

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Isolation of 28-pentyl-3-galloyl-betulinate and 11-hydroxy friedelane from the plant *Argyrea speciosa*.

Aiswarya G^{1*}, Rajesh Gupta², Kambhoja S³.

¹J.D.T. Islam College of Pharmacy, Jamiath Hill, Vellimadukunnu, Kozhikode, Kerala, India

²Sri Sai College of Pharmacy, Dalhousie road, Badhani, Pathankot, Punjab, India.

³Dept. of Pharmacognosy, The Oxford College of Pharmacy, Bangalore-78, India.

ABSTRACT

The present study was undertaken to isolate phytoconstituents of *Argyrea speciosa*. Two compounds were isolated from *Argyrea speciosa* by soxhale extraction and purification has been done by column chromatography method. The spectral analysis revealed the two isolated compounds, as compound I (28-pentyl-3-galloyl-betulinate) and compound II (11-hydroxy friedelane) and they are steroidal moieties.

Key words- *Argyrea speciosa*, soxhale extraction, 28-pentyl-3-galloyl-betulinate, 11-hydroxy friedelane

***Corresponding author**

E-mail: aiswaryageorge_d@yahoo.com

INTRODUCTION

Argyreia speciosa belongs to the family Convolvulaceae distributed through out India up to an altitude of 400m. It is cultivated in Rajasthan, Konkan, Deccan, near the costs of N. Kanara, Belgaum, and Mysore. The chemical constituents found are lysergic acid amide, isolysergic acid amide [1]. The leaves of *Argyreia speciosa* contain 1-triacontanol, epifriedelinol [2] and flavone glycosides [3]. Pharmacologically *Argyreia speciosa* are used in skin diseases, antiphlogistic, gleet gonorrhea, chronic ulcer [4], immunomodulatory [5], antiarthritic, anti inflammatory [6,7], antidiabetic activities and in male subfertility [8]. The present study was undertaken to isolate compounds of *Argyreia speciosa*.

MATERIALS AND METHODS

Dried leaves of *Argyreia speciosa* were obtained and authenticated by Dr. Harish Botanist. Alva's education foundation (R). Alva's Health centre complex Moobdidri-574227.D.K.

Preparation of extract

The powdered plant material was successively extracted by using Soxhlet extractor. solvent recovered and the extract is concentrated and percentage yield was calculated (Table 1). The solvents were recovered by using simple distillation method. The charged drug from the central compartment was removed, dried, recharged and extracted by using next solvent of higher polarity than the first solvent. By using Soxhlet extractor exhaustive extraction with a series of solvents of increasing polarity was done. Solvents used with increasing polarity are Petroleum ether, Benzene, Chloroform, Methanol and finally water. The percentage yield of each extracts was reported.

Preliminary phytochemical screening

The crude extract obtained by successive extraction from petroleum ether, benzene, chloroform, methanol and aqueous extraction are subjected to preliminary phytochemical screening using standard procedure to determine the presence of various phytochemicals (Table 2).

Optimization of TLC solvent system

Different solvent systems were tried for optimization the TLC system to identify the constituents presents in the extract. The chromatograms are shown in plate 1 and 2. The detail is tabulated in table no. 3 for pet ether extract. Among these solvent system pet-ether: benzene 0.1:1 was found to be better system for pet-ether extract as these gave more number of separate bands.

Isolation of phytoconstituents

The constituents of pet-ether extract were isolated by column chromatography and identification and purity determination were done by thin layer chromatographic techniques.

Column chromatography

The pet. ether extract was subjected to column chromatography using different solvent systems. The fractions collected were further chromatographed, to know the no. of constituents present. Silica gel G as used as stationary phase. Column chromatography was done by using a glass column. The dimension of the column was 5X15 cm in height and 4 cm in diameter. The column was packed with silica gel by wet packing method wherein a padding of cotton was placed at the bottom of the column and then it was filled with eluting solvent of the lowest polarity (pet ether). Then the required amount of stationary phase (silica gel) was poured into the column to form a bed of silica. The extract was then poured on to the bed of silica, a layer of cotton covered it again and more amounts of solvents were poured over it, the column was then eluted gradiently. The general principle applied in column chromatography consisted of following steps:

Pre-column preparation: The pre-column preparation included adsorption of the selected extract/ fraction, charging and saturation of the column.

a. Adsorption of the extract: The extract selected for fractionation was adsorbed on stationary phase in ratio 1:1.

b. Charging of column: A glass column was selected and rinsed with the solvent. A cotton layer was placed at the bottom and the column was charged with the solvent and stationary phase. The silica gel was used in the ratio (1:2) of the extract to make the gel bed for complete separation. The solvent was eluted up to the level of column bed and the dried extract was charged in the column. Another layer of cotton was placed over the charged matter to prevent the disturbance of the extract bed while pouring the eluting solvent from the top.

c. Saturation of the column: The charged column was left for 4 hrs. for complete saturation and removal of air bubbles to make the bed static.

