Phytochemical Analysis of Albizia procera
7. PHYTOCHEMICAL ANALYSIS OF ALBIZIA PROCERA

7.1 PHYTOCHEMICAL SCREENING AND METABOLITE ANALYSIS OF ALBIZIA PROCERA

7.1.1 Introduction

Plants contain a wide variety of chemical compounds broadly classified as primary metabolites, secondary metabolites and semantides. Phytochemical characterization of plant material is important and it relates to the therapeutic actions. Natural products are believed to be an important source of new chemical substances with potential therapeutic applicability. Therefore phytochemical evaluation of plant is essential to find out the relationship between the biological activity and the chemical structure of the biologically active phytochemicals. Different chemical tests were performed for establishing profile of the extract for its chemical composition, the following chemical tests for various phytoconstituents in the petroleum ether, chloroform, ethyl acetate, ethanol and hydro alcoholic extracts were carried out as described below (Harborne, 1974). The extracts were hydrolysed with dil. HCl, the following tests were performed.

7.1.2 Materials and Methods

7.1.2.1 Chemicals used

Analytical grade chemicals were obtained from Loba, Hi-media, S.D. Fine chemicals, E.Merck, Qualigens and Sigma chemicals (USA).

7.1.2.2 Preparation of extracts

Shade dried and coarsely powdered leaf and bark of Albizia procera (2.5kg) was subjected to successive extraction in solvent of increasing polarity
Phytochemical Analysis of Albizia procera

(Petroleum ether, Chloroform, Ethyl acetate, Ethanol and Hydro alcohol) by using cold maceration technique for 72, 48 and followed by 24 hours. Solvents were filtered, distilled and dried in a vacuum desiccator to obtain the following extracts.

(i) Petroleum ether extract of leaf and bark of Albizia procera (PELE, PEBE).
(ii) Chloroform extract of leaf and bark of Albizia procera (CLLE, CLBE).
(iii) Ethyl acetate extract of leaf and bark of Albizia procera (EALE, EABE).
(iv) Ethanolic extract of leaf and bark of Albizia procera (ETLE, ETBE).
(v) Hydro alcoholic extract of leaf and bark of Albizia procera (HALE, HABE).

7.1.2.3 Chemical analysis

The chemical profiling of a herbal drug reflects its chemical nature and the quantity the active principles that are responsible for its therapeutic activity.

7.1.2.3.1 Qualitative phytochemical evaluation

Different chemical tests were performed for establishing profile of the extract for its chemical composition, the following chemical tests for various phytoconstituents in the petroleum ether, chloroform, ethyl acetate, ethanol and hydro alcoholic extracts were carried out as described below (Harborne, 1974).

The extracts were hydrolysed with dil. HCl, the following tests were performed.

a) Test for Alkaloids

1. Dragendroff’s Test: In a test tube containing 1ml of extract, few drops of Dragendroff’s reagent was added and the color developed was noticed. Appearance of orange color indicates the presence of alkaloids.

2. Wagner's Test: To the extract, 2ml of Wagner's reagent was added. The formation of a reddish brown precipitate indicates the presence of alkaloids.
3. Mayer's Test: To 2ml of extract, 2ml of Mayer's reagent was added. A dull white precipitate revealed the presence of alkaloids.

4. Hager's Test: To the 1ml of extract, 3ml of Hager's reagent was added. The formation of yellow precipitate confirmed the presence of alkaloids.

b) Test for Steroids

Libermann Burchard Test: To 1ml of extract, 1ml of glacial acetic acid and 1ml of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution becomes red, then blue and finally bluish green, indicating the presence of steroids.

c) Test for Coumarins

To 1ml of extract, 1ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

d) Test for Tannins

1. To 5ml of extract, ferric chloride was added, formation of dark blue or greenish black color showed the presence of tannins.

2. To 5ml of extract, potassium dichromate solution was added, formation of a precipitate showed the presence of tannins and phenolic compounds.

3. The extract was mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of tannins.

e) Test for Saponins

To 1ml of the extract, 5ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.
f) **Test for Flavonoids**

1. Shinoda Test: To the extract, a few magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added, formation of red color shows the presence of flavones.

2. Zinc-Hydrochloric Acid Reduction Test: To few mg of powder zinc dust and concentrated hydrochloric acid was added. Formation of magenta color showed the presence of flavonoids.

