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Qualitative and Quantittaive Phytochemical Assessment of *Albizia Amara* Using Different Solvents

[1]Dr. P. Gowsalya,[2]Dr. R. Archana, [3] S.Saranya

[1]Associate professor,Dept.Of.Science and Humanities,Surya Engineering College,Erode.Tamilnadu.
[2] Assistant.Professor,Dept.Of.Biochemistry,Excel Siddha Medical College &Research Centre,Namakkal Dt.Tamilnadu.
[3] Dept.Of.Biomedical Engineering,Surya Engineering College,Erode.Tamilnadu.
E.mail.ID: gowsiguruvishnu@gmail.com

ABSTRACT

The present study focuses on the phytochemical analysis of *Albizia Amara* leaf extracts prepared using different solvents such as ethanol, methanol, acetone, and hexane. The objective was to identify and evaluate the major classes of bioactive compounds present in the plant. Qualitative screening revealed the presence of key phytoconstituents, including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds, with notable variation depending on solvent polarity.Quantitativeanalysis indicated that methanolic and ethanolic extracts contained higher concentration of phenolic and flavonoid compounds compared to non-polar extracts. One of the prominent components was further isolated and purified using column chromatography for characterization. The findings suggest that *Albizia Amara* is a rich source of secondary metabolites with potential applications in the development of the oral therapeutic agents and pharmaceuticals.

Keywords: *Albizia Amara*, phytoconstituents, plant extracts, column chromatography.

INTRODUCTION

Plants have been an integral part of traditional medicine for centuries and continue to serve as valuable sources of bioactive compounds doe modern drug discovery[26].Phytochemicals,the secondary metabolites produced by plants, play a crucial role in defense mechanisms against pathogens, environmental stress, and herbivory.These compounds

Including alkaloids, flavonoids, phenols, tannins, saponions, and terpenoids are known for their diverse pharmacological activities such as antioxidants, antimicrobial, anti-inflammatory, and anticancer effects[10]Systematic phytochemicals analysis is therefore essential to identify and characterize these bioactive constituents, which can contribute to the development of novel therapeutic agents.

Albizia Amara (Fabaceae).Commonly known as “Arappu”in traditional Indian medicine, is a deciduous tree widely distributed in tropical and subtropical regions of Asia and Africa. Various parts of the plant, including the bark, leaves, and pods, have been traditionally used to treat ailments such as fever, diarrhea, skin infections and inflammation[16].Previous studies have indicated that *Albizia Amara* contains several biologically active compounds, yet comprehensive phytochemical profiling of its leaf extracts using different solvents remains limited.

The present aims to perform a detailed phytochemical analysis of *Albizia Amara* leaf extracts prepared using solvents of varying polarity, including ethanol, methanol, acetone, and hexane. This comparative approach helps determine the influence of solvent polarity on the extraction effiencyand diversity of phytochemicals. Furthermore, major phytoconstituents were isolated and purified using column chromatography for potentials bioactivity evaluation. The findings are expected to provide a scientific basis for the traditional use of *Albizia Amara* and support its potential application in pharmaceutical and nutraceutical development. [1]

MATERIAL AND METHODS

COLLECTION OF PLANT SAMPLE:

Albizia Amara(leaves)were collected from in and around Erode district, TamilNadu,

India. The freshly collected leaves were washed with water and dried under shade at room

temperature. The dried leaves were powdered and it was stored in sterile containers for further use.


EXTRACTION OF PLANT MATERIAL:

AQUEOUS EXTRACTION:

10 gram of dried powder of*Albizia Amara* was suspended in 100ml cold distilled water and mixture was soaked for 24hours.The suspended solid was filtered through What man No.1 filters paper and kept in water bath at 80˚C for 2hours.The dried crude extracts were stored at 4˚C for further use [5].

SOLVENT EXTRACTION:

5 gram of dried powder of *Albizia Amara* suspended in 100ml of solvents (Ethanol, Methanol, Acetone, Hexane) and the mixture was soaked for 24hours.The suspended solid was filtered through What man No.1 filter paper and kept in water bath at 80˚C for 2hours.

The dried crude extracts were stored at 4˚C for further use.

