https://doi.org/10.48047/AFJBS.6.7.2024.3805-3811

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

PHYTOCHEMICAL SCREENING AND EXTRACTION OF LEAVES OF ACACIA CATECHU (L.) WILLD AND ACACIA AURICULIFORMIS A.CUNN

Trishul S. Thorat and Tanuja V Kadre*
Dr. A.P.J.Abdul Kalam University, Indore, (M.P.) – India
[1, 2*]Dr. A.P.J.Abdul Kalam University, Indore, (M.P.) – India
***Corresponding Author-**Tanuja V Kadre
*Dr. A.P.J.Abdul Kalam University, Indore, (M.P.) – India
Email ID-tanujavkadre@gmail.com

Article History

Volume 6, Issue 7, 2024
Received: 09 March 2024
Accepted: 19 April 2024
doi:
10.48047/AFJBS.6.7.2024.3805-3811

Abstract
By considering of longtime infectious diseases and synthetic drugs side effects, many researchers had put their attention on semi-synthesis of medicinal compounds to battle with miscellaneous diseases. *Acacia catechu* (L.) Willd belongs to the family Leguminosae known as Khair or Cutch tree, possesses diverse pharmacological actions, and has been widely used in Asia and different parts of the world. And Acacia auriculiformis Cunn. is a valuable, vigorously growing, evergreen tree, belonging to same family to that of A.catechu. In the present investigation, two Acacia species viz., acacia catechu (L.) Willd and Acacia auriculiformis A.Cunn were evaluated for quality parameters. Herein, phytochemical screening and estimation of leaves extract of both plants were reported.
Keywords: Acacia, Appearance, Screening, Estimation

Introduction

Acacia catechu has been widely used in Ayurveda for many years for the treatment and prevention of different diseases and disorders. It is an indigenous species of India and some other Asian countries. This species can be found in almost all regions of South and Southeast Asia, such as, India, Indonesia,Thailand and Myanmar. In India, the tree can be found in the states of Tamil Nadu, Rajasthan,Gujarat and Maharashtra. It is also found in East African countries. It has potential to grow in almost all kind of habitats. It is highly adaptable and invades disturbed habitats, urban ecosystem and unoccupied habitats.[1]

Acacia auriculiformis thrives in tropical lowlands, particularly along the banks of rivers, whereit often dominates the area. It can also be found in small depression pockets and open forests, which are sometimes dominated by other species of Acacia and eucalyptus. Additionally,

itoccurs in rainforests near river banks, behind coastal dunes, or in mangroves. In Papua NewGuinea, A. auriculiformis is abundant on the leaves and floodplains of the Morehead andBensbach rivers on the Plateau. It is also sparsely present in riparian habitats and monsoonforests. This species is planted for its wood and pulp, and it is also used as an ornamental tree,from where, it can escape from its natural habitat and invade other regions. These trees canoften be found in disturbed areas and along roadsides, and it has been known to invade scrub,hammocks, and pineland habitats in Florida. In the present study extraction and phytochemical screening of leave extract of both the Acacia species were estimated and reported. [2]

Material and Methods

Collection and Documentation of Plant herbs

The leaves of *Acacia catechu* (L.) Willd and *Acacia auriculiformis* A.Cunnwere collected in the months of July-December 2019 from the Southern region of India and identified & authenticated by Dr. Smruti Sohani, Professor, Faculty of Life Sciences, SAGE University, Indore (M.P.) and was deposited in our Laboratory. Voucher specimen no. SU/LS-ACL36 & SU/LS-AAL37 was allotted.

Successive Extraction of selected herbs

Sample were shattered and screened with 40 meshsize.The shade dried coarsely powdered plant material (300gms) was loaded in Soxhlet distillation assembly and was extracted with using various solvents namely,n-hexane, petroleum ether, benzene, chloroform, ethyl acetate, ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotary evaporator. The residues were then stored in desiccators and percentage yield were calculated. [3]

Preliminary phytochemical screening of extracts

The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of various phytochemical constituents present in the extracts.
The standard procedures were adopted to perform the study. [4-6]

Tests for carbohydrates

Molisch's test

To the Sample 2-3 drops of 1% alcoholic naphthol solution and 2 ml of conc. sulphuric acidwas added along the sides of the test tube. Appearance of purple to violet ring at the junction of two liquids shows the presence of carbohydrates.

Fehling test

To the sample add Fehling reagent, appearance of brick red precipitate shows presence of carbohydrates.

Test for glycosides

Legal's test

To the sample add 1 ml of pyridine and few drops of sodium nitroprusside solutions and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Borntrager's test

Sample was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, showing the presence of glycosides.

Baljet's test

To the sample add picric acid, orange color shows presence of glycosides.