Elution: The charged column was then eluted with different mobile phases with gradual increase in polarity. The fractions collected and the solvent recovered by simple distillation. All the concentrated fractions were subjected to TLC for the identification of the desired bands.

Column Requirements: -

- | | |
|------------------|--|
| Stationary phase | – silica gel G (60-120 mesh) |
| Mobile phase | – Pet ether, Benzene, Chloroform, MeOH, EtOH |

Charged material – Pet-ether extract.
Volume of each fraction – 25 ml.

Visualization – Iodine, 10% Ethanolic sulphuric acid, anisaldehyde sulfuric acid.

Procedure

The column was first eluted with 100% pet ether. The polarity of mobile phase was gradually increased with Benzene, Chloroform, MeOH, and EtOH. The fractions collected were concentrated. The desired concentrated fractions were screened for phytoconstituents. The desired concentrated and dried fractions were kept in container with suitable label and kept for further use. Total 111 fractions were collected from column. The details of collected fraction are given in flow chart no 1.

Purification of compounds

Purification of compound I

The fraction 1 to 20 of column (pt-ether), after concentration, it has formed white ppt, remaining solution was decanted. The precipitate has shown single spot with the mobile phase pet-ether: benzene, 0.5:0.8. The chromatogram is shown in plate 3

Purification of compound II

The fraction 30 and 31 of column (pt-ether: benzene), on concentration it has formed white precipitate and purified by adding 90% ethanol, shown single spot with the mobile phase pet-ether: benzene, 1:0.2. The chromatogram is shown in plate 3

Characterization of the isolated compounds

The isolated compounds (compound I and II) were characterized by: - $^1\text{H-NMR}$, LCMS and IR analysis.

IR spectroscopy of the isolated compounds

The presence of functional group in the isolated compounds was determined by IR spectroscopy.

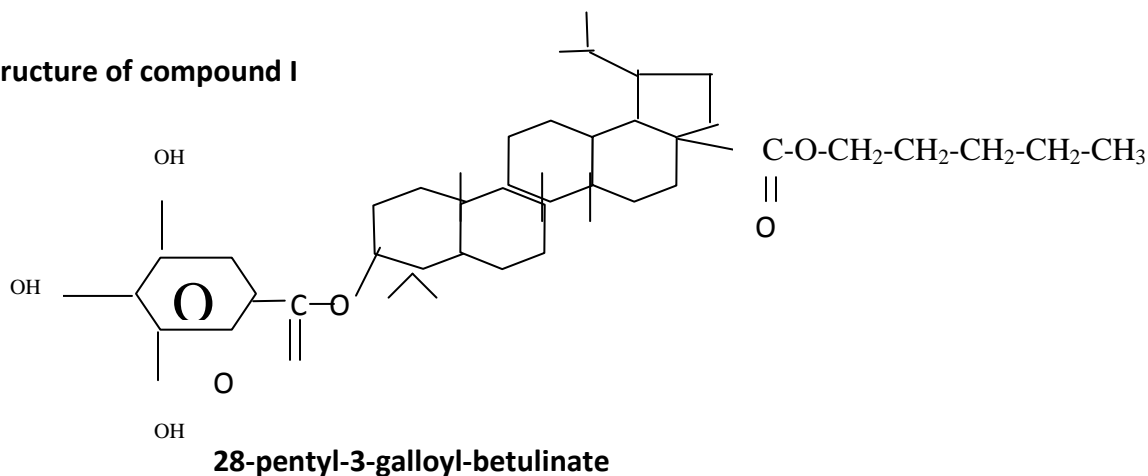
NMR Spectroscopy of the isolated compounds

The isolated compounds were subjected to $^1\text{H-NMR}$ analysis. By using the solvent – DMSO-D_6 + CDCl_3 MIX. The analysis was done at sophisticated Instrument Facility at QUEST, Research and Training Institute, Bangalore.

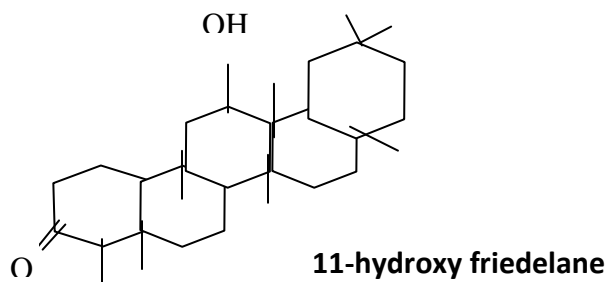
LCMS Spectroscopy of the isolated compounds

The mass spectra of isolated compound I and II also has been done.

