



## Isolation Of Active Components Derived From Whole Plant Of *Borreria hispida* (Linn.)

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*The objective of the present investigation was to isolate the active components present in whole plant of Borreria hispida. The plant were extracted with various solvents (pet. ether, ethyl acetate and methanol), methanol was found to be more active among them. The preliminary phytochemical results revealed that flavonoids and amino acids as active constituents in methanolic extract of Borreria hispida. The methanolic extract of Borreria hispida was undergone column chromatography with different solvent fractions. Hence, two compounds were isolated from methanolic extract of Borreria hispida with the compound 1 was eluted with benzene: Chloroform 70:30, v/v and compound 2 were eluted with eluted with ethyl acetate: methanol 50:50, 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 1-amino-1-ethoxypropan-2-ol (C<sub>5</sub>H<sub>13</sub>O<sub>2</sub>N), and compound 2 was characterized as 3,5,7-trihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one(C<sub>15</sub>H<sub>12</sub>O<sub>6</sub>). Therefore, further biological investigations need to be carried out isolated compounds present in this plant.*

**Key words:** *Borreria hispida*, column chromatography, FT-IR, NMR.

## INTRODUCTION

*Borreria hispida* is belongs to the family Rubiaceae. It is widely distributed in throughout India, up to 900m in hills and on all dry lands as a weed. The seed of *Borreria hispida* is used as PPAR-alpha gene expression, antioxidant redox status, protein metabolism in STZ diabetic rats. Potential role of *Borreria hispida* in ameliorating cardiovascular risk factor<sup>1</sup>. Therefore, the objective of the present investigation was to isolation of active components derived from whole plant of *Borreria hispida* by using FT-IR, NMR and mass spectrophotometric methods

## EXPERIMENTAL SECTION

### Plant material

The whole plant of *Borreria hispida* (Linn), were collected from Naserath, Tuticorin 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 *Borreria hispida* (Linn), 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<sup>0</sup>C) by hot continuous percolation method in Soxhlet apparatus<sup>2</sup> for 24 hours. Then the marc was subjected to Ethyl acetate (76-78<sup>0</sup>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<sup>0</sup>C 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 *Borreria hispida* was subjected to the following chemical tests such as tests for Alkaloids<sup>3</sup>, test for Carbohydrates<sup>3</sup>, tests of Glycosides<sup>3</sup>, tests for Phytosterol<sup>4</sup>, test for Coumarins<sup>4</sup>, test for Flavonoids<sup>5,6</sup>, test for Tannins and Phenolic compounds<sup>7</sup>, tests for Proteins and Amino Acids<sup>3</sup>, test for Saponins<sup>3</sup>, test for Fixed Oils<sup>3</sup>.

## **TLC characterization of methanolic extract of *Borreria hispida***

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<sub>f</sub> values. The methanolic extract of *Borreria hispida* 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<sup>0</sup>C for 1 hour, cooled and protected from moisture. Before using, the plates were activated at 110<sup>0</sup>C for 10 minutes.

## **Separation of components**

The methanolic extracts of *Borreria hispida* 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 methanolic extract of *Borreria hispida* by using Column Chromatography**

The 20gms of methanolic extract of *Borreria hispida* 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 methanolic extracts of *Borreria hispida* were recorded using a Nicolet 170SX. The spectral resolution for the Nicolet 170SX was  $0.25\text{cm}^{-1}$ , and the spectral data were stored in the database at intervals of  $0.5\text{ cm}^{-1}$  at  $4000\text{-}2000\text{ cm}^{-1}$ , and of  $0.25\text{ cm}^{-1}$  at  $2000\text{-}400\text{ cm}^{-1}$ . Liquid samples were measured with liquid film method, and solid samples were measured by using KBr disc methods.

#### **$^1\text{H}$ NMR**

$^1\text{H}$ NMR spectra of the compounds isolated from the methanolic extracts of *Borreria hispida* 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  $^1\text{H}$  NMR chemical shifts were referred to TMS in organic solvents and TSP in  $\text{D}_2\text{O}$ .

