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RESEARCH ARTICLE

Preliminary Phytochemical Screening of *Cajanus cajan* Linn.

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ABSTRACT:
The seeds and leaves of *Cajanus cajan* Linn.which is a shrub cultivated in Central India are used in medicine belong to the family Fabeaceae. Its application in anti-ulcer, wound healing, hepatoprotective, anti-asthamatic ailments is practiced by the villagers. From extensive literature survey it was revealed that no reports were available on macroscopic as well as pytochemical standardization parameters of *Cajanus cajan* Linn.in order to check the identity and purity of the drug. 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 respect tochemical tests and thin layer chromatography. Thus it was thought worthwhile to explore this videly cultivated plant on the basis of it's standardization parameters .The study will provide referential information for the better understanding the plant to be used as medicine for the treatment of the various disease.

KEYWORDS: *Cajanus cajan*, Fabeaceae,botanical evaluation,phytochemicals,extractive values

1.0 INTRODUCTION:
Plants are erect shrub which is indigenous to south asia, cultivated in India. Vernacularly called as kandipappu in Telugu and Tuvar in Hindi. Other synonym of the plant is *Cajanus indicus.* Branches provided with silky hair, leaves are compound, pollinate, leaflet oblong-lanceolate , entire, densely silky beneath, flowers are yellow in terminal panicles or corymbs racemes, fruits pods, tipped with the persistent lower half of the style, seeds vary in colour from yellow and red to brown or black. The leaves and seeds are used in medicine. This species is used as medicine in China and Brazil.[1,2]

2.0 MATERIALS AND METHODS:
The plants collected,identified and its extracts in different solvents are used for preliminary phytochemical studies.

2.1 Collection and Identification of plant material:
The plants of *Cajanus cajan* was collected from Neelbad (Bhopal ,India) were identified by the botanist Dr. Zea Ul Hasan, Department of botany, Saifia Science College (Barkatulla University) Bhopal (M.P.) and a voucher specimen of plant (No.226/Bot/Safia/2011) has been deposited in herbarium for further reference.

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2.3 sampling of plant material:
The leaves are 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 phytochemical screening and physical evaluation.

2.4 Extraction of phytochemical constituents:
For preliminary phytochemical analysis, extract was prepared by weighing 100 gm of the dried powdered leaves were defatted by Pet.ether and were subjected to hot successive continuous extraction in soxhlet apparatus with different solvents with increase in polarity, ethyl acetate, ethanol and finally with water. The extracts were filtered in each step, concentrated and the solvent was removed by vaccum distillation. The extracts were dried in the vaccum dessicator and the residues were weighed. The presence or absences of the primary and secondary phytoconstituents were detected by usual prescribed methods.[3,4]

2.5 Moisture content:
The moisture content of the all Four extract was found by 10 mg extract was titrated with standard karl fischer reagent and the end point will be found by karl fischer apparatus. Results are given in Table -1

2.6 Ash value:
Dried leaves were incinerated to determine the ash content.[5]as given in Table-2

Table-1 Moisture content test by using Carl-Fisher Reagent:

S. No.	Extract	Quantity taken	Moistur content
1.	Pet. Ether	10mg	Nill
2.	Ethyl acetate	10mg	1 %
3.	Ethanol	10mg	Nill
4.	Aqueous	10mg	1%

Table- 2 Evaluation of Ash Value of Leaves of *Cajanus cajan*

S/No.	Parameters	%
1.	Ash value	14.5

2.7 Extractive values:

Alcohol soluble extractive value:

Accurately weighed 5 gm coarse and air dried drug material was macerated with 100ml ethanol (99%) in a stoppered flask for 24 hrs. with frequent shaking for 6 hrs. It was then filtered rapidly through filter paper taking precautions to prevent excessive loss of ethanol. The volume was made up to 100ml with ethanol. The residue was evaporated in a flat bottom shallow dish, dried at 105 ^{0}C, weighed and kept in a desiccator. Average extractive value in percentage w/w (on dry basis) was calculated with reference to air dried drug (Table-3).

Water soluble extractive value:

5 gm coarse and air dried drug material was macerated with water in a stoppered flask for 24 hrs. with frequent shaking for first 6 hrs . The extract was filtered rapidly through filter paper taking precaution to prevent excessive loss of solvent. The residue was evaporated in a flat bottom shallow dish, dried at 105 ^{0}C weighed and kept in a desiccator. Average extractive value in percentage w/w (on dry weight basis) was calculated with reference to air dried drug (Table-3).

Table- 3 **Extractive values of Leaves of *Cajanus cajan***

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

Table- 4 **Phytochemical screening of Cajanus cajan linn. :**

S. No.	CHEMICAL TEST	PET. ETHER	ETHYL ACETATE	ETHANOL	WATER
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	-	++	-	+

Table -4 **Rf Values of different solvent system of different extract of *Cajanus cajan* Linn R_f Values for Petrolieum ether extract by TLC:**

S. No.	Solvent system	Solvent front height (cm)	No. of spots	Spot height (cm)	R_f Value
1.	Benzene:Ethanol (9:1)	6	1	5	0.83
2.	Methanol:Benzene (5:5)	5	-	-	-
3.	Benzene:Acetic acid (9:1)	5	1	4.2	0.84
4.	Chloroform:Acetone (7:3)	6	-	-	-
5.	Benzene:Ethyl acetate:Acetic acid (7.5:2.4:0.1)	5	1	4	0.8
6.	n-Butenol:Acetic acid:Water (12:3:5)	5	1	3.6	0.72

Table-5 R_f Values for Ethyl acetate extract by TLC:

