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ABSTRACT

Objective: Natural products from medicinal plants, either as isolated compounds or as standardized plant extracts exhibit promising source of medicinal activity against various diseases. The aim of the present work was to make an attempt of isolation of bioactive principle and characterization of the isolated compound, from the medicinal plant Melia dubia

Methods: The extraction was done by a cold percolation method and the compound was separated and isolated by chromatography technique such as thin layer chromatography (TLC), column chromatography and high-performance liquid chromatography (HPLC). The isolated compound was crystallized and the structural characterization of the isolated compound was made using UV-Visible, FT-IR, 1H-NMR, GC-MS and MS techniques which confirmed the structure of the isolated compound.

Results: The separated and isolated compound was characterized by both physical and spectral methods like Ultraviolet-Visible spectroscopy (UV-Visible), Fourier transform infrared spectroscopy (FT-IR), Proton Nuclear Magnetic Resonance Spectroscopy (1H-NMR), Gas chromatography-mass spectrometry (GC-MS), and Mass spectrometry(MS). Based on the studies, organizational characteristics of one bioactive principle were deciphered. The results revealed that the isolated species is 2-Chlorobenzimidazole and it agreed well with the reported value and spectra for 2-Chlorobenzimidazole.

Conclusion: The above results obtained in this research work clearly indicated the promising occurrence of 2-chlorobenzimidazole in Media dubia plant leaves. The future scope of these studies may guide us to view the biological activity of the isolated compound.

Keywords: Melia dubia, Extraction, Isolation, Characterization, 2-Chlorobenzimidazole

INTRODUCTION

The genus Melia dubia from the Meliaceae family is mainly distributed in India, Sri Lanka, Malaysia, Australia and Angola [1]. Melia dubia tree grows up to 25 m tall. It is flowering between March to April and fruits during April [2]. A medicinal plant Melia dubia is deemed to be a chemical factory as it contains a multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oil and is confirmed by the photochemical screening tests. Quantitative analysis sees the presence of secondary metabolites such as alkaloids (0.98 mg/g), flavonoids (2.29 mg/g), saponins (5.7 mg/g) and terpenoids (1.1 mg/g). Proximate analysis and physicochemical analysis reveals that the percentage composition of Moisture(73.22%), Crude Protein (7.25 mg/g), Lipid (5.27%), Vitamine-B (40.8%), Vitamin-C (0.28%), Ash (99%), Acid insoluble ash (22.68%), Water soluble ash (36.65%). Alcohol soluble extractive (31.89%), Water-insoluble extractive (19.65%). Mineral composition of Melia dubia Leaves contains carbon 0.89%, Nitrogen 1.82%, Phosphorus 0.26%, Potassium 4.59%, Sodium 0.11%, calcium 6.59%, Magnesium 4.25%, Sulphur 0.52%, Zinc 1.69%, Copper 0.06%, Iron 159.32%, Manganese 11.20%, Boron 0.05%, Molybdenum 0.02% [3]. The results of the above analysis indicate the richness of bioactive compounds in Melia dubia plant leaves and it encourages continued development of separation methods and bioassays for their isolation and characterization.

A survey of the literature revealed that various parts of Melia dubia exhibits pharmacological activities like hepatoprotective activity, antulcer activity, antimicrobial activity, anti-inflammatory activity, anti-inflammatory activity, antifluorant activity, analgesic activity, anti-urolithiatic activity, antidiabetic activity, larvicidal activity, anticancer activity, antibacterial activity, ovicidal and biopesticidal activity was reported[1,4]. We herein report the isolation and identification of compounds from Melia dubia leave extract. The structure of the isolated compound was elucidated unambiguously by extensive spectral methods such as UV-Visible, FT-IR, 1H-NMR, GC-MS and MS.

MATERIALS AND METHODS

Chemicals and reagents

All the chemical and glassware (Hexane, Chloroform, Methanol, Ethanol, Iodine, DMSO, Double distilled water, precoated TLC silica gel 60 F) used for extraction and isolation were of analytical grade purchased from Ponnani and Co Chemicals, Trichy, Tamil Nadu, India.

Collection of plant material

The leaves of Melia dubia were collected from in and around areas in trichy and lies on the geographical coordinates of 10° 48’ 18” N, 78° 41’ 8” E at winter time and the plant is authenticated in the Rapinat Herbarium, St. Joseph’s College (Autonomous) Trichy, Tamil Nadu, India. The voucher specimen number of the Melia dubia leaves was GD002. The specimen copy of Melia dubia was showed in fig. 1.