Structure of compound I



Structure of compound II



Determination of solubility of isolated compounds

The isolated compounds were analyzed for their solubility in different solvents. Their solubility profiles are tabulated in table no.4

Determination of melting point of isolated compounds

Melting point of compound I and II was done in Thermonic apparatus to determine its identity and purity. The results are tabulated in table No.5

RESULTS AND DISCUSSION

The crude extract obtained by successive extraction from petroleum ether, benzene, chloroform, methanol and aqueous extraction are subjected to preliminary phytochemical screening using standard procedure, which revealed the presence of steroids saponins flavanoids and sugars as shown in Table No. 2. Two compounds have been isolated from the petroleum ether extract by column chromatography. The further spectroscopic analysis revealed the structure of the two compounds and they are found to be steroidal moieties

PLATE 1
TLC OF EXTRACTS



PLATE 2
TLC OF PET-ETHER EXTRACT



Solvent system (PE:BZ-0.1:1)

Solvent system (PE:BZ-0.1:1)

PLATE 3
TLC OF COMPOUND 1



PLATE 4
TLC OF COMPOUND 2

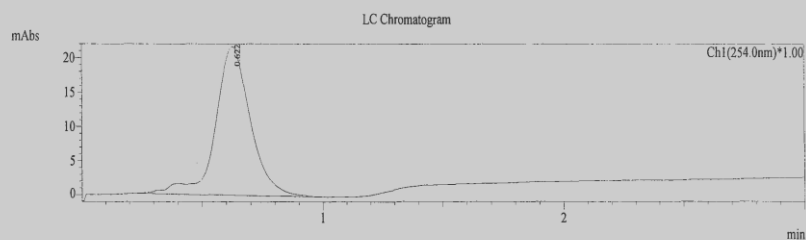


Solvent system (PE:BZ-0.5:0.8)

Solvent system (PE:BZ-1:0.2)