#### **$^{13}\text{C}$ NMR**

$^{13}\text{C}$ NMR spectra of the compounds isolated from the methanolic extracts *Borreria hispida* 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 methanolic extracts of *Borreria hispida* 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 *Borreria hispida* (Linn.) were subjected to screening for its phytochemical constituents. The phytochemical screening results are shown in Table 1. The petroleum ether extract of *Borreria hispida* contains phytosterols, fixed oils & fats. Ethyl acetate extracts containing Alkaloids, Carbohydrates, Phenolic compounds & tannins, protein and amino acid compounds, Saponins and fixed oils & fats. The Methanolic extracts containing Alkaloids, Carbohydrates, glycoside, Phenolic compounds, Saponins, Tannins, Protein and amino acid & flavonoids.

**Table 1 - Phytochemical analysis of various extracts of whole plant of *Borreria hispida* (Linn.)**

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 *Borreria hispida*. The methanolic extract of *Borreria hispida* was found more active among them. Therefore, the methanolic extract of *Borreria hispida* was subjected to the TLC chromatographic profile and column chromatographic separation. The methanolic extract of *Borreria hispida* 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 *Borreria hispida***

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 methanolic extract of *Borreria hispida* was subjected to column chromatographic separation using normal phase silica gel column. The dark brown solid (20 g methanolic extract of *Borreria hispida*) 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 (125 mg) was eluted with benzene: Chloroform 70:30, v/v, and compound 2 (168mg) was eluted with ethyl acetate: methanol, 50:50 v/v.

### Characterization of compound 1

The spectral data IR,  $^1\text{H}$ NMR &  $^{13}\text{C}$ NMR 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 149°C. The IR spectrum of the compound 1 was analysed from the IR data. A broad band at  $3405\text{cm}^{-1}$  is due to the presence of  $-\text{OH}$  &  $-\text{NH}$  groups. Whereas a strong band at  $1096\text{cm}^{-1}$  indicates the presence of carbonyl is due to  $-\text{C}-\text{O}-\text{C}-$  stretching.  $^1\text{H}$ NMR &  $^{13}\text{C}$ NMR Spectra of this compound are given in the fig.  $^1\text{H}$ NMR &  $^{13}\text{C}$ NMR data and the corresponding assignments are given in the structure proposed to the compound (Fig 1&2). The mass spectral analysis of compound 1 led to the molecular peak  $m/z$  119, which indicated the molecular formula  $\text{C}_5\text{H}_{13}\text{O}_2\text{N}$ . Thus, the compound 1 was characterized as 1-amino-1-ethoxypropan-2-ol was given in Fig 3.

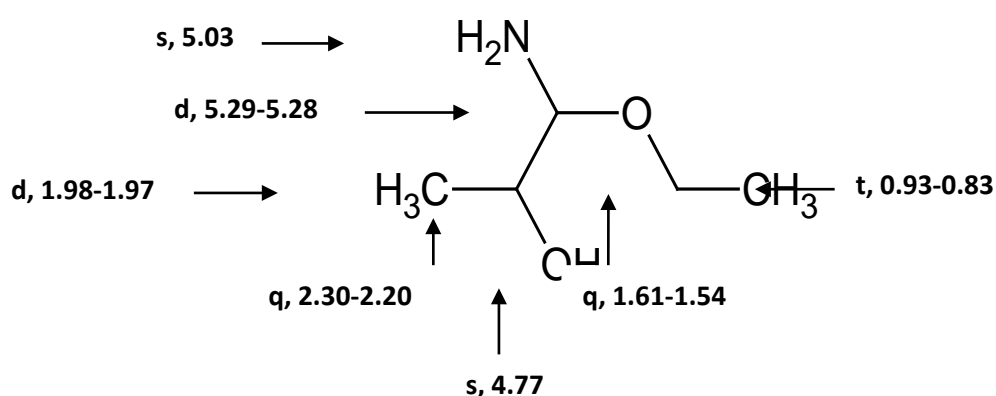


Fig 1:  $^1\text{H}$ NMR spectral data of compound 1 and corresponding assignments