3. Ferric Chloride Test: To few mg of the powder, a small quantity of ethanolic solution and few drops of neutral ferric chloride were added. Blackish red color indicated the presence of flavonoids.

4. Alkaline Reagent Test: To few mg of the powder, a few drops of dilute sodium hydroxide were added. An intense yellow color, which became colorless on addition of a few drops of dilute acid, indicated the presence of flavonoids.

g) **Test for Phenols**

To 1ml of the extract, 2ml of distilled water was added followed by few drops of 10% aqueous ferric chloride. Appearance of blue or green color indicates the presence of phenols.

h) **Test for Proteins**

1. Biuret Test: To few mg of powder, 1ml of forty percent sodium hydroxide solution and two drops of 1% copper sulphate solution were added. Formation of violet color indicated the presence of proteins.

2. Xanthoprotein Test: To few mg of powder, 1ml of concentrated nitric acid was added. As a white precipitate was formed, it is boiled and cooled.
Then, 20% of sodium hydroxide or ammonia was added. Orange color indicated the presence of aromatic amino acids.

3. Tannic Acid Test: To the powder, 10% tannic acid was added. Formation of white precipitate indicated the presence of proteins.

i) Test for Carbohydrates

1. Molish’s Test: To the powder, 1ml of α-naphthol solution and concentrated sulphuric acid through the sides of the test tube were added. Purple or reddish violet color ring at the junction of the two liquids revealed the presence of carbohydrates.

2. Fehling’s Test: To the powder equal quantities of Fehling’s solution A and B were added and on heating, formation of a brick red precipitate indicated the presence of carbohydrates.

3. Benedict’s Test: To the 5ml of Benedict’s reagent, few mg of powder was added and boiled for two minutes and cooled. Formation of a red precipitate showed the presence of carbohydrates.

j) Test for Gum

The extract mixed with water leads to the thickening of the substance, indicates the presence of gum.

k) Test for Fixed oil

A small quantity of powder/ extract was pressed between the filter paper. Formation of grease spot indicates the presence of fixed oils and fats.
7.1.2.3.2 Quantitative evaluation of primary metabolites

This involves the quantification of carbohydrates, proteins in ETBE and ETLE of Albizia procera using standard methods.

**Estimation of Carbohydrate** (Morris et al., 1948)

**Principle**

The Carbohydrates present in the extract were reduced by anthrone reagent and the formation of green color was estimated at 630nm, which is the basis of determination of the total carbohydrate content of plant extract.

**Requirements**

- **Anthrone reagent** - This was prepared by dissolving 0.2gm of anthrone in 100ml of sulphuric acid, made by adding 500ml of concentrated acid to 200ml of distilled water. It was allowed to stand for 30-40 minutes with occasional shaking until the solution was perfectly clear.

- **Stock glucose solution** - 10mg of glucose was weighed accurately and dissolved in distilled water and the volume was made up to 10ml in standard flask (concentration 1mg/ml).
- **Working standard solution** - 1ml of Stock Solution was diluted to 10ml with distilled water in a standard flask (concentration 100µg/ml).

- **Preparation of sample** - About 100mg of the extract was hydrolyzed by boiling with 100ml of 2.5N HCl for 3hrs and then cooled to room temperature. This mixture was then neutralized using solid sodium carbonate until the effervescence ceased and its volume was made up to 100ml. This was centrifuged at 3000rpm for 15minutes. The supernatant was decanted off and used for estimation.

**Procedure**

From the working standard solution 0.2, 0.4, 0.6, 0.8 and 1ml of solutions were pipetted out into a series of test tubes. 1ml of supernatant of sample extract was pipetted out in triplicates. The volume in all test tubes was made up to 1ml with distilled water. A test tube with 1ml of water was used as the blank. 4ml of anthrone reagent was added to each test tube including the blank. It was heated for 8 minutes in a water bath and cooled. The absorbance of the developed green color was read at 630nm. A standard graph of glucose was plotted from which the carbohydrate content of the extract was determined in terms of glucose equivalents.

**Estimation of Total protein** (Lowry et al., 1953)

**Principle**

The aromatic amino acids present in proteins react with Folin-ciocalteu reagent, which contains phosphomolybdic acid and tungstate to produce a blue
colored complex which absorbs maximally at 620nm, which is the basis of
determination of the total protein content of plant extract.

