5 spots in methanol with  $R_f$  values 1.3, 1.5, 4.3 in aqueous; 1.5, 1.7, 3.9 in acetone; 1.4, 1.8, 4.4 in benzene; 1.4, 1.7, 4.2 in diethyl ether; 1.6, 1.9 in chloroform and 1.6, 2.0, 3.5, 3.8, 4.3 in methanol extracts of *C.cajan* Figure B.

The results of fluorescent studies of dried leaf and seed powder of *C.cajan* with different chemical reagents are given in Table 3 and Figure C. Under visible light and UV light the powdered leaf samples are treated with various solvents. Among the different solvents tested, acetone, methanol, diethyl ether, petroleum ether, ethanol did not show any fluorescence in both seed as well as leaf

powder whereas, distilled water, benzene, chloroform, HCl, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, CH<sub>3</sub>COOH, 5% FeCl<sub>3</sub>, 5% I<sub>2</sub>, 1N NaOH, picric acid and 1N NaOH + methanol showed characteristic colouration in both seed as well as leaf powder (Table 3).

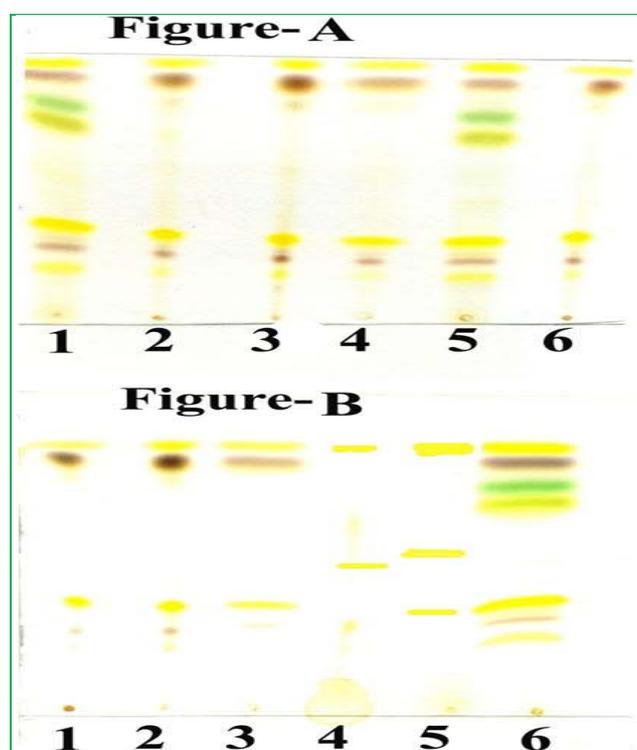
### DISCUSSION

Phytochemical investigation of seed extracts of *C.cajan* showed the presence of alkaloids, glycosides, fats and

oils, phenols, sterols, resins and lignins. Fresh and dry leaf extracts showed the presence of alkaloids, tannins, glycosides, flavonoids, lignins, resins, phenols, saponins and steroids. These results are supportive with other studies performed with *C.cajan* leaf extracts, where the same secondary metabolites were found<sup>24, 25</sup>. They reported that the dry leaf extracts of aqueous and ethanol extracts showed the presence of alkaloids, tannins, glycosides and saponins.

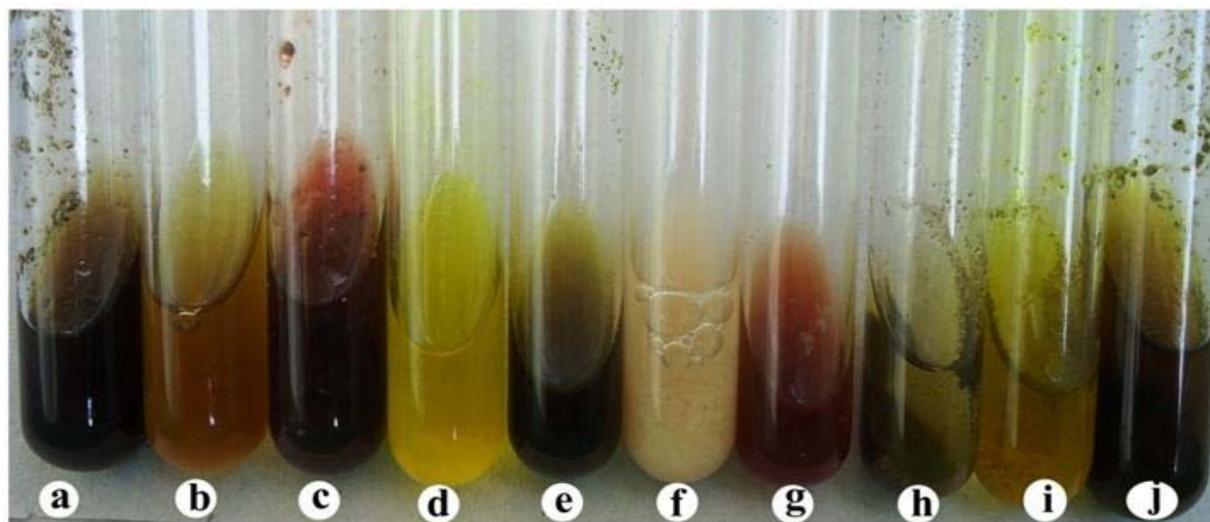
**Table 3:** Fluorescent analysis of seed and leaf powder of *C.cajan*

