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Research Article

Phyto-chemical Evaluation of extracts of *Cajanus cajan* Linn.

Subrata Das[1], Kamalesh Purkait[1], Tamal Maity[2], Pranabesh Chakraborty[2]

[1] Women's Polytechnic (Govt. of West Bengal), Chandernagore, Hooghly-**712136**

[2] Bengal School of Technology, Sugandha, Delhi road, Chinsurah, Hooghly-712102

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ABSTRACT

Phytochemicals are chemical compounds that occur naturally in plants (phyto means plant in Greek).Some is responsible for color and other has organoleptic properties. Some phytochemicals with physiological properties are elements. Some phytochemicals are biologically active which provides protective health benefits. The seeds and leaves of *Cajanus cajan* Linn. belonging to the family Fabeaceae, which is a shrub cultivated in Central India are used as medicine for the treatment of ulcer, wounds, liver ailments and asthma by the villagers. From extensive literature survey it is revealed that no reports were available on macroscopic as well as phytochemical standardization parameters of *Cajanus cajan* Linn. The present study aimed to establish methods for quality control of drugs, botanical evaluation which comprises of macroscopy, physicochemical parameters like loss on drying, extractive values, ash values and to investigate the phytochemicals present in the extracts in the preliminary level with chemical analysis and thin layer chromatography. The study will provide referential information for the better understanding of the plant to be used as medicine for the treatment of the various diseases.

Keywords: Botanical evaluation, *Cajanus cajan*, Extractive values**,** Fabeaceae, Phytochemicals

INTRODUCTION

Cajanus cajan (L) Millsp. (In Sanskrit: Adhaki, Hindi: Arhar, English: Pigeon pea, Bengali: Tur) is a perennial herb belonging to the family of fabaceae. Pigeonpea [*Cajanus cajan* (L.) Millsp.] is the oldest food crop abundantly used and ranks fifth as an important edible legumes of the world (1,2). Pigeonpea is a multipurpose plant as it is extensively eaten as a dal. *Cajanus cajan* normally grows well in tropical and sub-tropical environments (3). The important producers of *Cajanus cajan* in the world includes India, followed by Uganda, Tanzania, Kenya, Malawi, Ethiopia, and Mozambique in Africa; the Dominican Republic, Puerto Rica, and the West Indies in the Caribbean region and and Latin America; Burma, Thailand, Indonesia, and the Philippines in Asia; and Australia (3). Pigeonpea is rich in proteins, carbohydrates, and certain minerals. The protein content normally ranges between 17.9 and 24.3 g/100 g (2) for whole grain samples, and between 21.1 and 28.1 g/100 g for split seed. (8) In India leaves of pigeonpea are used for rearing silkworms; green pods are used as a vegetable; husk, green leaves and tops are used as fodder and green manure. (9) Amongst its many medicinal uses, *C. cajan* is indicated in the relief of pain in traditional Chinese medicine and as a sedative. (10) *Cajanus cajan* is also used traditionally in Ayurveda, and Siddha. The plant is used for treatment of stomach problems, syphilis, anaemia, dizziness, epilepsy, cough, constipation, sore throat, worm infestation, insomnia , wounds, diabetes .(4-7) . The important phytochemical constituents are 2'-o-methylcajanone; 5,2'-dihydroxy-7,4'-dimethoxyisoflavone; 5,2',4'-trihydroxy-7 methoxyisoflavone; 5,2'-dihydroxy-7,4' dimethoxyisoflavone;5,7,2',4'-tetrahydroxyisoflavone; 5,7,4'-trihydroxyisoflavone;7-hydroxy-4'-methoxyisoflavone;alpha-copaene;alpha-himachalene; alpha-humulene; beta-himachalene; cajaminose; cajanin; cajaninstilbene acid; cajaquinone; concajanin; gamma-himachalene; lupeol; orientin; phytic acid; pinostrobin; vitexin (11).The chemical constituents are responsible for the different types of pharmacological activities such as Hypoglycaemic activity, Hepatoprotective activity, Nephroprotective activity**,** Anticytotoxic activity, Immunomodulatory activity, Antioxidant activity, Anti-osteoporotic activity etc.(12,13).