Requirements

- **Alkaline copper sulphate solution** - 50ml of 2% w/v sodium carbonate in
  0.1N sodium hydroxide solution and 1ml of 0.5% copper Sulphate in 1%
  w/v potassium sodium tartrate were mixed prior to its use.

- **Folin-ciocalteu reagent** - The reaction was mixed with distilled water (1:2).

- **Stock protein solution** - 50mg of bovine serum albumin was weighed
  accurately and dissolved in distilled water and made up to 50ml in a
  standard flask.

- **Working standard solution** - 1ml of the stock solution was diluted to
  10ml with distilled water in a standard flask (concentration 100µg/ml).

- **Preparation of sample** - About 100mg of the extract was hydrolyzed by
  boiling with 100ml of 2.5N HCl for 3hrs and then cooled to room
  temperature. This mixture was then neutralized using solid sodium
  carbonate until the effervescence ceased and its volume was made up to
  100ml. This was centrifuged at 3000rpm for 15minutes. The supernatant
  was decanted off and used for estimation.

Procedure

From the working standard Solution 0.2, 0.4, 0.6, 0.8 and 1ml of solutions
were pipetted into a series of test tubes. 1ml of supernatant of sample extract was
pipetted out in triplicates. The volume in all test tubes was made up to 1ml with
distilled water. A test tube with 1ml of distilled water was used as the blank. 5ml
of the alkaline copper sulphate solution was added to each test tube including the
blank. It was mixed well and allowed to stand for 10 minutes. To this 0.5ml of prepared solution, Folin-ciocalteu reagent was added and mixed well and incubated at room temperature in the dark for 30 minutes. Absorbance of developed blue color was read at 660nm. A standard graph of protein was plotted from which the protein content of extract was determined in terms of bovine serum albumin equivalents.

7.1.2.3.3 Secondary metabolites

Secondary metabolites like phenols, tannins content of ETBE and ETLE of Albizia procera was quantified by the following methods.

Estimation of total phenols (McDonald et al., 2001)

Principle

The Phenolic hydroxyl groups present in the phenolic compound of the plant reacts with Folin’s phenol reagent, to produce a blue color, which absorbs maximally at 765nm, which is the basis of determination of the total phenol content of plant extract.

Requirements

- **Folin’s phenol reagent** - The reaction was mixed with distilled water (1:2).
- **Sodium carbonate (7.5% w/v)** - 7.5gm of the accurately weighed sodium carbonate was dissolved in distilled water and volume was made up to 100ml in a standard flask.
- **Stock gallic acid solution** - Accurately weighed 10mg of gallic acid was dissolved in Methanol and made up to 10ml in a standard flask (concentration:1mg/ml)
- **Working standard solution** - 1ml of the stock solution was diluted to 10ml with methanol in a standard flask. 1ml of this solution contains 100µg of gallic acid.

- **Preparation of test extract** - 10mg of extract was weighed and dissolved in 10ml of methanol water (70:30). From this 1ml was used for estimation.

**Procedure**

From the working standard solution 0.2, 0.4, 0.6, 0.8 and 1ml of solutions were pipetted out into a series of test tubes. 1ml of sample extract with duplicates was pipetted out in two other test tubes. The volume in all the test tubes was made up to 1ml with methanol. 1ml of distilled water was used as blank. To all the test tubes 5ml of Folin’s phenol reagent (1:2) followed by 4ml of 7.5% sodium carbonate were added and kept at room temperature for 1.5hr with occasional shaking. A blue color was formed and its color intensity was read at 765nm. A standard graph of gallic acid was plotted, from which the total phenol content of the extract was determined in terms of gallic acid equivalents.

**Estimation of Tannins** (Giner-Chavez, 1996)

**Principle**

The polyhydroxyl phenolic compounds of tannins present in plant extract reacts with Folin’s phenol reagent to produce a blue color, which absorbs maximally at 640nm which is the basis of determination of the total tannin content of plant extract.
Requirements

- **Folin’s phenol reagent (1:2)** - The reagent was mixed with distilled water (1:2).

- **Sodium carbonate (35% w/v)** - A supersaturated solution of sodium carbonate was prepared by dissolving accurately weighed 35gm of Sodium Carbonate in distilled water and the volume was made up to 100ml with distilled water in a standard flask.

- **Stock tannic acid solution** - Accurately weighed 10mg of Tannic Acid was dissolved in distilled water and made up to 10ml in a standard flask (concentration:1mg/ml).

