Research Article

Isolation of Active Constituents Derived From Whole Plant of *Bauhinia Purpurea*

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ABSTRACT

The aim of the present investigation was to isolate the active components present in whole plant of *Bauhinia purpurea.* The plant were extracted with various solvents (pet. ether, ethyl acetate and methanol), ethyl acetate fraction was found to be more active among them. The preliminary phytochemical results revealed that flavonoids and amino acids as active constituents in ethyl acetate extract of *Bauhinia purpurea*. The ethyl acetate extract of *Bauhinia purpurea* was undergone column chromatography with different solvent fractions. Hence, two compounds were isolated from ethyl acetate extract of *Bauhinia purpurea* with the compound 1 was eluted with benzene: Chloroform 90:10 v/v and compound 2 were eluted with eluted with ethyl acetate: methanol 80:20 v/v. The structures of the two isolated compounds were characterized by using FT-IR, NMR and Mass spectrophotometric methods. Thus, the compound 1 was characterized as ethyl 2-amino-5-hydroxy-3,6,6-trimethyl heptonate ($C_{12}H_{25}NO_3$) and compound 2 was characterized as 3,6,7-trihydroxy-2-(3-methoxyphenyl)-4*H*-chromen-4-one($C_{16}H_{12}O_6$).Therefore, further biological investigations need to be carried out isolated compounds present in this plant.

Keywords: *Bauhinia purpurea,* column chromatography, FT-IR, NMR, Mass.

INTRODUCTION

Bauhinia purpurea (Leguminosae) is a medium sized deciduous tree, sparingly grown in India. This plant is used traditionally in dropsy, pain, rheumatism, convulsions, delirium, and septicemia[1]. The bark of the plant is used as an astringent in the treatment of diarrhea. Its decoctions are recommended for ulcers as a useful was solution[2]. They are reported to exhibit various pharmacological activities such as CNS activity, cardiotonic activity, lipid-lowering activity, anti-oxidant activity, hepatoprotective activity, hypoglycemic activity, etc[3].Even through, traditionally, leaves of *Bauhinia purpurea* (Linn) were extensively used for the treatment of variety of wounds[4], and no scientific data in its support is available. Hence, the objective of the present investigation was to isolation of active components derived from whole plant of *Bauhinia purpurea* by using FT-IR, NMR and mass spectrophotometric methods

EXPERIMENTAL SECTION

Plant material

The whole plant of *Bauhinia purpurea*, were collected from Nagercoil, Kanyakumari District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Bauhinia purpurea* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Extraction

The powdered plant materials were successively extracted with Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[5] for 24 hours. Then the marc was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then marc was subjected to

Methanol for 24 hours. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained. All the three extract were stored in screw cap vial at 4^0C until further use.

Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical screening for the detection of various plant constituents present. The various extracts of *Bauhinia purpurea* was subjected to the following chemical tests such as tests for Alkaloids[6], test for Carbohydrates[6], tests of Glycosides[6], tests for Phytosterol[7], test for Coumarins[7], test for Flavonoids[8,9], test for Tannins and Phenolic compounds[10], tests for Proteins and Amino Acids[6], test for Saponins[6], test for Fixed Oils[6].

TLC characterization of Ethyl acetate extract of *Bauhinia purpurea*

The principle of separation is either partition or adsorption. The constituent which is having more affinity for mobile phase moves with it, while the constituent which is having more affinity for stationary phase gets adsorbed on it. This way various compounds appear as a band on the TLC plate, having different R_f values. The ethyl acetate extract of *Bauhinia purpurea* was subjected to thin layer and high performance thin layer chromatographic studies for the separation and identification of their components.

Preparation of plates

100g of silica gel G was weighed and made into a homogenous suspension with 200 ml of distilled water to form slurry. The slurry was poured into a TLC applicator, which was adjusted to 0.25 mm thickness on flat glass plate of different dimensions (10X2, 10X5, 30X5, 20X10 cm etc,). The coated plates were allowed to dry in air, followed by heating at 100-105°C for 1 hour, cooled and protected from moisture. Before using, the plates were activated at 110°C for 10 minutes.