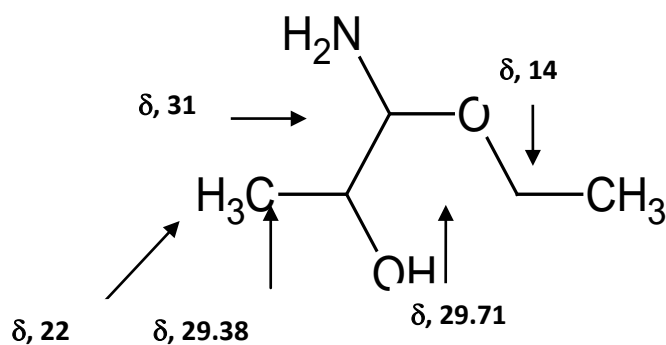


Fig 2:  $^{13}\text{C}$ NMR spectral data of compound 1 and corresponding assignments

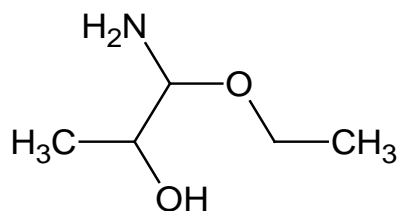


Fig 3: Structure of Compound 1(1-amino-1-ethoxypropan-2-ol)

### Characterization of compound 2

The spectral data IR,  $^1\text{H}$ NMR &  $^{13}\text{C}$ NMR 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  $225^\circ\text{C}$ . The IR spectrum of the compound 2 was analysed from the IR data. The presence of  $-\text{NH}$  group known from the absorption at  $3320\text{cm}^{-1}$ . Absorption at  $2920\text{cm}^{-1}$  shows the presence of  $-\text{C}-\text{H}$  (aromatic) group. A strong band at  $1164\text{cm}^{-1}$  is due to the presence of  $-\text{C}=\text{O}$  group. The presence of  $-\text{C}=\text{C}$  (aromatic) indicates in the absorption at  $1611\text{cm}^{-1}$ . The  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR chemical shift values of the compound 2 was found to be 3,5,7-trihydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (Fig 4&5). The mass spectral analysis of compound 2 led to the molecular peak  $m/z$  404, which indicated the molecular formula  $\text{C}_{15}\text{H}_{12}\text{O}_6$ . Thus, the compound 2 was characterized as 3,5,7-trihydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one was given in Fig 6. This is the first report of occurrence of this compound in this plant.

d, 6.18



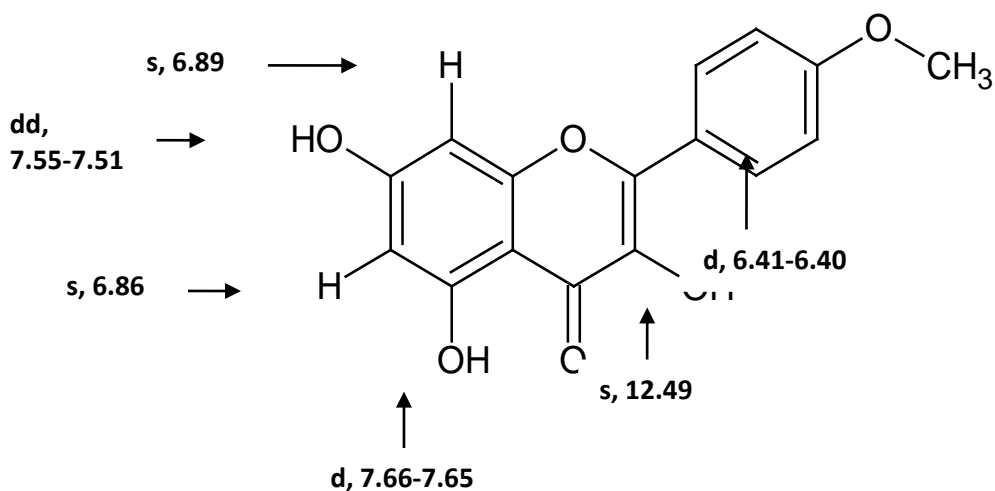


Fig 4:  $^1\text{H NMR}$  spectral data of compound 2 and corresponding assignments