*Corresponding author: Subrata Das

Materials and method:

Collection and Identification of plant material:

The leaves of *Cajanus cajan* Linn. was collected from Hooghly (West Bengal, India) and identified by the Office of the Scientist-'F' Central National Herbarium, Botanical Survey of India, Botanical Garden, Howrah-711103 (West Bengal) and a voucher specimen of plant **(No.: CNH/Tech. II/2016/39).**

Sampling of plant material:

The fruits were collected and separately dried in shade at room temperature, grinded coarsely in mixer, kept in the small plastic bag and preserved in air tight containers. The coarsely powdered dried leaves were used for the Phyto-chemical screening and physical evaluation. 20g of the semi-powdered sample was mixed with 200ml of ethanol in a beaker and kept for 72 h at room temperature. It was then filtered, and the filtrate was concentrated to dryness in a rotary evaporator and stored in refrigerator at 4°C for further analysis.

Preparation of plant extract:

Extraction method:

The powdered plant was extracted by maceration process with the solvent medium. The powdered plant material was placed in a container full of menstrum and kept for seven or more days, shaken frequently until complete extraction took place. The maceration was performed at room temperature. Hot or cold water, Ethanol, Methanol, Ethyl acetate, Methyl acetate and their combinations were used as the solvent medium for extraction.

Physiochemical analysis

Powder plant materials were subjected to determination of various physicochemical parameters such as moisture content (%LOD), total ash, acid insoluble ash, and water soluble ash. Water and alcohol soluble extractive values were determined by cold maceration. (14, 15)

Phytochemical screening

Preliminary phytochemical screening of the extracts were carried out for detection of the presence of different phytoconstitutents such as alkaloids, glycosides, flavonoids, phenolic compounds, saponins, tannins etc. present in pegionpea leaves. The qualitative chemical tests were performed according to the standard procedures. (14, 15)

Quantitative Phytochemical Screening

Total Flavonoid Content and total saponin content of the drug extracts was performed according to standard procedure. Values Are Presented As Mean ± Standard Deviation of Triplicates**.**

Thin layer chromatographic (TLC) analysis

The chemical fingerprint of the extracts was determined by thin layer chromatography using prepared by spreading silica gel G-60 F-254 (0.25 mm thick) on glass plate using Distill water as solvent; these plates are activated in oven at 110^{o}C for half hour. All four extracts are applied separately and run in different solvent system of varying polarity. The TLC plate was air-dried and spots were visualized under ultraviolet light (254 & 365 nm) and Iodine chamber for different spot of chemical constituent these plates are developed in. Rf value calculated for different extracts of *Cajanus cajan* Linn. The TLC plate was air-dried and spots were visualized under ultraviolet light (254 & 365 nm). The Rf values of the spots were also recorded. (16)

Fourier Transform Infrared (FTIR) Fingerprint Analysis

Fourier transform infrared (FTIR) spectrophotometer was used to identify the characteristic functional groups in the seed extracts. The Ethanol extract and Methanol extract (5mg), respectively, were thoroughly mixed with potassium bromide (KBr) in a mortar and pressed at pressure of 6 bars within 2min in order to prepare a thin translucent sample discs. The FT-IR spectrum was obtained using Perkin Elmer 2000 spectrophotometer system with a scan range from400 to 4000 cm−1 and analysed using Bruker OPUS software. (17)

Assignment of FTIR spectra of lignin

Peak location range (cm^{-1})	**Assignment**
3412 -3460	O-H stretching
3000 -2842	C-H stretch in methyl and methylene group
1738 -1709	C=O stretch in unconjugated ketone, carbonyl and ester groups
1675 -1655	C=O stretching in comjugated p -subst. Aryl ketones
1593 -1605	Aromatic skeleton vibrations plus C=O stretching; S>G: Gcondensed> G ether ified
1505 -1515	Aromatic skeleton vibrations (G>S)
1460 -1470	C-H deformations (asym in –CH3 and –CH2-)
1422 -1430	Aromatic skeleton vibrations combined with C -H in plane deformations
1365 -1370	Aliphatic C -H stretching in CH3 and phen. OH
1325 -1330	Condensed S and G ring (G ring bound via position 5)
1266 -1270	G ring plus C+O stretching
1221 -1230	C-C + C -O + C=O stretching (Gcondensed>Getherifie d)
1166	Typical for HGS lignins; C=O in ester groups (conj.)
	Aromatic C -H in -plane deformation (typical of G unit; Gcondensed> G etherified)
1125 -1128	Typical of S unit; also secondary alcohol & C=O strt.
1086	C-O deformation in sec. alcohol & aliphatic ether
	Aromatic C-H in - plane deformation (G>S) plus C -O deform.in primary alcohols plus C -H stretching (unconjugated)-HC=CH - out of plane deformation. (trans)
966 -990	C-H out of plane (aromatic ring)
915 -925	C-H out of plane in positions 2, 5 and 6 (G units)
853 -858	C-H out of plane in positions 2 and 6 of S units
834 -835	C-H out of plane in positions 2, 5 and 6 of G units