Separation of components

The ethyl acetate extracts of *Bauhinia purpurea* was dissolved in methanol separately and spotted using a capillary tube on TLC plates 2 cm above from the bottom of the plate. The selection of solvent systems was based on increasing the order of polarity. The different spots developed in each system were detected by means of iodine staining.

Isolation of ethyl acetate extract of *Bauhinia purpurea* by using Column Chromatography

The 20gms of ethyl acetate extract of *Bauhinia purpurea* was admixed with 20gms silica gel (60/120 meshes) to get uniform mixing. 200gms of silica gel (70/325 meshes) was taken in a suitable column and packed very carefully without air bubbles using hexane as filling solvent. The column was kept aside for 1 hour and allowed for close packing. Admixture was then added at the top of the stationary phase and started separation of compounds by the eluting with various solvent mixtures with increasing order of polarity. All the column fractions were collected separately and concentrated under reduced pressure. Finally the column was washed with ethyl acetate and methanol.

Characterization of isolated Compounds

FT-IR

IR spectra of the compounds isolated from the ethyl acetate extract of *Bauhinia purpurea* were recorded using a Nicolet 170SX. The spectral resolution for the Nicolet 170SX was 0.25cm^{-1}, and the spectral data were stored in the database at intervals of 0.5 cm^{-1} at 4000-2000 cm^{-1}, and of 0.25 cm^{-1} at 2000-400 cm^{-1}. Liquid samples were measured with liquid film method, and solid samples were measured by using KBr disc methods.

^{1}HNMR

^{1}HNMR spectra of the compounds isolated from the ethyl acetate extract of *Bauhinia purpurea* was recorded using a JEOL AL-400 (399.65 MHz). The measuring conditions for the most of the spectra were

as follows: flip angle of 22.5-30.0 degrees, pulse repetition time of 30s. The long pulse repetition time and small flip angle is used to ensure precise relative intensities. The ^{1}H NMR chemical shifts were referred to TMS in organic solvents and TSP in D_2O.

^{13}CNMR

^{13}CNMR spectra of the compounds isolated from the ethyl acetate extract *Bauhinia purpurea* was recorded with a Bruker AC-200 (50.323 MHz). The measuring conditions for the most of the spectra were as follows: a pulse flips angle of 22.45-45 degrees, a pulse repetition time of 4-7 seconds, and a resolution of 0.025-0.045 ppm. The spectra whose spectral codes started with "CDS" were reconstructed from peak positions, intensities, and line widths by assuming all resonance peaks were Lorenz lines. The chemical shift was referred to a TMS for all solvents.

Mass Spectrum

Mass spectra of the compounds isolated from the ethyl acetate extract of *Bauhinia purpurea* was recorded with JEOL JMS-700 by the electron impact method where an electron is accelerating voltage 75eV and an ion accelerating voltage of 8-10nV. The reservoir inlet systems were used. The dynamic range for the peak intensities were 3 digits and the accuracy of the mass number was 0.5.

RESULTS AND DISCUSSION

The various extracts of *Bauhinia purpurea* were subjected to screening for its phytochemical constituents. The phytochemical screening results are shown in Table 1. The petroleum ether extract of *Bauhinia purpurea* was contains phytosterols, fixed oils & fats. Ethyl acetate extracts containing Alkaloids, Carbohydrates, glycosides, Phenolic compounds & tannins, coumarins, protein and amino acid compounds. The Methanolic extracts containing Alkaloids, Carbohydrates, glycoside, Phenolic compounds, Saponins, Tannins, coumarins, Protein and amino acids & flavonoids.