QUALITATIVE PHYTOCHEMICAL ANALYSIS:

Preliminary phytochemical screening was carried out following standard procedures by [10,22].

ALKALOIDS: (WANGNER'S TEST):

Approximately 0.5g of each extract was diluted with 10ml of acid alcohol and heated to boiling; cooled and then filtered by what man filter paper then added 2ml of dilute ammonia and 5ml of chloroform. Shaken gently to extract the alkaloid base, the chloroform layer was extracted with 10ml of Acetic acid and 1ml of wangner'sreagent (1.27g of iodine and 2g of potassium iodide) was added to the resultant extract. Formation of cream or reddish brown precipitate was indicates the presence of Alkaloids[10].

FLAVONOIDS: (ALKALINE REAGENT TEST):

To 1 ml of test solution, 5 drops of 5% sodium hydroxide was added. An increase in the intensity of yellow colored solution is seen which becomes colorless on the addition of few drops of 2M hydrochloric acid.

PHENOLS: (FERRIC CLORIDE TEST):

To 2 ml of the diluted leaf extract added few drops of 10% aqueous ferric chloride solution and mixed well. Formation of blue or green or violet color indicates the presence of phenols.

TANNINS: (FERRIC CHLORIDE TEST):

To 2ml of test solution, few drops of 5% ferric chloride solution were added. The blue color indicates the presence of hydrolysable tannis, while the green color indicates the presence of condensed tannins.

SAPONINS: (FOAM TEST):

5ml of the test solution was taken in a graduated cylinder and shaken well for five minutes. Stable foam was formed.

TEST FOR CARBOHYDRATES:

The presence of carbohydrates in solvent extracts was determined by different methods such as, Fehling's test, Benedict's test, Molisch's test and iodine test.

BENEDICT'STEST:

To 5 ml of Benedict's qualitative reagent added 0.5ml of the leaf extract and mixed well. Boiled it for 5 minutes in a boiling water bath. Appearance of yellow, green, red, or reddish brown color precipitate showed that presence of reducing sugar.

GLYCOSIDES TEST: (KELLERKILLIANI TEST):

To 2ml of the diluted leaf extract added 1 ml of aqueous sodium hydroxide solution. Formation of yellow color shows the presence of glycosides.

STEROIDS :(SALKOWSKI'S TEST):

To 2 ml of the leaf extract (prepared in chloroform) added 2 ml of concentrated sulphuric acid drop by drop along the sides of the test tube. Red color formed in the chloroform layer, shows the presence of steroids.

TERPENOIDS:

5 ml of each plant extract was mixed with 2ml of chloroform and 3ml of concentrated sulfuric acid (98%).Formation of a reddish brown colored ring between the interfaces indicated the presence of terpenoids.

PROTEINS :(NINHYDRIN TEST):

Few drops of Ninydrin reagent and 1ml of extract were added. Appearance of blue color shows the presence of protein.

TRITERPENOIDS: (SALKOWSKI TEST):

Approximately 2mg of dry extract was shaken with 1ml of chloroform and few drops of concentrated sulfuric acid were added along the sides of the test tube. Formation of red brown color at the interface indicates the presence of triterpenoids.

ANTHOCYANIN:

2 ml of aqueous extract was added to 2 ml of 2N HCl and NH3. The appearance of pink red turns into blue violet indicates presence of Anthocyanin.

PHYTOSTEROLS: (LIEBERMANN- BURCHARDS TEST):

The extract was (2mg) dissolved in 2ml of acetic anhydride and heated to boiling, cooled and 1 ml of concentrated sulfuric acid was added along the sides of the test tube. A brown ring was formed at the junction and the upper layer turned to dark green color indicates the presence of phytosterols.

ANTHRAQUINONE GLYCOSIDES: (HYDROXYANTHRAQUINONETEST):

To 1ml of extract, few drops of 10% potassium hydroxide solution were added. The appearance of red color confirms the test.

PHYTOSTEROLS: (LIEBERMANN- BURCHARDS TEST):

The extract was (2mg) dissolved in 2ml of acetic anhydride and heated to boiling, cooled and 1 ml of concentrated sulfuric acid was added along the sides of the test tube. A brown ring was formed at the junction and the upper layer turned to dark green color indicates the presence of phytosterols.