Fig. 1: Specimen copy of Melia Dubia
Preparation of extracts

500g of dry powder of plant leaves were first soaked at room temperature, in hexane for 36 h. The extract was filtered using Whatmann filter paper. This was repeated for two more days and similar extracts were pooled together and concentrated at below 40°C under reduced pressure using a rotary evaporator. The residual plant material was extracted successively with chloroform and methanol in the same manner as followed for hexane. All the concentrated extracts were subjected to thin layer chromatography for further isolation process [5].

Isolation by thin layer and column chromatography

Since bioactive compounds occurring in plant material consist of multi-component mixtures, their extraction, identification and determination still creates problems. Practically most of them need to be purified by the combination of several chromatographic techniques and various other purification methods to isolate bioactive compounds.

The pre-coated silica gel thin layer chromatogram sheet was used solely for TLC. The chloroform extract was spotted at 2 cm from the edge of the sheet. The chromatogram is developed with a mixture of a suitable solvent system (trial-and-error method) and dried at room temperature. The spots were visualized in an iodine chamber. The Rf values of the coloured spots were recorded. Solvent systems for use as mobile phases in column chromatography can be determined from previous TLC experiments, literature, or experimentally. A glass column was packed with silica gel. Concentrated extract was ground with a small amount of silica gel and loaded on to the top of the column that was eluted with solvents by increasing polarity. A total of eleven fractions was collected, and the fraction with high quantity was subjected to HPLC for further purification level [5-7].

Purification by high-performance liquid chromatography (HPLC)

It is a joint practice in isolation of these bioactive compounds that a number of different separation techniques such as TLC, column chromatography, flash chromatography, Sephadex chromatography and HPLC should be used to obtain pure compounds. HPLC is the most frequently utilized method for isolation and purification of compounds from fractions or extracts.

High yield fraction purity was tested by using HPLC on a C-18 column (model HPLC system shimadzu CLASS-VP V6.14AP2) with an ultraviolet detector using a diode array detector (DAD) operating at 280 nm. An isocratic solvent system, consisting of methanol: water (7:3) was invoked as a mobile phase at a flow rate of 0.8 ml/min. Ultraviolet detection was carried out with a double array detector (shimadzu). The purity of the compound was analyzed by analytical HPLC and the highly purified compound was collected by preparative HPLC [8].

Characterization of the isolated compound

The pure compound was subjected to numerous spectroscopic analysis viz. UV-Visible, FT-IR, 1H-NMR, GC-MS, and MS to elucidate the structure together with its physical characteristics.

UV-visible analysis

The UV-visible spectrum of the isolated compound was recorded on Shimadzu spectrophotometer at room temperature. The wavelength of the maxima of the absorption spectrum so obtained was recorded (in nm). UV spectral measurement is important in the identification of various plant constituents. A UV-Visible spectrum of the isolated compound was carried out by methanol as a solvent.

FT-IR analysis

Characterization of the chemical structure was obtained by using Fourier transform infrared (FT-IR) technique. About 1 mg of the isolated compound was combined with spectroscopic grade KBr and well ground before preparing the pellet. The IR spectrum was taken in the wave number range of 500 to 4000 cm⁻¹.

1H-NMR spectra

Proton NMR (1H-NMR) is a plot of signals arising from absorption of RF during a NMR experiment by the different protons in a compound under study as a function of frequency (chemical shift). Nuclear magnetic resonance spectral studies were finished with the purified compound utilizing Buckner NMR 200MHz spectrophotometer. DMSO and TMS were used as the solvent and internal standard respectively [9, 10].

GC-MS analysis

Mass spectrometry is a powerful analytical technique for the identification of unknown compounds, quantification of known compounds and to elucidate the structure and chemical properties of molecules. Through MS spectrum, the molecular weight of the sample and its fragmentation can be determined. The GC-MS analysis was performed on a combined GC-MS instrument (ITQ900 Model of Fisher Scientific make) using a HP-5 fused silica gel capillary column. The method to perform the analysis was designed for both GC and MS. 1 μl aliquot of sample was injected into the column using a PTV injector whose temperature was set at 275°C. The GC program was initiated by a column temperature set at 60°C for 5 min, increased to 300°C at a rate of 8°C/min, held for 10 min. Helium was invoked as the carrier gas. The mass spectrometer was operated in E1 mode of the mass source was set at 200°C. The chromatogram and spectrum of the peaks were visualized. The particular compounds present in the samples were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of standard and technology mass spectral database (NIST-MS, 1998) library [11-13].