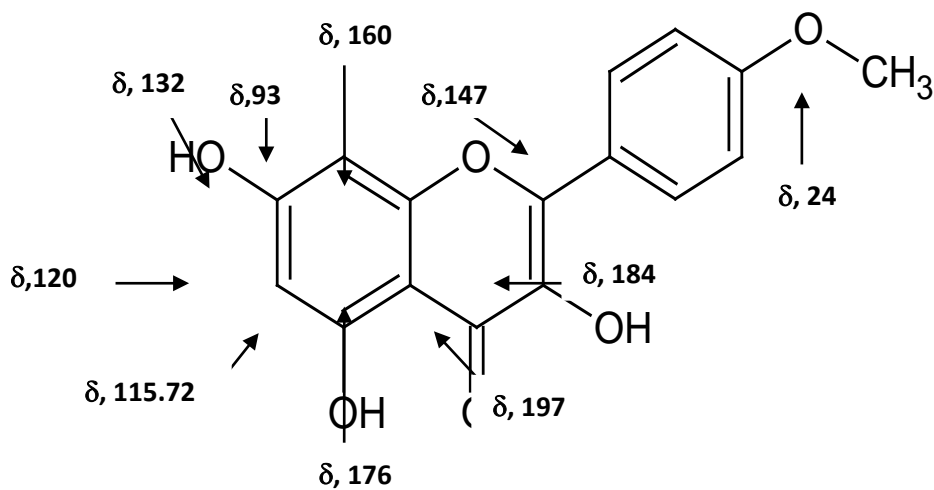


Fig 5:  $^{13}\text{C NMR}$  spectral data of compound 2 and corresponding assignments

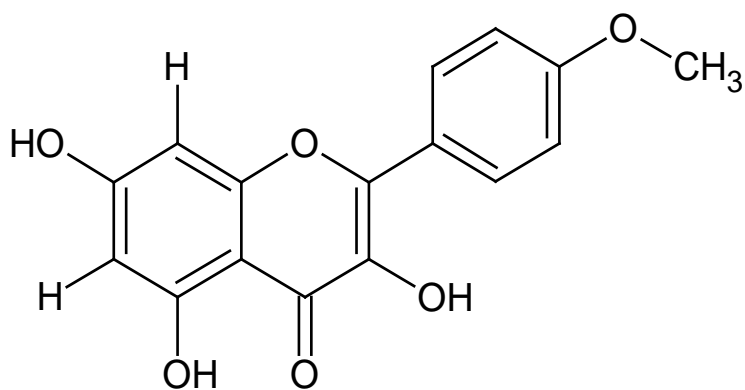


Fig 6: Structure of Compound 2(3,5,7-trihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one)

## CONCLUSION

From the above reports, three compounds were isolated from methanolic extract of *Borreria hispida* (Linn.) such as 1-amino-1-ethoxypropan-2-ol (C<sub>5</sub>H<sub>13</sub>O<sub>2</sub>N) and compound 2 was characterized as 3,5,7-trihydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one(C<sub>15</sub>H<sub>12</sub>O<sub>6</sub>). This is the first report of occurrence of 3,5,7-trihydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one(C<sub>15</sub>H<sub>12</sub>O<sub>6</sub>) in this plant. Furthermore, biological investigations are required for these isolated compounds.

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## REFERENCES

1. Vasanthi, Hannah R PhD; Mukherjee, Subhendu MS; Lekli, Istvan MFarm; Ray, Diptarka MS; Veeraraghavan, Gayathri PhD; Das, Dipak K PhD. Potential Role of *Borreria hispida* in Ameliorating Cardiovascular Risk Factors. *Journal of Cardiovascular Pharmacology*:2009. 53 ; 6: 499-506
2. Harborne JB. *Phytochemical methods* 11<sup>th</sup> Edn. In Chapman & Hall. New York, 1984; 4-5.
3. Evans WC *An index of medicinal plants. A Text book of Pharmacognosy*. 14<sup>th</sup> ed.1997; 7(5): 12-14.
4. Finar G. *Plants of economic importance. Medicinal Plants and Medicine in Africa*. Spectrum Books Ltd. Ibadan. 1986; 78:150-153.
5. Dey PM and Harborne JB. *Methods in Plant Biochemistry*: Academic Press; London 1987.
6. Evans WC. *Pharmacognosy*, 13<sup>th</sup> Ed, Balliere-Tindall; London 1989..
7. Mace Gorbach SL. *Anaerobic bacteriology for clinical laboratories*. *Pharmacognosy*, 1963; 23: 89-91.