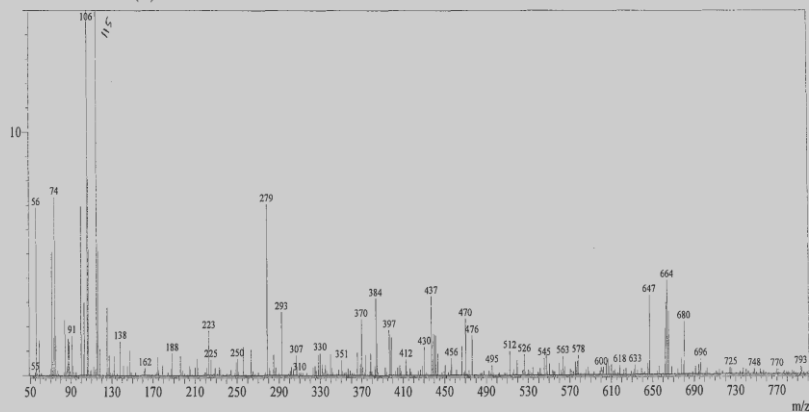
LCMS-2010A DATA REPORT SHIMADZU

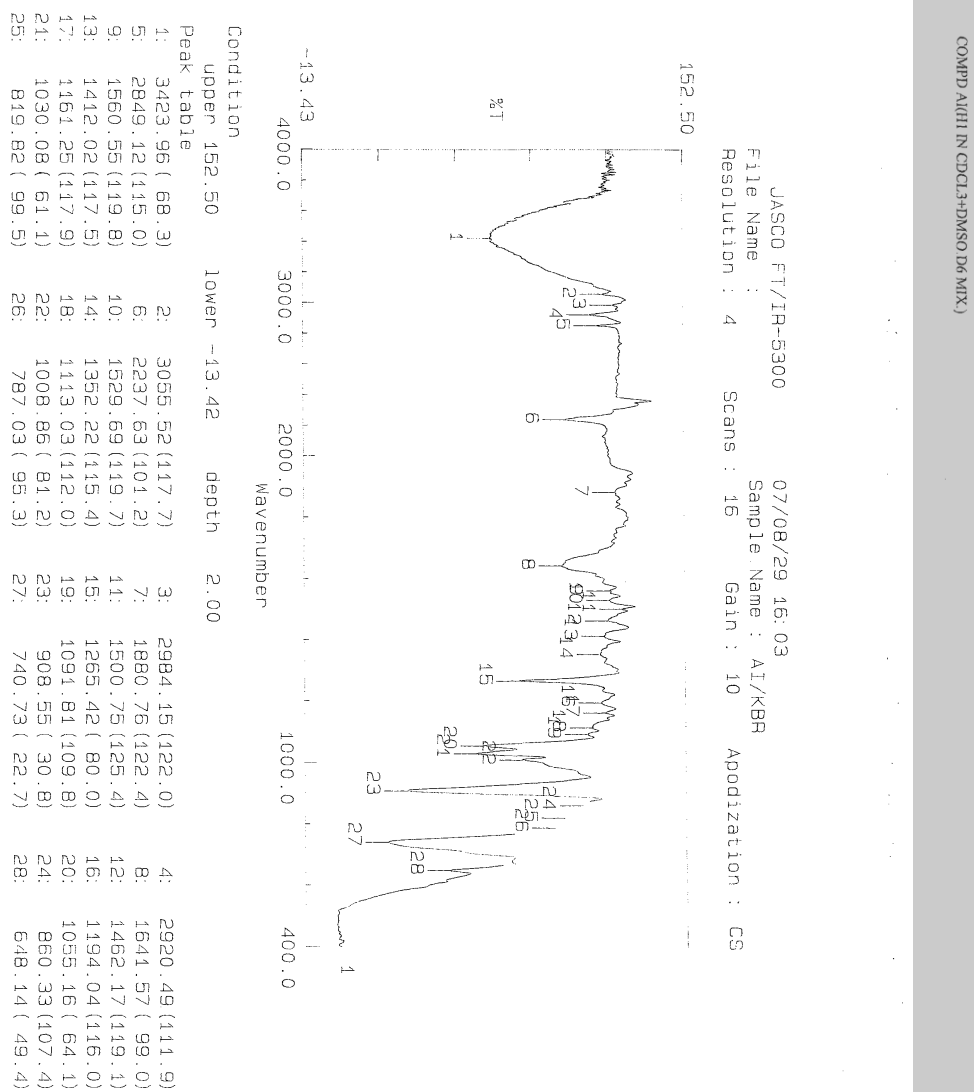
Date and Time : 8/28/2007 13:34:31 PM
 User : Admin
 Vial # : 41
 Sample : COMPD-AI
 Inj. Volume : 5.000
 Data Name : C:\LCMSsolution\User\Data\COMPDAI-APCI-POS1.qld
 Method Name : C:\LCMSsolution\User\Method\JAY-4-APCI.qlm