Organic solvents	Seed powder		Dry leaf powder	
	Visible light	Fluorescence in UV light	Visible light	Fluorescence in UV light
Distilled water	Cream	Cream	Yellowish	Yellowish
Acetone	White	White	Colourless	Colourless
Ethanol	White	White	Colourless	Colourless
Benzene	Mud brown	Light brown	Yellow	Light yellow
Chloroform	Mud brown	Light brown	Light yellow	Yellow
Diethyl ether	White	White	Colourless	Colourless
Methanol	White	White	Colourless	Colourless
Petroleum ether	White	White	Colourless	Colourless
Glacial acetic acid	Mud brown	Light brown	Colourless	Colourless
Sulphuric acid	Reddish	Reddish brown	Black	Black
Nitric acid	brown	Reddish	Greenish	Light green
Hydrochloric acid	Reddish	brown	yellow	Purple
5% FeCl <sub>3</sub>	Brown	Mustard yellow	Brownish	Black
5% I <sub>2</sub>	Niger brown	Grey	Black	Dark green
Picric acid	Grayish black	Yellow	yellow	Mustard yellow
1N NaOH	Muddy brown	Intense yellow	Mustard yellow	Dark green
1N NaOH+Methanol	Brick red orange	brown	Green Yellowish green	Dark green



**Figure A:** TLC analysis of fresh leaf extracts of *C.cajan* (1. Aqueous extract; 2. Acetone extract; 3. Benzene extract; 4. Chloroform extract; 5. Diethyl ether extract; 6. Methanol extract)

**Figure B:** TLC analysis of seed extracts of *C.cajan* (1. Aqueous extract; 2. Acetone extract; 3. Benzene extract; 4. Chloroform extract; 5. Diethyl ether extract; 6. Methanol extract)



**Figure C:** Fluorescent analysis of seed and leaf powder of *C.cajan* (a.  $H_2SO_4$  + leaf powder; b. 5%  $FeCl_3$  + seed powder; c.  $H_2SO_4$  + seed powder; d. 1N  $NaOH$  + seed powder; e. 1N  $NaOH$  + methanol + leaf powder; f. Distilled water + seed powder; g.  $HNO_3$  + seed powder; h.  $HNO_3$  + leaf powder; i. Picric acid + leaf powder; j. 5%  $I_2$  + leaf powder)

Alkaloids are present in seed extracts, fresh and dry leaf extracts of *C.cajan*. Antitumor, anti inflammatory and antimicrobial properties are due to the presence of alkaloids<sup>26</sup>. Flavonoids are present in fresh and dried leaf extracts but absent in seed extracts. Antiparasitic, antibacterial and antifungal activities are due to the presence of flavonoids.<sup>27, 28</sup> Tannins are present in fresh and dry leaf extracts but absent in seed extracts. Tannins are used as tanning agents as they possess astringent, antioxidant and antimicrobial activities.<sup>29</sup> Bactericidal and fungicidal properties are due to the presence of tannins.<sup>30</sup> Antihelmenthic activity was reported due to the presence of phenolics (flavonoids & tannins).<sup>31</sup> In addition to their industrial uses such as foaming agents and detergents, saponins have a wide range of medicinal applications.<sup>32</sup> Since the plant contains high amounts of these bioactive compounds, it is reliable to possess large number of medicinal values.

TLC analysis of fresh leaf extract resulted in identification of 6 spots in benzene and chloroform extract each, 5 spots in diethyl ether and aqueous extract each, 7 spots in methanol and acetone extract (Figure A). TLC analysis in seed extracts, resulted in identification of 3 spots in aqueous, acetone, benzene and diethyl ether extract each, 2 spots in chloroform extract, 5 spots in methanol extract (Figure B). This shows presence of phytochemicals in varying quantities with the respective extracts.

Fluorescent study of seed and leaf powder of *C.cajan* using different chemical reagents showed different colouration under visible light and UV light. One of the important feature of fluorescence is that UV light induces a fluorescent nature in many natural products (e.g. Alkaloids like berberine) where fluorescence is not seen in natural day light. Among various solvents tested, acetone, methanol, diethyl ether, petroleum ether, ethanol did not

show any fluorescence in both seed as well as leaf powder. Whereas, distilled water, benzene, chloroform,  $HCl$ ,  $H_2SO_4$ ,  $HNO_3$ ,  $CH_3COOH$ , 5%  $FeCl_3$ , 5%  $I_2$ , 1N  $NaOH$ , picric acid and 1N  $NaOH$ +methanol showed characteristic colouration in both seed as well as leaf powder (Table-3). These results are supportive with fluorescent studies performed with *C.cajan* leaf powder.<sup>33</sup> They reported that the leaf powder of *C.cajan* in various solvents like acetone, ethanol, methanol, did not show any fluorescence while nitric acid, chloroform and  $HCl$  showed characteristic colouration. Some of substances may be often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent, hence we can often assess qualitatively some crude drugs using fluorescence as it is the most important parameter of pharmacognostical evaluation.<sup>34</sup>

## CONCLUSION

Phytochemical analysis of seed, fresh and dry leaf extracts of *C.cajan* showed the presence of various bioactive compounds in all the extracts in varying quantities. The quality and quantity of phytochemicals is based on the selection of solvent system and the procedure selected for extraction. TLC analysis revealed the presence of various types of phytochemicals based on the number of spots. The phytoconstituents of different extracts of *C.cajan* would be helpful in treating many diseases and the fluorescent analysis of powdered drug play an important role in the determination of quality and purity of the drug.

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