Test for alkaloids

A small portion of the sample was stirred separately with few drops of dilute hydrochloric acid and was tested with various reagents for the presence of alkaloids. The reagents are

- Dragendroff's reagent- Reddish brown precipitates
- Wagner's reagent- Reddish brown precipitates
- Mayer's reagent- Cream color precipitates
- Hager's reagent- Yellow color precipitate

Test for proteins and free amino acids

Small quantities of the sample was dissolved in few ml of water and treated with following reagents.

- Million's reagent: Appearance of red color shows the Presence of protein and free amino acid.
- Ninhydrin reagent: Appearance of purple color shows the Presence of Proteins and free amino acids.
- Biuret's test: Equal volumes of 5% sodium hydroxide solution & 1% copper sulphate solution was added. Appearance of pink or purple color shows the presence of proteins and amino acids.

Test for tannins and phenolic compounds:

A small quantity of the sample was taken separately in water and test for the presence of phenol compounds and tannins was carried out with the following reagents.

- Dilute Ferric chloride solution (5%) - Blue color or green color
- 10% lead acetate solution - White precipitates

Test for flavonoids

Alkaline reagent test

To the test solution add few drops of magnesium hydroxide solution, intense yellow colour is formed which turns to colorless on addition of few drops of dilute acid indicates presence of flavonoids.

Shinoda's test

Small quantities of the sample was dissolved in alcohol, to them piece of magnesium followed by conc. hydrochloric acid drop wise added and heated. Appearance of pink, crimson red, green to blue color shows the presence of flavonoids**.**

Tests for fixed oils and fats

Spot test

A small quantity of sample was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

Saponification test

Few drops of 0.5 N alcoholic potassium hydroxide were added to a small quantity of sample along with a drop of phenolphthalein, the mixture was heated on a water bath for 1-2 hours, formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Tests for steroids and triterpenoids

Libermann-burchard test

Treat the sample with few drops of acetic anhydride, boil and cool. Then add con. Sulphuric acid from the side of test tube, brown ring is formed at the junction two layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoids.

Salkowski test

Treat the sample with few drop of conc. sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

Test for mucilage and gums

- Small quantities of sample was added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitates was dried in oil and examined for its swelling property for the presence of gum and mucilage.
- To the sample add ruthenium red solution, pink color shows presence of mucilage.

Test for waxes

To the test solution add alcoholic alkali solution, waxes get saponified.

Quantitative Estimation of Extract

The plant extract was estimated for flavonoids content, phenol content and terpenoids content as per method described. [4-6]

Total flavonoids content: Determination of total flavonoids content was based on aluminum chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol.10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Total phenol content: The total phenol content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol.2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for color development. The absorbance was measured at 765 nm using a spectrophotometer.

Total terpenoids content: The qualitative phytochemical analysis for the presence of terpenoids was determined by the methods described. 0.8 g of plant sample was taken in a test

tube and 10 ml of methanol was poured in it. The mixture was shaken well and filtered to take 5 ml extract of plant sample. Then 2 ml of chloroform was mixed in extract of selected plant sample and 3 ml of sulphuric acid was added in selected sample extract. Formation of reddish brown color indicates the presence of terpenoids in the selected plants. The previously prepared sample for qualitative analysis was transferred from assay tube to Colorimetric cuvette [95% (v/v) Methanol will be used as blank] to read the absorbance at 538 nm. For the standard curve 200µl of previously prepared Linalool solution in methanol will be added to 1.5 ml Chloroform & serial dilution must be done [dilution level-100mg/200µl to 1mg/200µl Linalool Conc.] In case of serial dilution total volume of 200µl will be made up by addition of 95% (v/v) Methanol. Alternatively, the total terpenoids content were determined by the method described. 100 mg of plant powder were taken and soaked in ethanol for 24 hour. The extract was filtered and the filtrate was extracted with petroleum ether using separating funnel. The ether extract was treated as total terpenoids.

Results and Discussion

The two*Acacia*species viz., *Acacia catechu* (L.) Willd and *Acacia auriculiformis* A.Cunn were chosen for the present investigation. Air dried coarsely powdered leaves of the plant were extracted with various solvent i.e., n-hexane, petroleum ether, benzene, chloroform, ethyl acetate, ethanol and water. The percentages of extract obtained were reported in table 1 for *Acacia catechu* (L.) Willd and table 2 for *Acacia auriculiformis* A.Cunn**.** In table 3 and 4 the active phytochemicals present in these extract were reported.

Table 1: % Yield estimation of leaf extracts of Acacia catechu (L.) Willd in various solvents.