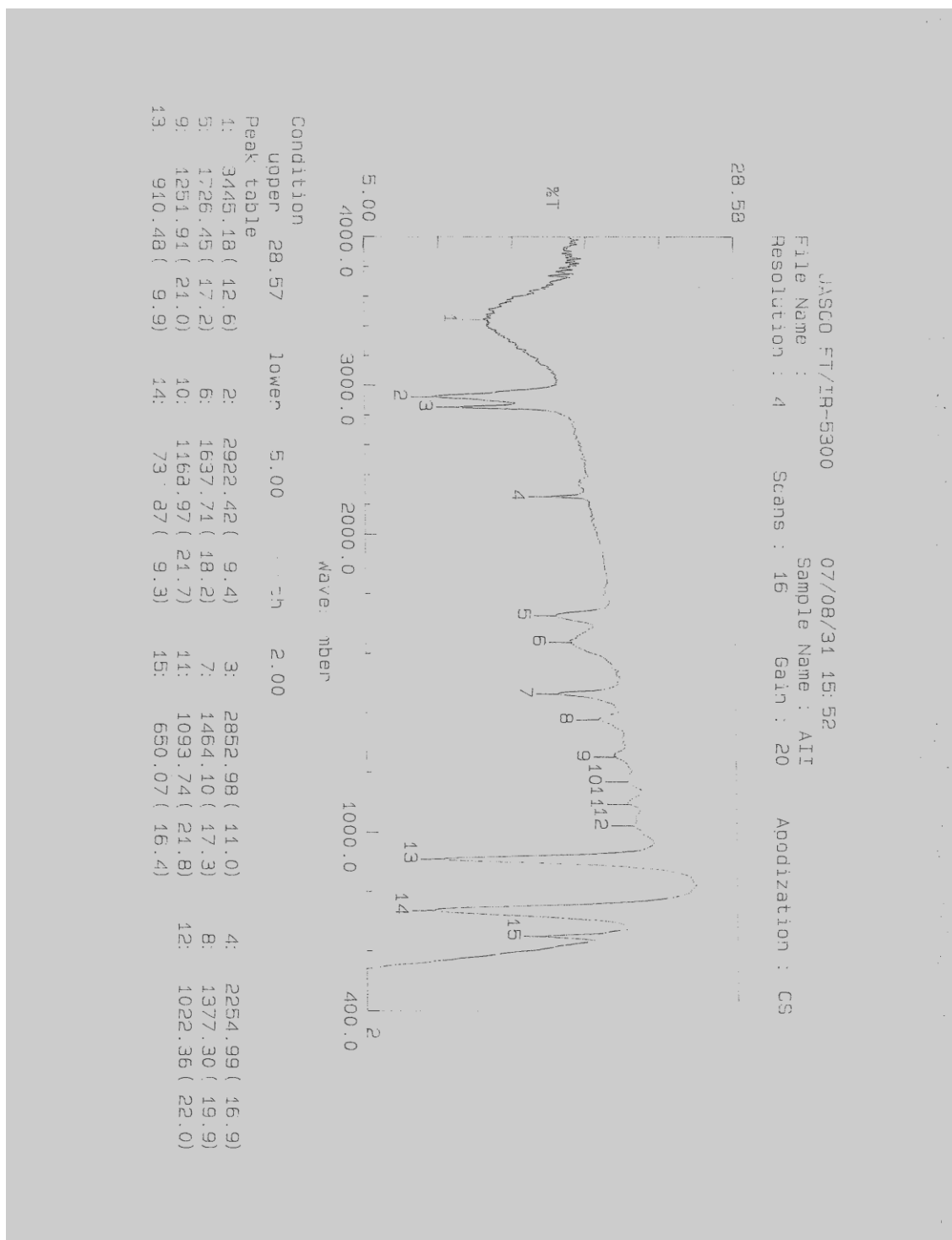


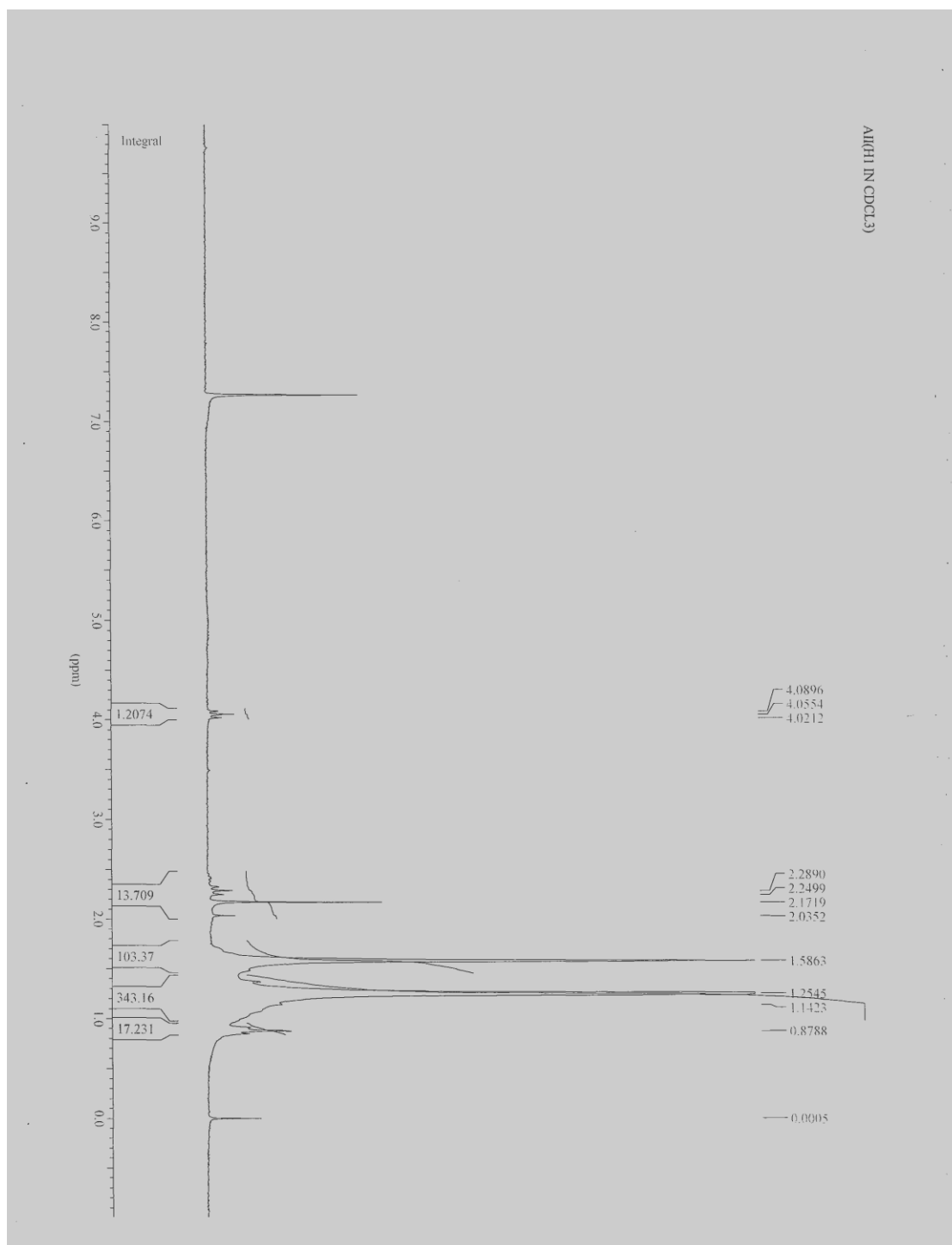
MS Spectrum

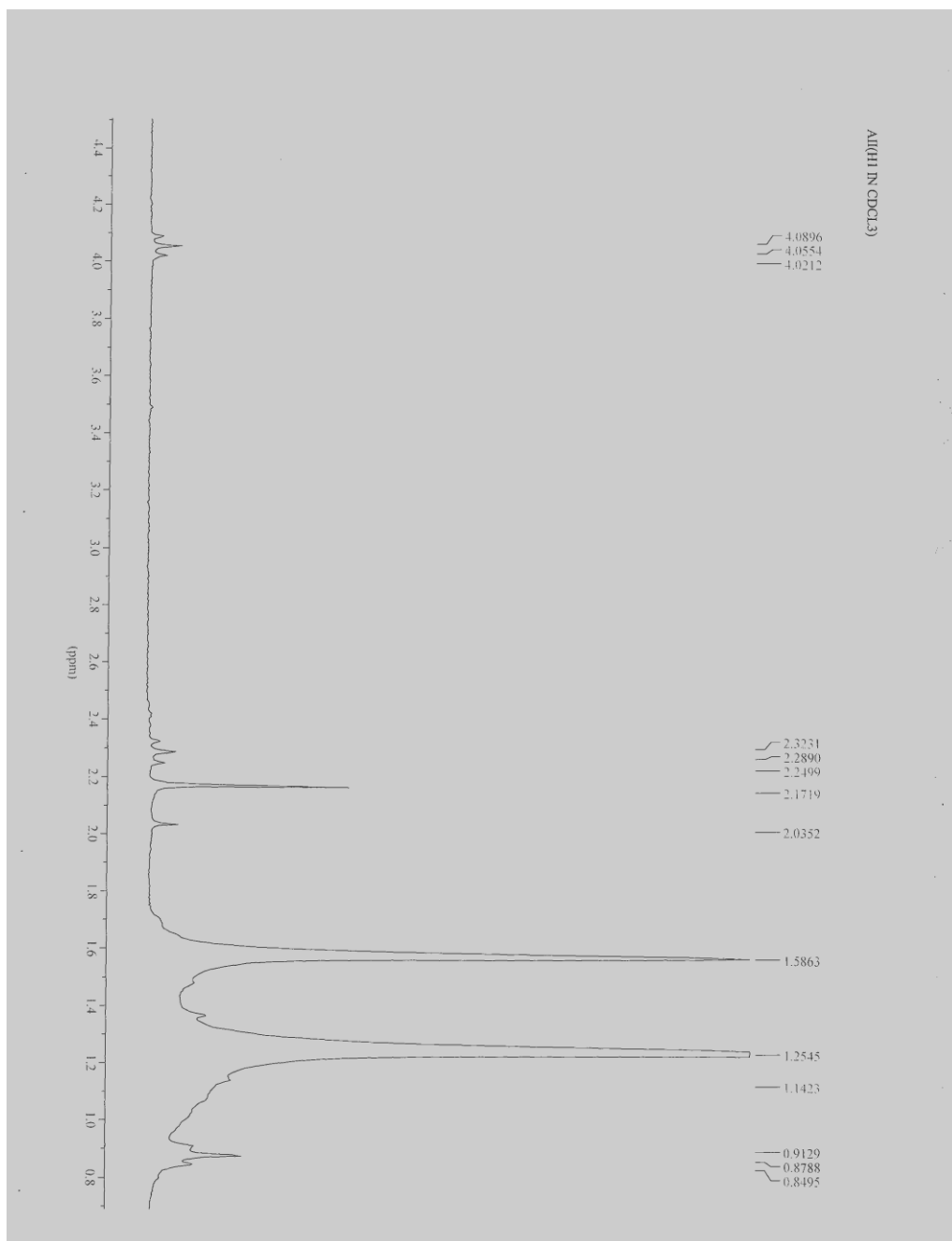
Line#1 R Time:0.710(Scan#43) Positive
 MassPeaks:409 BasePeak:115.05(88224)
 RawMode:Single 0.710(43)
 BG Mode:Peak Start 0.577(35)