Fluorescence analysis:

The fluorescence analysis was done to identification where the herbs were fluorescence. The fluorescence character of the were dried leaves powder (40 mesh) was studied both in daylight and UV light (366nm) and after treated with different reagents like Sodium hydroxide 1(N), Hydrochloric acid 1(N), Ferric chloride 1(N), Methanolic NaOH.(14)

Results:

Table 1: Phytochemical screening of *Cajanus cajan:*

S. No.	**CHEMICAL TEST**	**WATER EXTRACT**	**METHANOL EXTRACT**	**ETHYL ACETATE EXTRACT**	**ETHANOL EXTRACT**
	1. CARBOHYDRATE				
A	Molish test	+	++	+	-
B	Fehling test	+	++	+	-
C	Pholoroglucinol test	-	-	+	-
D	Tollen's test	++	+ ++	+	-

E	Cobalt chloride	-	+	-	-
F	Iodine test	+	+	+	-
G	Tannic acid test	+	++	-	-
H	Gum test	-	-	+	-
I	Mucilage test	-	-	+	-

2. PROTEIN

A	Biuret test	+	+++	+	-
B	Millon's test	-	+	+	-
C	Sulpher test	++	++	++	+
3.	**AMINO ACID**				
A	Nihydrin test	-	+++	++	-
B	Tyrosine test	+	+	+	-

4. FATS AND OILS

A	Filter paper test	+++	++		

5. STEROID

A	Salkowski reaction	-	-	-	-
B	Libermann-Burchard reaction	++	+++	-	-
C	Libermann's reaction	++	+++	-	-

6. GLYCOSIDES

A	**Cardiac glycoside**				
A	Legal's test	++	+++	-	-
B	Keller-Killani test	+	++	+	-
B	**Anthraquinone glucoside**				
A	Borntrager's test	+	+	++	+
B	Modified Borntrager's test	++	++	-	+
C	**Saponin glycoside**				
A	Foam test	+	+	+	++
D	**Flavonoids**				
A	Shinoda test	-	-	++	+
B	Lead acetate test	-	-	+++	+

7. ALKALOIDS

A	Dragendorff's test	-	+	-	-
B	Mayer's test	-	+	-	-
C	Wagner's test	+	++	-	-

8. PHENOLIC COMPOUNDS

A	5% $FeCl_3$ solution	-	+	-	+
B	Lead acetate test	++	++	-	+
C	Acetic acid solution	-	++	-	+

(+++) = Maximum
(++) = Average
(+) = Minimum
(-) = Nil

Table 2: Moisture content test by using Carl-Fisher Reagent:

S. No.	Extract	Quantity taken	Moisture content
1.	Methyl acetate	1.5 mg	Nil
2.	Ethyl acetate	1.5 mg	1 %
3.	Ethanol	1.5 mg	Nil
4.	Methanol	1.5 mg	Nil
5.	Aqueous (Hot)	1.5 mg	1%
6.	Aqueous (Cold)	1.5 mg	1%

Table 3: Evaluation of Ash Value of Leaves of *Cajanus cajan*

Sr. No.	Parameters	Percentage (%)
1	Ash value	14.5
2	Water soluble ash value	95.04
3	Acid insoluble ash value	0.73

Table 4: Extractive values of Leaves of *Cajanus cajan*

Sr. No.	Solvent used	Average extractive value in % w/w on dry weight basis
	Ethanol(Absolute)	6
	Water	12

Table 5: Quantitative Phytochemical Screening of the Dried *Cajanus cajan*

Sr. No.	Solvent system	Rf value
1	Benzene : Ethanol (9:1)	0.58, 0.7, 0.8
2	Benzene : Acetic acid (9:1)	0.47,0.52
3	Chloroform : Acetone (7:3)	0.42,0.58,0.6

Table 6: Rf Values of different solvent system of different extract of *Cajanus cajan*