- **Working standard solution** - 1ml of the stock solution was diluted to 10ml with distilled water in a standard flask. 1ml of this solution contains 100µg of Tannic Acid.

- **Preparation of extract** - 10mg of extract was weighed and dissolved in 10ml of Methanol Water (70:30). From this 1ml was used for estimation.

Procedure

From the working standard solution 0.2, 0.4, 0.6, 0.8 and1 ml of solutions were pipetted out into a series of test tubes. 1ml of sample extract with duplicates was pipetted out in two other test tubes. The volume in all the test tubes was made up to 1ml with distilled water. 1ml of distilled water was used as blank. To all the test tubes 0.5ml of Folin’s phenol reagent (1:2) followed by 3ml of 35% sodium carbonate solution was added and kept at room temperature for 5 minutes. A blue color was formed and its color intensity was read at 640nm. A standard graph of tannic acid was plotted, from which the total tannin content of extract was determined in terms of tannic acid equivalents.
7.1.3 Results and Discussion

7.1.3.1 Preliminary phytochemical screening

The preliminary phytochemical studies of the leaf and bark of *Albizia procera* have revealed the presence of phytoconstituents such as tannins, alkaloids, steroids, carbohydrates, saponin, flavonoids and phenols which was shown in Table 7.1.

7.1.3.2 Metabolite analysis

Primary metabolite and secondary metabolite values were tabulated. Tannins in vascular plants occur as two types, condensed or hydrolysable tannins. Tannins are powerful antioxidants often characterized by reducing power and free radical scavenging activities (Zang and Lin, 2008). The preliminary phytochemical studies of the bark and leaf parts of *Albizia procera* have revealed the presence of phytoconstituents such as alkaloid, steroid, carbohydrates, tannins, flavonoids, phenols and significant content of carbohydrate and tannins in the plant. The observation was tabulated and result was represented in the Table 7.2.
Table 7.1 Preliminary phytochemical screening of leaf and bark of Albizia procera

<table>
<thead>
<tr>
<th>S.No</th>
<th>Reagent</th>
<th>PELE</th>
<th>PEBE</th>
<th>CLLE</th>
<th>CLBE</th>
<th>EALE</th>
<th>EABE</th>
<th>ETLE</th>
<th>ETBE</th>
<th>HALE</th>
<th>HABE</th>
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<td>+</td>
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<td>12.</td>
<td>Fixed oil</td>
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PELE - Petroleum ether leaf extract; PEBE - Petroleum ether bark extract; CLLE - Chloroform leaf extract; CLBE - Chloroform bark extract; EALE - Ethyl acetate leaf extract; EABE - Ethyl acetate bark extract; ETLE - Ethanol leaf extract; ETBE - Ethanol bark extract; HALE - Hydro alcoholic leaf extract; HABE - Hydro alcoholic bark extract; + → Present; - → Absent.
Table 7.2 Metabolite analysis of leaf and bark of Albizia procera

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Metabolite</th>
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<th>Bark mg% (w/w)</th>
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<td>12.3</td>
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<td>Total Protein</td>
<td>8.5</td>
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<td>Tannins</td>
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7.2 QUANTIFICATION OF BIOCHANIN BY HPTLC FROM ETBE OF ALBIZIA PROCERA (Sethi., 1996)

7.2.1 Introduction

Densitometric HPTLC has been widely used for the phytochemical evaluation of the herbal drugs, due to its simplicity and minimum sample clean up requirement. Hence, a densitometric HPTLC method has been developed in the present work for quantification of phytocompounds. HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials thus serving as a reference standard for the quality control of the extracts. It allows the analysis of a large number of compounds both efficiently and cost effectively.

7.2.2 Materials and Methods

7.2.2.1 Preparation of biochanin A standard solution

A stock solution of standard Biochanin A (10mg/10ml) was prepared by transferring 10mg of Biochanin A, accurately weighed, into a 10ml volumetric flask, dissolving in 10ml acetone. From this working solution was prepared by diluting the stock 10 times using acetone. 2–10µl of the standard solution was spotted on the plate with the concentration ranging from 200ng – 1000ng.
7.2.2.2 Preparation of ETBE of *Albizia procera*

Accurately weighed 50mg dried ETBE of *Albizia procera* was dissolved in 1ml of 70% ethanol. It was then sonicated for 10 minutes and the contents of the flask were filtered through Whatman No.1 paper (Merck, Mumbai, India). 2–10µl of the prepared extract solution was spotted on the plate with the concentration ranging from 100µg – 500µg.