Table 1: Phytochemical analysis of various extracts of wholeplant of *Bauhinia purpurea*

S.No.	Test	Petroleum ether	Ethyl acetate	Methanol
I	Alkaloids	-	+	+
II	Carbohydrates and glycosides	-	+	+
III	Phytosterols	+	-	-
IV	Fixed oil and fats	+	-	-
V	Saponins	-	+	+
VI	Phenolic compounds and tannins	-	+	+
VII	Protein and Amino Acid	-	+	+
VIII	Coumarins	-	+	+
IX	Flavonoids	-	+	+

+ Positive; - Negative

Petroleum ether, ethyl acetate and methanol were used individually as solvent for the extraction of *Bauhinia purpurea*. The ethyl acetate extract of *Bauhinia purpurea* was found more active among them. Therefore, the ethyl acetate extract of *Bauhinia purpurea* was subjected to the TLC chromatographic profile and column chromatographic separation. The ethyl acetate extract of *Bauhinia purpurea* dissolved in their mother solvent was taken in a capillary tube and spotted on TLC plates 2cm above its bottom. Most of the sample for application were between 0.1 – 1%. The applied spots were of equal size as far as possible and diameter ranging from 2-3mm. The solvent system for methanolic extracts was developed by trial and error method using various solvents which were differing in polarities.

Table 2: TLC profiles of methanolic extracts of *Bauhinia purpurea*

S.No	Solvent system	No. of Spot	Rf Value
1.	Benzene : Chloroform (90:10)	2	0.72, 0.35
2.	Benzene : Chloroform (80:20)	2	0.72, 0.26
3.	Benzene : Chloroform (70:30)	2	0.57, 0.37
4.	Ethyl acetate: Methanol (70:30)	2	0.88, 0.65
5.	Ethyl acetate: Methanol (50:50)	3	0.68,0.47, 0.34

The ethyl acetate extract of *Bauhinia purpurea* was subjected to column chromatographic separation using normal phase silica gel column. The dark brown solid (20 g ethyl acetate extract of *Bauhinia purpurea*) was adsorbed on silica gel (20 g) and transferred to a column of silica gel (200g equilibrated with benzene). Two compounds were isolated in column chromatography with different solvents such as compound 1 (130 mg) was eluted with benzene: Chloroform 90:10, v/v, and compound 2 (150mg) was eluted with ethyl acetate: methanol, 80:20 v/v.

Characterization of compound 1

The spectral data IR, ^{1}HNMR & ^{13}CNMR and Mass of the compound 1 are good in agreement with the structure proposed for the compound. The melting point of the compound 1 was found as 230ºC. The IR spectrum of the compound 1was analysed from the IR data. Absorption at 3412cm^{-1} shows the presence of –OH group, whereas the strong band at 1734cm^{-1} indicates the presence of carbonyl group of aliphatic compound. The absorption at 1173cm^{-1} reveals the presence of –C-N stretching. The ^{1}HNMR and ^{13}CNMR chemical shift values of the compound 1 was found to be ethyl 2-amino-5-hydroxy-3,6,6-trimethyl heptonate (Fig 1&2). The mass spectral analysis of compound 1 led to the molecular peak *m/z* 434, which indicated the molecular formula $C_{16}H_{12}O_7$. Thus, the compound 1 was characterized as ethyl 2-amino-5-hydroxy-3,6,6-trimethyl heptonate was given in Fig 3.

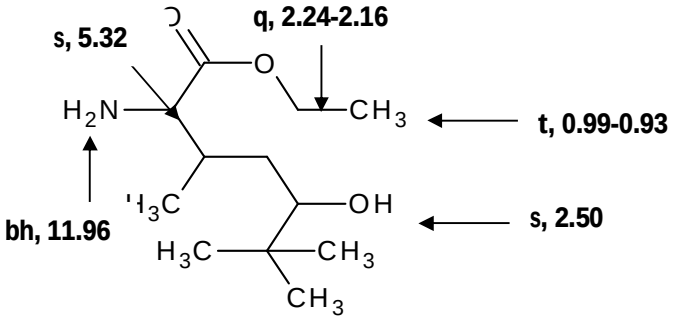
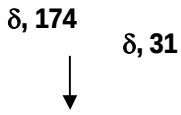