Table 6.1: Rf Values for Methyl Acetate extract by TLC:

Parameter	Result
Saponins (mg/g)	377 ± 0.02
Flavonoids (mg/g)	235 ± 0.02

Values Are Presented As Mean ± Standard Deviation of Triplicates

Table 6.2: Rf Values for Ethyl acetate extract by TLC:

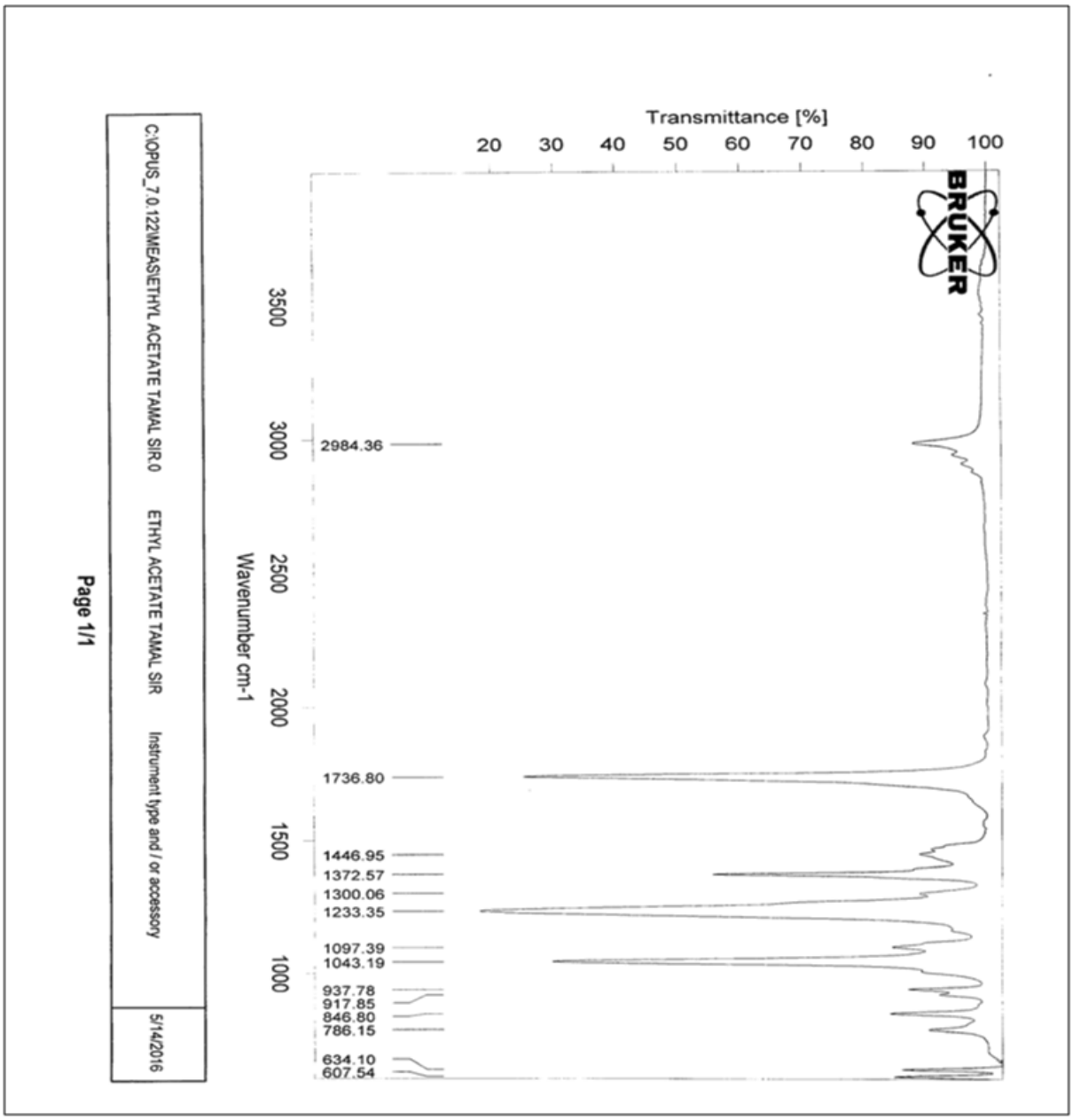
Sr. No.	Solvent system	Rf value
1	Methanol : Benzene (5:5)	0.89,0.53
2	Benzene : Acetic acid (9:1)	0.63
3	Chloroform : Acetone (7:3)	0.73,0.63