RESULTS AND DISCUSSION

Isolation and purification of a compound

The TLC result of chloroform extract exhibited many spots and high yield compared to the hexane and methanol extracts. The TLC result of chloroform extract is displayed on fig. 2. Therefore chloroform extract is employed to isolation steps by developing column chromatogram. Eleven fractions came off from column chromatography and fractions showing similar spots were pooled together and Rf values were recorded. Among eleven fractions the fourth fraction shows high quantity than the others. The eluent used in TLC and column chromatography was hexane: ethyl acetate (7:3). The elution of the column was given in fig. 3.
High-performance liquid chromatography (HPLC) of CD-1

HPLC analysis was performed for the fourth fraction and the spectrum was shown in fig. 4. The HPLC chromatogram of fraction four has shown only one peak predominant. The most predominant peak in 251,923,319 areas and 76,282,52 heights is observed at the retention time 6.283 (Rt min). In the HPLC chromatogram of fraction four apart from the dominant peak, few inconspicuous peaks were also detected having percent area greater than ten percent, which may be attributed to the presence of certain impurities in very small concentration along with the fraction. The purified form of the isolated compound was collected with the help of preparative HPLC and it was named as compound-1.

Characterization of compound-1 (CD-1)

The isolated compound was crystallized by ethanol and the structure was elucidated by physical and spectral methods.

Physical characteristics

Compound CD-1 was a brown color and the melting point was observed to be 309°C. It showed a Rf value of 0.82 in TLC in the solvent system of hexane: ethyl acetate (7:3). It was soluble in chloroform, hexane, benzene, ethyl acetate and ethanol. The spot turned into a brown color in an iodine chamber.

Spectral characterization of the compound-1 (CD-1)

The UV-Visible spectrum of CD-1 was done by dissolving CD-1 in ethanol and the spectrum exhibited maximum absorption at 262 nm. The characteristic spectrum of CD-1 indicates the characteristics of imidazole. The absorption spectrum and FT-IR spectrum of compound-1 is illustrated in fig. 5 and 6 respectively.

A sharp peak at 3423.65 cm⁻¹ indicates the presence of N-H group in the isolated compound-1. Similarly, the peak at 1627.92 cm⁻¹ clearly indicates the presence of C=O group and the absorption range at 2922.16 cm⁻¹ was due to C-H bond vibration in CD-1. The peak at 831.32 cm⁻¹ is responsible for vibrations of the C-Cl bond in CD-1.
The $^1$H-NMR spectrum of the CD-1 showed the following details. Proton NMR in DMSO showed peaks at Delta (6.50-7.90 ppm) of the 9m,4H,aryl protons which is responsible for the aromatic protons and of 12.259(s,1H, -NH-) is due to the NH proton in the imidazole ring. The peak at 2.5 ppm indicates four proton unsymmetrical patterns in the aromatic ring and the spectrum is shown in fig. 7.
The GC-MS spectrum of CD-1

Compound 1 was tested by GC-MS and this compound gave a single peak confirming the presence of only one compound. The retention time of the compound was 5.96 min with more predominance. GC-MS data showed major parent molecular ion (M+1) peak at m/z=151.71(100%) which corresponds to the molecular weight of 2-chlorobenzimidazole. The elemental analysis of this compound-1 reveals that C-55.10%, H-3.30%. CI-23%, N-18.36% is present. The elemental analysis and mass spectrum indicated the chemical formula as C<sub>7</sub>H<sub>5</sub>ClN<sub>2</sub>.

![Fig. 9: Mass spectrum of CD-1](image)

The structural characterization of the isolated compound was determined by both physical and spectral methods like UV-Visible, FT-IR, <sup>1</sup>H-NMR, GC-MS, and MS. The chemical formula and name of the isolated compound were C<sub>7</sub>H<sub>5</sub>ClN<sub>2</sub> and 2-chlorobenzimidazole respectively. This was confirmed by the above-mentioned characterization techniques. The compound 2-chlorobenzimidazole is involved in many synthesis processes and it was used as a building block to synthesize 4-amino 6-benzimidazole-pyrimidines. The bioactive compounds in plants may be acting independently or in synergy, with one another to produce the biological activities. The future scope of these studies may guide us to do the biological activity of the isolated compound.

**AUTHOR CONTRIBUTION**

All the authors contributed equally to the successful completion of this research work.

**CONFLICT OF INTERESTS**

There is no conflict of interest

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How to cite this article

- G Dayana Jeyaleela, S Irudaya Monisha, J Rosaline Vimala, An Anitha Immaculate. Isolation of 2-Chlorobenzimidazole from Melia dubia leaf extract and its structural characterisation. Int J Pharm Pharm Sci 2017;9(10):67-72.