7.2.2.3 Chromatographic condition

- **Sample**: ETBE of *Albizia procera*
- **Standard**: Biochanin A
- **Stationary phase**: Silica gel GF254
- **Mobile phase**: Chloroform:acetone: Formic acid (7.5:1.65:0.85)
- **Scanning wavelength**: 265nm
- **Sample concentration**: Sample (50mg/ml); Standard (100µg/ml)
- **Applied volume**: Track 1 – Standard Biochanin (10µl)
  
  Track 2 – ETBE of *Albizia procera* (10µl)

- **Development mode**: Ascending mode

7.2.2.4 Preparation of stock solution

Chromatography was performed on 10cm x 10cm aluminium foil plates coated with 0.2 mm thickness silica gel 60F$_{254}$ HPTLC layers (E.Merck, Darmstadt, Germany). The standard and samples were applied on the plates as 6mm wide bands. The space between two spots was made 6mm (10 mm from the bottom and 10 mm from the sides) by means of an automated TLC sample applicator under a flow of nitrogen gas providing delivery speed 150µl/s from the syringe using Camag Linomat 5 automated TLC applicator (ATS 5) (Camag,
7.2.3 Results and Discussion

7.2.3.1 Detection and quantification

After the sample application was completed, the plate was developed in a Camag Twin through glass tank presaturated with a mobile phase of Chloroform: acetone: Formic acid (7.5:1.65:0.85) for 15min. The TLC runs were performed under laboratory conditions of 25±2°C and 60% relative humidity. After development, the plates were removed from the chamber, dried in air for 5 min and spots were visualized under UV light using a Camag UV viewer cabinet. The densitometric scanning at 265nm was performed with a Camag TLC Scanner-3 operated by win CATS software1.4.1. The slit width was 6mm x 0.45mm and wave length at $\lambda_{\text{max}}$ 265nm. Evaluation was based on the peak area with linear regression. The Rf of 0.82 and 0.89 showed the presence of biochanin A in the standard and samples. The identification of biochanin A was confirmed by superimposing the UV spectra of samples and standards within the same Rf window. The percentage of biochanin A present in *Albizia procera* extract was found to be 0.57%w/w.
Figure 7.1 HPTLC chromatogram of ETBE of *Albizia procera*
Figure 7.2 HPTLC chromatogram of standard biochanin A

Figure 7.3 HPTLC chromatogram of standard calibration curve
Table 7.3 HPTLC chromatogram of standard biochanin A

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<tr>
<th>Track</th>
<th>Peak</th>
<th>Start Rf</th>
<th>Start Height</th>
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<th>Height %</th>
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<td>0.6</td>
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7.3 ISOLATION OF DOCOSANOIC ACID BY COLUMN CHROMATOGRAPHY

7.3.1 Introduction

The Pharmacological action of any plant is due to the presence of phyto constituents. Isolation and characterization of the phyto constituents help us to identify the lead molecule responsible for the therapeutic potential of the plant extracts. Hence the isolation and characterization of bioactive constituents was carried out.

Chromatography is the separation technique of a mixture into individual components using a stationary phase and mobile phase. Column chromatography is a method used to purify individual chemical compounds from mixture of compounds. The principle of separation is by adsorption. “When a mixture of components dissolved in the stationary phase is introduced into the column, the individual components move with different rates depending upon their relative affinities”.

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7.3.2 Materials and Methods

7.3.2.1 Isolation of compound

17.048g of ETBE was mixed with 36.015g of silica gel (60-120 mesh) to make an admixture. About 2.4 diameter column was packed with 81.684g of silica gel and 53.04g of admixture with hexane. The column was eluted with increasing solvent polarity from hexane to ethyl acetate. Analytical TLC was performed on precoated plastic sheets of silica gelG/UV-254 of 0.2mm thickness. The spot was detected in Iodine Chamber (Macherey-Nagar, Germany).