ANTHRAQUINONE GLYCOSIDES: (HYDROXYANTHRAQUINONETEST):

To 1ml of extract, few drops of 10% potassium hydroxide solution were added. The appearance of red color confirms the test.

QUANTITATIVE ESTIMATION:

ESTIMATION OF ALKALOIDS:

5 g of the sample was weighted into a 250ml beaker and 200ml of 10% acetic acidic ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentration a water bath to one quarter of the original volume concentrated ammonium hydroxide was added drop wise to the extract until the preparation was complete. The whole solution was allowed to settle down and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloids which was dried and weighed **(10).**

ESTIMATION OF FLAVONOID:

10g of the plant sample was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered using What man filter paperNo.42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight **.**

RESULT AND DISCUSSION:

QUALITATIVE PHYTOCHEMICAL ANALYSIS:

The preliminary phytochemical screening of the leaves was done to appraise the presence of bioactive components. They were summarized in (Table 1, Plate 1). From the results we came to know that the leaf extract of *Albizia Amara* showed positive test for Alkaloids, Flavanoids, Phenolics, Tannins, Saponins, Terpenoids, Protein, Triterpenoids, Phytosterols. Anthraquinones was not detected. Anthocyanins give positive result in methanol extract only. These results showed that the plant had a number of chemical constituents, which may be responsible for the many pharmacological actions. Some authors stated that the plants are that phytocompounds disturb cell wall or increase permeability of the cytoplasmic membrane and thereby facilitate the influx of antibiotics, inhibit penicillin binding protein or produce efflux pump inhibitor [24,23,27],.Many recent reports revealed that many phytochemicals have become important molecules in various drug and/or pesticide discovery program [17,20]reported that rediscovery of the connection between plants and health is responsible for launching new generation of botanical therapeutically, multi component botanical drugs, dietary

supplements and functional foods. Photochemical are otherwise known as primary antioxidants and has ability to protect the body from damage caused by free radicals inducing oxidative stress[18].

[13] revealed that Preliminary phytochemical screening of 50% ethanolic leaf extract *Albizia Amara* depicted in the presence of flavanoids, tannins, terpenoids, phenols and saponins. Many active components especially polyphenols such as flavanoids, phenol acids tannins possess therapeutic Effects[2]. Phenols and flavanoids are the major secondary constituents present in plants with extensive phyto pharmacological activity. Flavanoids are polyphenolic compounds and are antioxidants; they play a role in defense mechanism against reactive oxygen species beneficial to living beings [9]suggested that chloroform leaf extract containing alkaloids and steroids showed a higher antibacterial and antifungal activity. Tannin is one of the major active ingredients found in plant based medicines [11]it serves as caustics for cationic dyes (tannin dyes) used in the dyestuff industry and also in the manufacture of inks (iron Gallatin)

TABLE: 1

QUALITATIVE PHYTOCHEMICAL ANALYSIS

S.NO	PARAMETERS	ETHANOL EXTRACT	METHANOL EXTRACT	ACETONE EXTRACT	HEXANE EXTRACT
1	ALKALOIDS	+	+	+	-
2	FLAVONOIDS	-	+	+	+
3	PHENOLS	+	+	+	-
4	TANNINS	+	+	+	+
5	SAPONINS	+	+	+	-
6	CARBOHYDRATES	+	+	+	+
7	GLYCOSIDES	-	+	+	+
8	STEROIDS	+	-	+	+
9	TERPENOIDS	+	+	+	-
10	PROTEINS	-	-	-	+
11	TRITERPENOIDS	+	+	+	-
12	ANTHOCYANIN	-	+	-	-
13	PHYTOSTEROLS	+	+	+	+
14	ANTHRAQUINONE GLYCOSIDES	-	-	-	-