Fig. 1: ^{1}HNMR spectral data of compound 1 and corresponding assignments



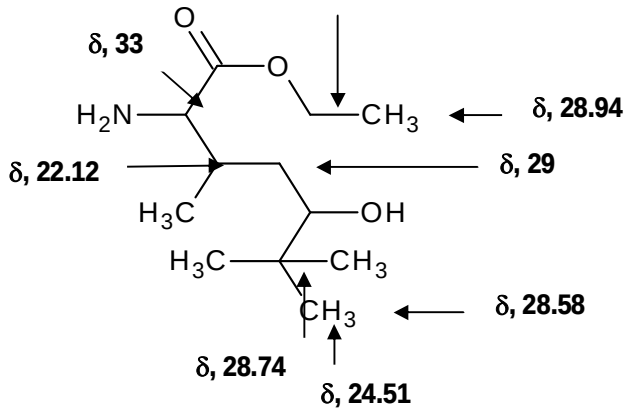


Fig. 2: ^{13}CNMR spectral data of compound 1 and corresponding assignments

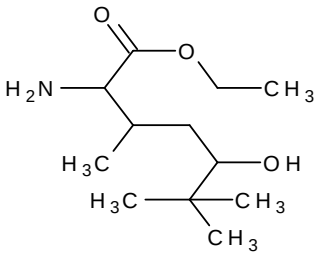


Fig. 3: Structure of Compound 1 (ethyl 2-amino-5-hydroxy-3,6,6-trimethyl heptonate)

Characterization of compound 2

The spectral data IR, ^{1}HNMR & ^{13}CNMR and Mass of the compound 2 are good in agreement with the structure proposed for the compound. The melting point of the compound 2 was found as 225ºC. The IR spectrum of the compound 2 was analysed from the IR data. The hydroxyl protons showed a sharp signal and two broad humps δ 13.05, 10.72 and 9.60ppm respectively for three protons. The signals for other aromatic protons occur at δ 8.33, 7.38, 6.83 and 6.50 ppm. The ^{1}HNMR chemical shift values of the compound 2 was found Two broad humps δ 13.05, 10.72 and 9.60ppm respectively for three protons. Aromatic protons occur at δ 8.33, 7.38, 6.83 and 6.50 ppm. ^{13}CNMR- signals at δ, 180, 157, 154, 152, 131, 130, 121, 115, 104, 93 and 59 ppm. The mass spectral analysis of compound 2 led to the molecular peak *m/z* 404, which indicated the molecular formula $C_{16}H_{12}O_6$. Thus, the compound 2 was characterized as 3,6,7-trihydroxy-2-(3-methoxyphenyl)-4*H*-chromen-4-one was given in Fig 4.

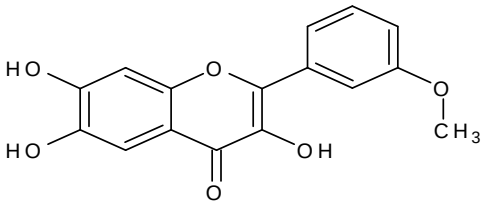


Fig. 4: Structure of Compound 2(3,6,7-trihydroxy-

CONCLUSION

On the basis of results revealed that, three compounds were isolated from ethyl extract of *Bauhinia purpurea*. Thus, the compound 1 was characterized as ethyl 2-amino-5-hydroxy-3,6,6-trimethyl heptonate ($C_{12}H_{25}NO_3$) and compound 2 was characterized as 3,6,7-trihydroxy-2-(3-methoxyphenyl)-4*H*-chromen-4-one ($C_{16}H_{12}O_6$). Therefore, further biological studies are required for these isolated compounds.

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