7.3.2.1.1 General information & results for UV

Instrument : Techcomp UV-Vis Spectrophotometer (Model 8500)
Solvent : Methanol
Mode : Absorbance
Wavelength : Full Scanning (190nm to 1100nm)
Sample Dilution : 10mg of sample in 10ml methanol
Sample absorbance @223nm : 3.1938
Table 7.4 Isolation of phytoconstituents from ETBE of Albizia procera

<table>
<thead>
<tr>
<th>S.No</th>
<th>Number of Fractions</th>
<th>% of Eluent Solvent</th>
<th>Volume of Solvent (ml)</th>
<th>TLC Mobile Phase (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-6</td>
<td>100% Hexane</td>
<td>300</td>
<td>EtoAc :Hexane (2:8)</td>
</tr>
<tr>
<td>2</td>
<td>7-18</td>
<td>15% EtoAc : 85% Hexane</td>
<td>600</td>
<td>EtoAc :Hexane (3:7)</td>
</tr>
<tr>
<td>3</td>
<td>19-23</td>
<td>25% EtoAc : 75% Hexane</td>
<td>250</td>
<td>EtoAc :Hexane (4:6)</td>
</tr>
<tr>
<td>4</td>
<td>24-57</td>
<td>35% EtoAc : 65% Hexane</td>
<td>1700</td>
<td>EtoAc :Hexane (5:5)</td>
</tr>
<tr>
<td>5</td>
<td>58-87</td>
<td>40% EtoAc : 60% Hexane</td>
<td>1500</td>
<td>EtoAc :Hexane (5:5)</td>
</tr>
<tr>
<td>6</td>
<td>88-103</td>
<td>50% EtoAc : 50% Hexane</td>
<td>800</td>
<td>EtoAc :Hexane (5:5)</td>
</tr>
<tr>
<td>7</td>
<td>104-125</td>
<td>60% EtoAc : 40% Hexane</td>
<td>500</td>
<td>EtoAc :Hexane (7:3)</td>
</tr>
<tr>
<td>8</td>
<td>126-150</td>
<td>65% EtoAc : 35% Hexane</td>
<td>600</td>
<td>EtoAc :Hexane (7:3)</td>
</tr>
<tr>
<td>9</td>
<td>151-175</td>
<td>70% EtoAc : 30% Hexane</td>
<td>1000</td>
<td>EtoAc :Hexane (9:1)</td>
</tr>
<tr>
<td>10</td>
<td>176-200</td>
<td>75% EtoAc : 25% Hexane</td>
<td>1200</td>
<td>EtoAc :Hexane (9:1)</td>
</tr>
</tbody>
</table>

7.3.2.1.2 Column details:

Column diameter : 2.4 cm

Column bed height : 20 cm

Column sample height : 12.5 cm

7.3.2.1.3 Packing of column

The column was prepared by wet packing method. In this the slurry was prepared with the eluent mixed to the stationary phase powder and then carefully poured into the column. Care must be taken to avoid air bubbles. The extract was loaded in the column and it was eluted with mixture of solvents of increasing polarity (hexane, chloroform, ethyl acetate, and methanol).
7.3.2.1.4 Purification

The methods used in purification were divided into analytical and preparative methods. Analytical methods aim to detect and identify the compound in a mixture, whereas preparative methods aim to produce large quantities of the compound for purposes like structural biology and industrial use.

7.3.2.1.5 Characterization

The compound obtained from column was first subjected to chemical test, TLC and spectroscopic studies

7.3.3 Results and Discussion

Carboxylic acids occur in many molecular forms, and the majority of the fatty acids found in lipids are monocarboxylic acids. In chemistry a fatty acid is a carboxylic acid with a long aliphatic tail (chain), which was either saturated or unsaturated. Most naturally occurring fatty acids had a chain of an even number of carbon atoms, from 4 to 28. Fatty acids are usually derived from triglycerides or phospholipids. When they are not attached to other molecules, they are known as "free" fatty acids. Fatty acids are important sources of fuel because, when metabolized, they yield large quantities of ATP. Many cell types can use either glucose or fatty acids for this purpose (Wolk et al., 2001).

In particular, heart and skeletal muscle prefer fatty acids. Despite long-standing assertions to the contrary, the brain can use fatty acids as a source of fuel in addition to glucose and ketone bodies. Polyunsaturated fatty acids are essential for correct development of young organisms and for maintenance of good health by humans. Some of the essential fatty acids are Dihomo-γ-linolenic acid,
Phytochemical Analysis of Albizia procera

Arachidonic acid, α-Linolenic acid, Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA).