LCMS-2010A DATA REPORT SHIMADZU

Date and Time : 8/31/2007 16:23:12 PM
 User : Admin
 Vial # : 54
 Sample : AII
 Inj. Volume : 5.000
 Data Name : C:\LCMSsolution\User\Data\AII-APCI-POS2.qld
 Method Name : C:\LCMSsolution\User\Method\JAY-4-APCI.qlm

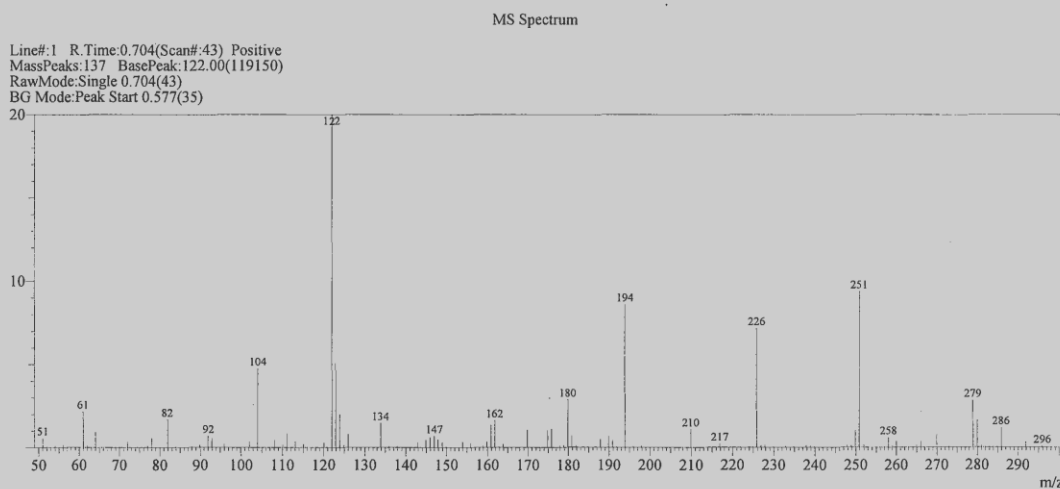
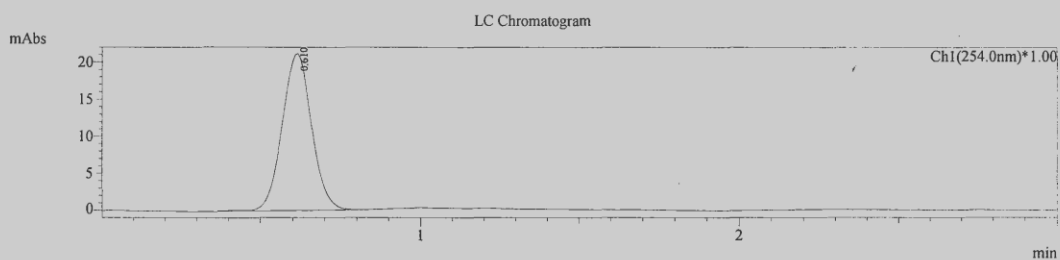


Table No: 1 Percentage yield of different extracts of *Argyreia speciosa*

Sl.No.	Extract	% Yield
1	Petroleum ether	1.75
2	Benzene	0.94
3	Chloroform	0.59
4	Methanol	6.93
5	Distill Water	4.80

Table No 2: Phytochemical screening of different extracts of *Argyreia speciosa*.

Phytoconstituents	Pet-ether	Benzene	Chloroform	Methanol	Ethanol	Water
1.Tannins	-	-	-	-	-	-
2.Alkaloids	-	-	-	-	-	-
3.Steroids	+	+	+	+	+	-
4. Glycosides						
Cardiac	-	-	-	-	-	-
Anthraquinone	-	-	-	-	-	-
Saponins	-	-	-	-	-	+
Flavonoids	-	-	-	-	+	+
Coumarin	-	-	-	-	-	-
5. Sugars	-	+	+	+	+	+

Table No: 3 Optimization of TLC of pet-ether extract

Solvent system	Separation	Detection
Chloroform : Methanol: water (70:30:4)	slight	Iodine vapour
Pet ether : Benzene (2:1)	Poor	10% Ethanolic sulfuric acid
Pet ether: Benzene(0.1:1)	Good (Clear separation with maximum band)	Anisaldehyde sulfuric acid