S. No.	Solvent system	Solvent front height (cm)	N0. Of spots	Spot height (cm)	R_f Value
1.	Benzene:Ethyl acetate:Ammonia (8:1:0.5)	6.2	5	5.9, 4.8, 3.7, 2.6, 1.0	0.95, 0.77, 0.59, 0.41, 0.16
2.	Methanol:Benzene (5:5)	5.4	2	5.2, 3.1	0.9, 0.5
3.	Benzene:Acetic acid (9:1)	6	3	5.6, 3.8, 3.7	0.91, 0.82, 0.64
4.	Chloroform:Acetone (7:3)	5.7	4	5, 4.2, 3.6, 1.5	0.83, 0.7, 0.6, 0.25
5.	Benzene:Ethyl acetate:Acetic acid (7.5:2.4:0.1)	6.4	2	5.4, 3.8	0.84, 0.59
6.	n-Butenol:Acetic acid:Water (12:3:5)	5.4	3	4.7, 3.8, 3.6	0.94, 0.76, 0.72

Table-5 R_f Values for Ethanolic extract by TLC:

S. No.	Solvent system	Solvent front height (cm)	No. of spots	Spot height (cm)	R_f Value
1.	Benzene:Ethanol (9:1)	5.2	-	-	-
2.	Methanol:Benzene (5:5)	6.5	2	5.7, 5.1	0.87, 0.78
3.	Benzene:Acetic acid (9:1)	5.3	1	5.0	0.94
4.	Chloroform:Methanol (7:3)	5.5	2	5.2, 4.9	0.94, 0.89
5.	Benzene:Ethyl acetate (8:2)	5	-	-	-
6.	n-Butenol:Acetic acid:Water (12:3:5)	5.4	3	5.1, 4.7, 4.5	0.94, 0.87, 0.83

Table-6 R_f Values for Aqueous extract by TLC:

S. No.	Solvent system	Solvent front height (cm)	No. of spots	Spot height (cm)	R_f Value
1.	Benzene:Ethanol (9:1)	5.0	-	-	-
2.	Methanol:Benzene (5:5)	5.5	-	-	-
3.	Benzene:Acetic acid (9:1)	5.3	1	3.2	0.60
4.	Chloroform:Acetone (7:3)	5.2	-	-	-
5.	Benzene:Ethyl acetate:Acetic acid (7.5:2.4:0.1)	5.1	2	4.2, 3.8	0.82, 0.74,
6.	n-Butenol:Acetic acid:Water (12:3:5)	5.4	1	3.6	0.72

2.8 Phytochemical Screening:

The fresh plants were collected. Leaves were separated and dried in shade and reduced to coarse powder. The powdered material was extracted with Chloroform, Methanol in Soxhlet apparatus for 48 hrs. The extract was filtered hot and solvent removed by distillation under reduced pressure.[6] The percentage yield was calculated and the extract was further subjected to Phytochemical tests for Alkaloids, Glycosides, Flavonoids ,Carbohydrates ,Tannins (Table-4).

2.9 Thin Layer Chromatography:

Thin Layer Chromatographic plates are prepared by spreading silica gel G 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. These plates are developed in Iodine chamber for different spot of constituent chemical. Rf value calculated for different extracts of *Cajanus cajan* linn. as per Table-5

3.0 RESULT AND DISCUSSION:

Properly treated leaves of *Cajanus cajan* Linn. was defatted with petroleum ether then extracted with Ethyl Acetate, Ethanol and water in soxhlet apparatus. The solvents are distilled from the extract by vacuum distillation at low temperature so that any Thermolabile chemical constituent does not change.

Moisture content is zero in pet. ether and ethanol extrct and 1% in ethyl acetate and water extract of the plant found out by Karl Fischer Reagent.(Table-1) Ash value of the leaves are determined as 14.5%(Table-2) Extractive values of dried leavesin absolute alcohol is 6% and in water12% (Table-3)

Phytochemical Screening shows maximum presence of Carbohydrates in Ethyl Acetate Extract, Pet. ether and ethanol extract shows less presence while water extract shows nil content of Carbohydrate. Protein and Amino acid is present considerably in ethyl acetate and ethanol extract while pet.ether and water extract shows little presence.

Fig.1 Pet. Ether ext.

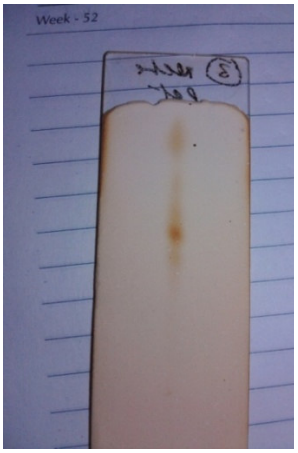

Fig.2 Ethyl acetate ext.

Fig.3 Ethanolic ext.

Fig.4 Aqueous ext.

Steroids, Glycosides, alkaloids and Phenolic compounds are present strongly in Pet. ether and ethyl acetate Extract while saponin glycosides and flavonoids show their presence in ethanol and water extract.(Table-4)

Thin Layer Chromatography of Pet. Ether, Ethyl acetate, Ethanol and Water Extracts have been performed in different solvent system of varying degree of polarity using silica gel G of TLC grade. Maximum no of constituent in different solvent system of varying polarity is present in ethyl acetate extract while less than half no. of compound present in ethanolic extract. Only four chemical constituent present in pet. ether and water extract of *Cajanus cajan* Linn.

4.0 CONCLUSION:

Looking to physicochemical data and phytochemical analysis of different extracts of *Cajanus cajan* Linn., it is concluded that Ethyl Acetate Extract and Ethanolic Extract are useful for further studies of Pharmacological parameters.

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