Fatty acids have a significant influence on the regulation of the activities of the cardiovascular system and oxygenation of tissues, and they have antiarrhythmic effect (reducing the risk of arrhythmia). They control the regulation of blood pressure, balance in blood coagulation and de-coagulation, and vascular stability. They regulate the contents of lipoproteins, in particular HDL, triglycerides, and specific proteins of lipoproteins.

They influence the adaptation of the body immunity to inflammatory processes, proliferation (regeneration and reproduction) of cells, activities of hormones and neuromediators, gene expression, and activities of many organs, such as brain, kidneys, lungs, and digestive tract, the feeling of pain, and many other physiological and biochemical processes.

Several fatty acids, free or esterified (methyl butyrate, ethyl octanoate, dodecanoic acid), belong to aroma compounds which are found in environmental or food systems. Among straight-chain fatty acids, the simplest are referred to as saturated fatty acids. They have no unsaturated linkages and cannot be altered by hydrogenation or halogenation.

In this context, this study was focused on isolation of a long chain saturated fatty acid from Albizia procera. The ETBE extract of Albizia procera was subjected to column chromatography in which the hexane elution gave a single isolated compound. This non polar compound was suspected to be the
expected fatty acid. The UV spectrum showed absorbance at 223nm which indicates that the compound did not contain much of chromophoric group. This was well supported by carbon and proton NMR.

The $^{13}$C NMR showed a peak at 177.43 δppm exclusively for –C=O of the acid group. The absence of peaks at 120.00-140.00 δppm showed that the fatty acid was a saturated one and did not contain any double bonds. Moreover the methylene carbons were observed at 22.33 – 31.67 δppm showed that they were aliphatic long chain carbons. The free methyl was seen at 12.65 δppm. Hence the fatty acid was a C-22 long chain fatty acid.

The $^1$H NMR showed a singlet at 8.0 δppm for acid proton. As observed in carbon NMR there was no aromatic peaks at 7.0 δppm and hence it was not an unsaturated fatty acid. The aliphatic protons were all found at 1.0 – 2.3 δppm and methyl protons were seen at 0.8 δppm. The mass spectrum showed a parent ion peak at 340 corresponds to Docosanoic acid and a base peak at 43. Hence the isolated long chain fatty acid was Docosanoic acid which was present in Albizia procera.

### Table 7.5 Infrared vibration of compound isolated from Albizia procera

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Bruker FT-IR Model: Alpha E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak (cm⁻¹)</td>
<td>Assignment</td>
</tr>
<tr>
<td>3355</td>
<td>-OH group</td>
</tr>
<tr>
<td>2924, 2857</td>
<td>-CH- stretching</td>
</tr>
<tr>
<td>1725</td>
<td>-C=O stretching</td>
</tr>
<tr>
<td>Below 1068</td>
<td>Bending vibration</td>
</tr>
</tbody>
</table>
Figure 7.4 UV spectrum of compound isolated from ETBE of *Albizia procera* $\lambda_{\text{max}} : 223 \text{ nm}$
Figure 7.5 Infrared spectrum of compound isolated from ETBE of *Albizia procera*
Figure 7.6 $^1$H NMR spectrum of compound isolated from ETBE of Albizia procera
Figure 7.7 $^{13}$C NMR spectrum of compound isolated from ETBE of Albizia procera
Figure 7.8 Mass spectrum of compound isolated from ETBE of *Albizia procera*
The isolated compound was identified as docosanoic acid. Several plants such as *Albizia procera* have been traditionally used as valuable medicinal herbs since the ancient times in the oriental countries. Current phytochemical and pharmacological studies revealed that they contain a variety of bioactive ingredient including terpene saponins, fatty acids and polysaccharides (Zhang et al., 2013). They exhibit extensively beneficial effects on immune system, CNS and cardio vascular system (Karmazyn et al., 2008).
Fatty acids, traditionally viewed as the source of energy have attracted interest for research and public health due to their effects on human health and diseases (Kris-Etherton et al., 1999). The unsaturated fatty acids including mono unsaturated and poly unsaturated fatty acids are health promoting and have significant metabolic and cardiovascular benefits (Gillingham et al., 2011). There is convincing evidence that fatty acids are used for treating several chronic diseases including hyperlipidemia, arrhythmia, rheumatoid arthritis and cancer (Ayalew-Pervanchon et al., 2007). Therefore fatty acids might substantially contribute to the whole beneficial effects of herbs.
7.4 REFERENCES


*Condensed tannins in tropical forages.*