Table 6.3: Rf Values for Alcoholic extract by TLC:

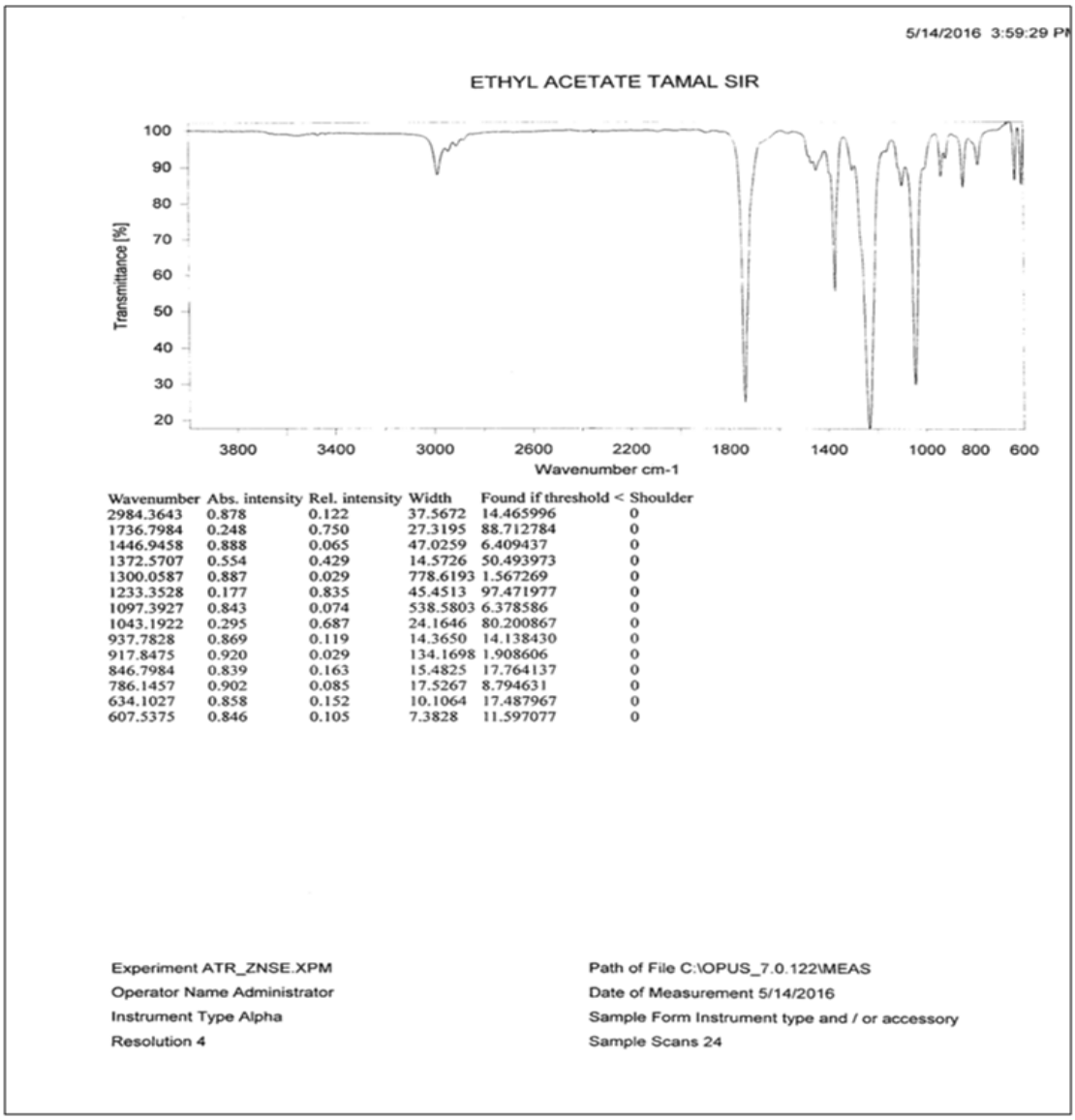
Sr. No.	Solvent system	Rf value
1	Benzene : Ethanol (9:1)	0.75
2	Chloroform : Acetone (7:3)	0.86,0.81