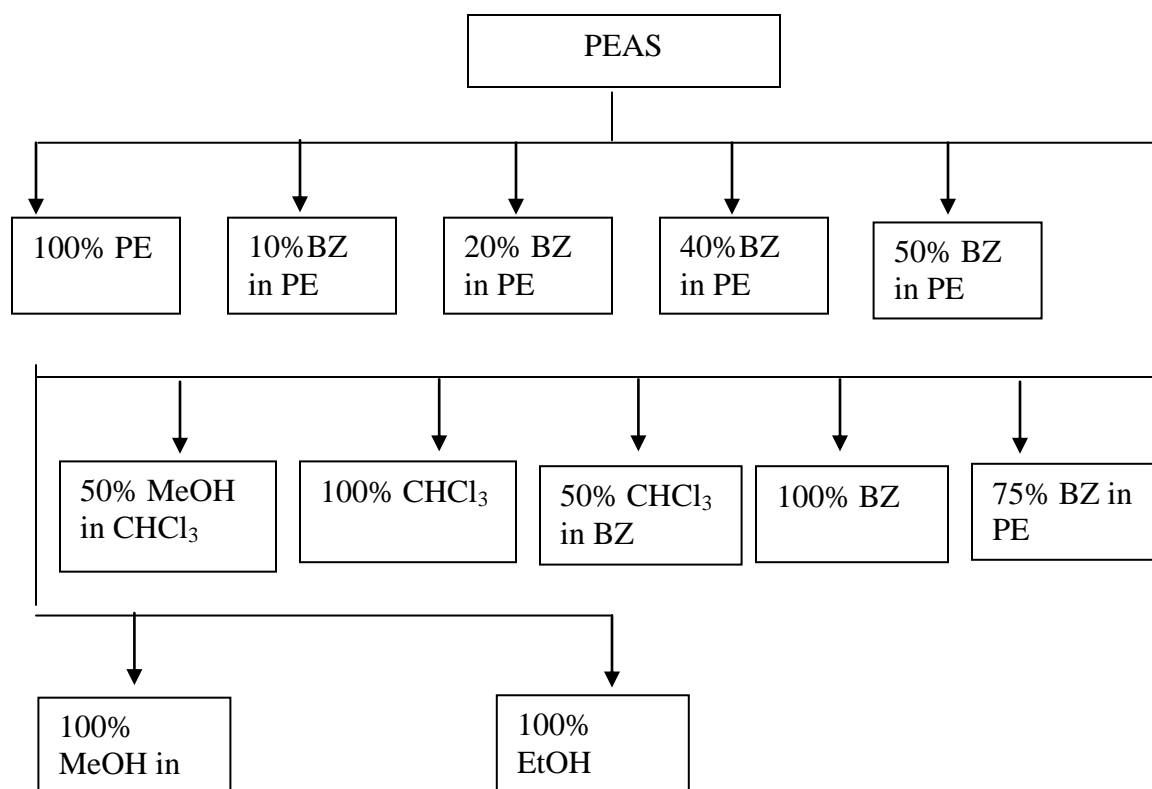
Table No: 4 Solubility profile of isolated compounds

Solvents	Compound I	Compound II
Pet ether	+++	+++
Benzene	+++	+++
CHCl ₃	+	-
MeOH	-	-
Water	-	

+++ Freely soluble, ++ partially soluble, - not soluble

Table No: 5 Melting point profile of isolated compounds

Isolated compound	Melting point (⁰ C)
Compound I	70
Compound II	78

Flow chart no.1: Flow chart of elution of column


PEAS- pet-ether extract of *Argyrea speciosa*, PE- pet-ether, BZ- benzene, CHCl₃-chloroform, MeOH- methanol, EtOH- ethanol. AS- *Argyrea speciosa*.

ACKNOWLEDGEMENTS

Authors are thankful to The Chairman, Director and Principal of The Oxford College of Pharmacy for providing all facilities to carry out research work. We also wish to thank Dr. Harish for identification and authentication of plant material. It's our gratitude to Mr. Jayanth and Ms Sashmita QUEST research and training institute, Bangalore for helping to do the antibacterial studies.



REFERENCES

- [1] Miller MD. J Assoc Off Anal Chem 1970; 53:123.
- [2] Sahu NP, chakravarti RN. Phytochem 1971; 10:1949.
- [3] Ahmad M, Jain N, Shafiullah, Khan MS, Ilyas M. J Chem Res Synop1993 ;(s):248.
- [4] Bakshi DN, Sensarma P, Pal DC. A Lexicon of Medicinal Plants in India. 2nd ed.Kolkata: Naya Prokash; 1999.
- [5] Gokhale AB, Damre AS, Saraf MN. J Ethnopharmacol 2003; 84:109-114.
- [6] Gokhale AB, Damre AS, Kulkarni KR, Saraf MN. Phytomedicine 2002; 9:433-7.
- [7] Khan IA, Khanum A. Herbal Wealth for Human Health antidiabetic Plants of India. 1st ed. Hyderabad: Ukaaz Publications; 2005.
- [8] Mukharjie K, Tripathi A, Kulkarni KS. Indian J Clin Practice 2003; 13:29-31.