PLATE:1 QUALITATIVE PYTOCHEMICAL ANALYSIS

The amount of phytochemicals which are found in the *Albizia Amara* extract was quantitatively determined by standard procedure. (Table 2 Plate 2). The amount of alkaloids content in the plant was 6.4% (64mg/g) and flavonoid content in the plant was 2% (20mg/g). Total protein content was determined by using standard method. The total protein content in *Albizia Amara* was 7700 µg/ml. The carbohydrate content was detected by standard method. The plant showed 35mg/ml of carbohydrate content. The loss on drying value of *Albizia Amara* powder was 0.81%.The ash value of plant powder was found to be 4.18%.According to [25]most phytochemicals used as natural antibiotics, which help the body in fighting microbial invasion and infections. Alkaloids have a wide range of pharmacological activities including antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine), antibacterial (e.g., chelerythrine) [7]and antihyperglycemic activities (e.g., piperine). Alkaloids have been equally exploited for their importance in traditional pharmaceutical usage. Other alkaloids possess psychotropic(e.g., psilocin) and stimulant activities (e.g., cocaine, caffeine, and nicotine) and have been used as recreational drugs.Although alkaloids carry out many metabolic activities in humans and other animals, they uniformly evoke a bitter taste [21]Flavonoids are known to have antioxidant effects and have been shown to inhibit the initiation, promotion, and progression of tumors [14]reduction of coronary heart disease has been reported to be associated with intake of flavonoid.

Apart from the antioxidant properties of flavonoid, other biological functions it possesses include protection against platelet aggregation, microorganisms, hepatotoxins, viruses, tumors, ulcers, free radicals, inflammation, and allergies[4].Ash value is useful in determining and purity of sample and also these values are important qualitative standards[3]Stability of natural products is greatly dependent on moisture contents. Less moisture

content is needed for prevention of chemical decomposition and microbial contamination in the natural products. Moisture content is estimated by calculating the loss of weight (of crude powder) on drying in oven. Moisture content of drugs could be at minimal level to avoid microbial growth during storage.[12]

The quality and purity of powdered crude drugs are determined by estimating ash value. All the organic matter traces are removed during ash test; their presence may interfere in analysis. Crude drug on incineration normally leaves an ash consisting of carbonates, silicates and phosphates of sodium, calcium, potassium and magnesium. The total ash of a crude drug determines that how much care is required in its preparation. Acid insolu*b*le ash test is performed if the crude drug has silica or calcium oxalate. Sulphated ash is less fusible than the ordinary ash that's why some analysts add sulphuric acid with powdered crude drug prior to performing ash test[8].

PLATE : 1 QUANTITATIVE PHYTOCHEMICAL ANALYSIS:

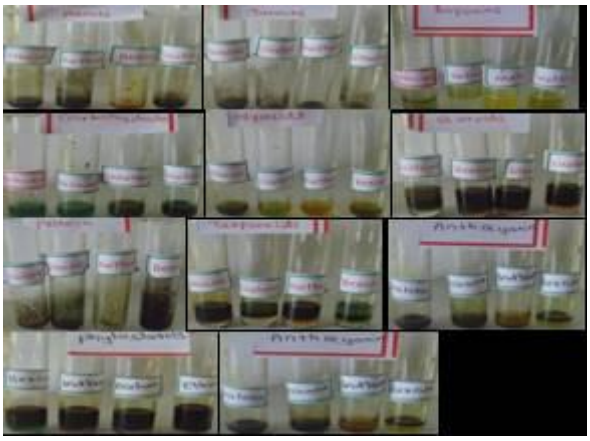

TABLE: 2

QUANTITATIVE PHYTOCHEMICAL COMPOSITION OF *ALBIZIA AMARA*

S.NO	PARAMETERS	PERCENTAGE%
1	ALKALOIDS	6.4%
2	FLAVANOIDS	2.0%
3	ASH	4.18%
4	MOISTURE	0.18%
5	PROTEIN	7700µg/ml
6	CARBOHYRATE	35mg/ml

PLATE : 2QUANTITATIVE ANALYSIS OF PHYTOCHEMICAL

CONCLUSION

The present study demonstrates that *Albizia Amara* leaves are a rich source of bioactive phytochemicals; particularly phenolics, flavonoids, tannins, alkaloids, saponins, and terpenoids.The type and polarity of the extraction solvent significantly influenced both the yield and diversity of phytoconstituents, with methanol and ethanol extracts exhibiting the highest concentrations of bioactive compounds. The isolation of major constituents via column chromatography provides a foundation for further characterization and bioactivity studies. Overall, the findings validate the traditional medicinal use of *Albizia Amara* and highlight its potential asa source of natural therapeutic into its pharmacological applications.

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