Table 7: Fluorescence analysis result

Experiment	Visible light(366nm)	UV light
Drug+NaOH	Brown	Dark blue
Drug+ HCl	Transparent colour	transparent colour
Drug+ FeCl3	Dark straw color	transparent colour



Graph 1:



Wavenumber	Abs. intensity	Rel. intensity	Width	Found if threshold <	Shoulder
2984.3643	0.878	0.122	37.5672	14.465996	0
1736.7984	0.248	0.750	27.3195	88.712784	0
1446.9458	0.888	0.065	47.0259	6.409437	0
1372.5707	0.554	0.429	14.5726	50.493973	0
1300.0587	0.887	0.029	778.6193	1.567269	0
1233.3528	0.177	0.835	45.4513	97.471977	0
1097.3927	0.843	0.074	538.5803	6.378586	0
1043.1922	0.295	0.687	24.1646	80.200867	0
937.7828	0.869	0.119	14.3650	14.138430	0
917.8475	0.920	0.029	134.1698	1.908606	0
846.7984	0.839	0.163	15.4825	17.764137	0
786.1457	0.902	0.085	17.5267	8.794631	0
634.1027	0.858	0.152	10.1064	17.487967	0
607.5375	0.846	0.105	7.3828	11.597077	0

Experiment ATR_ZNSE.XPM
Operator Name Administrator
Instrument Type Alpha
Resolution 4

Path of File C:\OPUS_7.0.122\MEAS
Date of Measurement 5/14/2016
Sample Form Instrument type and / or accessory
Sample Scans 24

Graph 2:

Discussion

The extensive survey of literature revealed that ***Cajanus cajan*** is an important medicinal plant with diverse pharmacological spectrum. ***Cajanus cajan*** is widely used in Ayurveda, and Siddha, etc. (4-7) Piliminary phytochemical creening shows the presence of phenolic comounds, glycosides, flavonoids in the alcoholic leaf extracts. Flavonoids like (isorhamnetin, luteolin, apigenin, quercetin) were found to possess antioxidant activity (18,19,20) Antioxidants are known to possess antiinflammatory, anticardiovascular disease, anticancer and antineurogenerative properties.

C. cajan leaf extracts showed varied physicochemical parameters such as total ash content of 14.5% while water soluble ash is greater than that of acid insoluble ash at 95.04 and 0.73 g/100 g, moisture content 1.00% respectively. Ash value is in detecting genuineness and purity of drug and the values are significant in quantitative standards (21). Pharmacognostic studies basically helps in identification of adulterants and also to identify closely related plant species (22).

The flavonoid contents in the analysed leaf extracts was found to be 235 mg GAE/g and saponin content was found to be 377mg/gm. Flavonoids are the polyphenolic compounds are important constituents

of plant due to their free radical scavenging ability because of their hydroxyl groups. Thus, the phenol content of plants may lead directly to their antioxidant activity (23). Saponins from plants areis used as mild detergents and in medicine, it is used in hypercholesterolaemia, hyperglycaemia, antioxidant, anti-cancer, anti-inflammatory and weight loss etc.(24) This compound has also been reported to have antihyper-cholesterol, anti-inflamatory, cardiac depressant properties(16) and appear to kill or inhibit cancer cells without killing the normal cells in the process(25) Saponins also have anti-fungal properties(26). Some saponins glycosides are cardiotonics and others used as contraceptives and precursors for other sex hormones (27).

Further evaluation needs to be carried out to explore the unknown and concealed areas and their practical and clinical applications which can be used in the treatment and welfare of the mankind.