S/No.	Solvent	Parameters			
		Nature of Extract	Color	pH	% Yield (w/w)
1.	n-Hexane	Semi solid	Light Green	6.9	1.12
2.	Pet.ether	Semi solid	Light Green	7.0	2.18
3.	Benzene	Semi Solid	Green	7.10	1.84
4.	Chloroform	Solid Powder	Green	7.04	3.42
5.	Ethyl acetate	Solid Powder	Dark Green	7.03	2.94
6.	Ethanol	Solid Powder	Blackish Green	7.11	6.84
7.	Water	Solid Powder	Blackish Green	7.02	12.72

Table 2: % Yield estimation of leaf extracts of *Acacia auriculiformis* A.Cunn in various solvents

S/No.	Solvent	Parameters			
		Nature of Extract	Color	pH	% Yield (w/w)
1.	n-Hexane	Semi solid	Green	7.0	1.89
2.	Pet.ether	Semi solid	Light Green	7.03	2.52
3.	Benzene	Semi Solid	Dark Green	7.05	3.17
4.	Chloroform	Solid Powder	Dark Green	7.02	3.55

5.	Ethyl acetate	Solid Powder	Blackish Green	7.01	4.12
6.	Ethanol	Solid Powder	Blackish Green	7.10	8.49
7.	Water	Solid Powder	Blackish Green	7.06	14.26

Table 3: Preliminary phytochemical screening of leaves extract of *Acacia catechu* (L.) Willd

Extract (As per solvent)	Qualitative Test									
	Carbohydrates	Glycosides	Alkaloids	Protein & Amino acid	Tannins & Phenolic compounds	Flavonoids	Fixed oil and Fats	Steroids & Triterpenoids	Waxes	Mucilage & Gums
n-Hexane	-	-	+	-	-	-	-	+	-	-
Pet.ether	-	-	+	-	+	+	-	+	-	-
Benzene	-	-		-	+	-	-	+	-	-
Chlorofor	-	+	+	-	+	-	-	+	-	-
Ethyl	-	+		-	+	+	+	+	-	-
Ethanol	-	+	+	+	+	+	+	+	+	+
Water	+	+	+	-	+	+	+	+	+	+

Table 4: Preliminary phytochemical screening of leaves extract of *Acacia auriculiformis* A.Cunn

Extract (As per solvent)	Qualitative Test									
	Carbohydrates	Glycosides	Alkaloids	Protein & Amino acid	Tannins & Phenolic compounds	Flavonoids	Fixed oil and Fats	Steroids & Triterpenoids	Waxes	Mucilage & Gums
n-H	-	-	-	-	-	-	-	+	-	-
Pet.ethe	+	-	+	-	-	+	-	+	-	-
Benzen	+	-		-	+	-	-	+	-	-
Chlorof	-	-	+	-	+	+	-	+	-	-
Ethyl	-	-		-	+	+	-	+	-	-
Ethanol	+	+	+	+	+	+	-	+	-	+
Water	+	+	+	+	+	+	-	-	+	+

The total flavonoids content, total phenol content and total terpenoids content in plant extract was determined and the results were mentioned in table 5.

Table No. 5: Estimation of total flavonoids, phenolics and terpenoids content in plantextract

S. No.	Extract	Total phenolic content (TPC) (mg/100mg of dried extract)	Total flavonoidcontent (TFC) (mg/ 100 mg of dried extract)	Total Terpenoid content (TTC) (mg/ 100 mg of dried extract)
1.	EEACL	1.813	1.911	1.145
2.	AEACL	2.112	2.345	1.932
3.	EEAAL	1.546	1.721	0.818
4.	AEAAL	1.903	2.217	1.245

Conclusion

Evaluation of quality control of medicinal plants is of great interest and importance to reveal the quality, safety and efficacy of medicinal plants. Ayurveda and traditional medicine systems treat diseases using these plants which have immense medicinal potential. But the lack of standardization parameters does not lead to accurate identification of the plant, so the development of quality control parameters is very interesting. Physico-chemical, extraction, preliminary phytochemical screening and quantitative estimation of phytoconstituents of selected plant materials were carried out and reported in this study.

References

1. Wickneswari R, Norwati M. Genetic Diversity of Natural-Populations of Acacia auriculiformis. Aust J Bot. 1993;41(1):65.
2. Kokate CK. "Practical Pharmacognosy.; 4th ed. Vallabh Prakashan : 2005.18, 112-121.
3. Khandelwal K.R., Practical Pharmacognosy, Thirteenth edition 2005, Nirali Prakashan, Pune, 149-156.
4. The Ayurvedic Pharmacopoeia of India. Part-I. Vol.-III. 1st ed. New Delhi: published by Gov. of India ministry of health and family welfare department of Homoeopathy. 1999; 225
5. Quality control method for medicinal plant material, 1st edition, published by World Health Organisation Geneva, Delhi; 2002.
6. Priyanka Karande, Qualitative and Quantitative Phytochemical Analysis of Acacia Catechu Willd
7. Lakshmi.T , Anitha Roy , Geetha R.V .The Pharma Research (T. Ph. Res.), (2011), 5(2); 273-293.
8. Rout S, Sahoo G, Mishra UN, Sheera A, Prusty AK. An Overview of Acacia catechu. Biot Res Today. 2021;3(8):691–3.