Reference

1. Morton JF, The pigeon pea (*Cajanus cajan* Millsp.), a high-protein, tropical legume. HortScience. 1976;11:11-19.
2. Salunkhe DK, Chavan JK, and Kadam SS. Pigeonpea as important food source. CRC Critical Review in Food Sci. and Nutrition. 1986;23(2):103-141.
3. Sinha SK. Food legumes: distribution, adaptability and biology of yield,. In: FAO plant production and protection paper 3. FAO, Rome. 1977
4. M. Brink, G. Belay Cereals and Pulses PROTA Foundation/ Backhuys Publishers Wageningen 2006 : 35
5. C.P. Khare Indian Medicinal Plants: An Illustrated Dictionary Springer-Verlag Berlin 2007 : 110
6. Duke, J.A. 1983. Handbook of energy crops. NewCROP (New Crops Resource Online Program), Purdue Univ. Center for New Crops and Plant Products. http://www.hort.purdue.edu/newcrop/duke_energy/Cajanus_cajun.html (accessed 24 July 2012).
7. Indian Medicinal Plants.(Vol-I):2007
8. Phatak, S.C., R.G. Nadimpalli, S.C. Tiwari, and H.L. Bhardwaj. Pigeonpeas: potential new crop for the southeastern United States. In: J. Janick and J.E. Simon, editors, New Crops. Wiley, New York. 1993:597–599. http://www.hort.purdue.edu/newcrop/proceedings1993/v2-597.html (accessed 24 July 2012).
9. Ambasta SP. The useful plants of India; 4th Ed. New Delhi: National Institute of Science Communication; 2004:94–5.
10. 10. Ahsan R, Islam M. *In vitro* antibacterial screening and toxicological study of some useful plants (*Cajanus cajan*) Euro J Sci Res. 2009;41:227–32.
11. Ogunbinu AO, Flamini G, Cioni PL, Adebayo MA, Ogunwande IA. Constituents of *Cajanus cajan* (L.) Millsp., *Moringa oleifera* Lam., *Heliotropium indicum* L. and *Bidens pilosa* L. from Nigeria. Nat Prod Commun. 2009 Apr; 4(4):573-8.
12. Ezike AC, Akaha PA,Okoli CC, Okpala CB, Experimental evidence for the antidiabetic activity of *Cajanus cajan* leave in rats, J Basic and Clinical Pharm.2010;1:25-30.
13. Jaiswal D, Rai PK, Kumar A Watal G, Study of glycemic profile of *Cajanus cajan* leaves in experimental rats. Indian J Clin Biochem. 2008;23:167-70.
14. Khandelwal Dr. K.R. "Practical Pharmacognosy",nirali prakashan, 20TH edition, Aug. 2010:25.1-25.
15. Kokate C., Purohit A. and Gokale S. Practical Pharmacognosy, Vallabh Prakashan, New Delhi,Edition 1994:10:112-120.
16. Harbone, JB., 19/3. Phytochemicals methods.London: Chapman and Hill.
17. Ashok kumar R and Ramaswamy M. Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants . Int.J.Curr.Microbiol.App.Sci (2014) 3(1): 395-406
18. Wu N, Fu K, Fu Y, Zu Y, Chang F, Chen Y, et al. Antioxidant Activities of Extracts and Main Components of Pigeonpea [*cajanus cajan* (L.) Millsp.] Leaves. Molecules. 2009;14:1032.
19. Lu LM, Zu Y, Fu Y, Zhang S, Yao L, Efferth T. Cajanol, a novel anticancer agent from Pigeonpea *[Cajanus cajan* (L.) Millsp.] Roots, induces apoptosis in human breast cancer cells through a ROS-mediated mitochondrial pathway. Chem Biol Interact. 2010;188:151–60.
20. Zhang D, Zhang S, Zu Y, Fu Y, Kong Y, Gao Y, et al. Negative pressure cavitation extraction and antioxidant activity of genistein and genistin from the roots of pigeon pea [*cajanus cajan* (L.) Millsp.] Sep Purif Technol. 2010;74:261–70.
21. Herodez SS, Hadolin M, Skerget M, Knez Z. Solvent extraction study of antioxidants from

balm (*Melissa officinalis*) Leaves. Food Chem. 2003;80:275–82.

22. Sumithra C. Importance of pharmacognostic study of medicinal plants: An overview. J Pharmacog Phytochem. 2014;2:69–73.

23. Tosun M, Ercisli S, Sengul M, Ozer H, Polat T, Ozturk E. Antioxidant properties and phenolic content of eight *Saliva* species from Turkey. Biol Res. 2009 ;41:175–81.

24. Ngbede J, Yakubu R A and Nyam D A, Med Well Res J Biolog Sci., 2008, 3(9), 1076-1078.

25. Okwu D E, Evaluation of chemical spices and flavouring agents, Global J Pure Applied Sci., 2001: 7: 455-459

26. Sodipo O A, Awanji M A, Kolawole F B and Oduntuga A A,. Bio Sci Res Commun., 1991:3:171.

27. Evans W C, Trease and Evans Pharmacognosy. 15th Edition, Elsevier, India, 2002: 27, 46, 183-184, 289-291, 411-413, 434, 485-486