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Original Article

A NEW ALKALOID FROM *DERRIS INDICA* (LAM) BENNETT SEED OIL: ISOLATION AND CHARACTERIZATION

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ABSTRACT

Objective: The aim of the study was to isolate alkaloid compound from seed oil of *Derris indica* (Lam) Bennett where relevant antimicrobial properties in traditional medicines.

Methods: The plant was selected based on their usage in traditional medicines and ethnopharmacological importance. Crude extract from *D. indica* seeds fractioned with different solvents through column chromatography. Isolated pure fraction was identified and characterized using UV, FTIR, ^{1}HNMR and Mass spectroscopy.

Results: *D. indica* seeds hexane extract on fractionation with ethyl acetate and methanol through column chromatography yielded a crystalline fraction. The fraction was identified as alkaloid group and characterized as a 2-(6-methoxyphenanthridin-8-yl) propan-2-ol. The compound is a new report from *D. indica* seed oil.

Conclusion: The usage of *D. indica* plant is much in traditional health care for treatment of diseases. Isolation of alkaloid compound from *D. indica* seeds in traditional herbal medicines may be found a good source of drug discovery.

Keywords: *Derris indica*, Seed, Phytochemical analysis, Alkaloid

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INTRODUCTION

The medicinal plants always played an important role in the global health and providing a new area of drug research [1]. Since from numerous years, there is increasing a great demand in developing and developed countries for plant-based medicines, food supplement, health products, pharmaceuticals, and cosmetics. Majority of peoples rely on natural products as they are non-toxic, have lesser side effects and easily available [2]. Secondary metabolites have been developed in nature form of different plant species, insects, fungi, algae, and prokaryotes during evolution in enormous diversity. Plant secondary metabolites, such as phenolic compounds, flavonoids, alkaloids, and tannins are reported to exert numerous biological properties [3, 4]. The alkaloids are among the most important active components in plants and important classes of secondary metabolites which are found to possess important pharmacological properties like analgesic, muscle relaxant, antioxidant, anticancer, antibacterial activities, etc. Hence, responsible for many therapeutic properties in natural medicine and found beneficial for certain life-threatening diseases [3, 5, 6]. Therefore, there is urgent need to discover new therapeutic compounds with diverse chemical structures and with novel mechanism of action is required for new and emerging infectious diseases [7].

Derris indica (Lam) Bennett, a medium-sized evergreen, fast growing, glabrous, deciduous nitrogen fixing, bio-diesel tree belonging to the family Fabaceae, widely distributed in the region of south-east Asia and Pacific Islands [8]. Seeds containing 30-40% oil, used for treating various diseases such as piles, skin diseases, bronchitis, whooping cough, fever, ulcers etc [8, 9]. *D. indica* plant has various types of chemical compounds [10]. There are several reports on presence of wide range of flavonoids in plant with antimicrobial activity [11-23]. Numerous compounds have been isolated and reported, such as karanjin, pongapin, kanjone, pongaglabrone, Pongamosides A, B and C, and a flavonol, glucoside and pongamoside D, Pongaglabol and pinnatin [14, 24-26]. Biologically active compounds obtained from plant sources have always been a great interest for scientists working on infectious and non-infectious diseases [27]. Therefore, the aim of the present investigation is to isolate alkaloid compound from the *D. indica* seeds.

MATERIALS AND METHODS

Plant material

Mature seeds of *D. indica* were collected from Gulbarga University campus and local areas of Kalaburagi district, Karnataka. The plant species initially identified by a field guide and later confirmed by herbarium with a voucher specimen (No. HGUG-206) of *D. indica* deposited in the herbarium centre, Department of Botany, Gulbarga University, Kalaburagi, Karnataka, India and also with literatures [28-30].

Extraction and isolation of compound

Seeds were washed with water, shade dried for 8-10 d at room temperature and pulverized using a grinder. The seed powder was stored in air tight container for further extraction. Dried seed powder was subjected to Soxhlet extractor for extraction with 500 ml of n-hexane for 48h at 40 °C and filtered. Further, extract was subjected to thin-layer chromatography (TLC) on silica gel-G plates (Hi-media, Mumbai, India) using solvent system ethyl acetate and methanol (7:3 v/v) used as mobile phase to separate alkaloid on stationary phase. After development, Dragendroff's reagent test was carried out for detection of alkaloid.

For isolation of compound, obtained extract was dissolved in hexane and consecutively, 10g of soluble fractions was subjected to column chromatography (Silica gel-H, 60-120Mesh, Hi-media, Mumbai, India) using solvents, n-hexane, hexane, petroleum ether, chloroform, ethyl acetate, methanol (Sd-fine, Mumbai, India) to afford 200 ml fractions each. Subsequently, elution was carried out by gradual changing the concentration (90:10, 80:20, 70:30, 60:40, 50;50, 40:60, 30:70, 20:80 and 10:90 v/v) of above mentioned solvents. During elution flow rate was set to 5-10 drops/min. using vacuum evaporator each fraction was concentrated and subjected to

TLC. Spots was visualized by spraying with 40% H_2SO_4 (v/v) followed by heating at 80 °C for 15 min and TLC plate was detected under visible light. Fraction-2, yielded single spot on TLC was collected. Purity of compound was confirmed by performing TLC studies by using different solvent system as mobile phase. Plates were sprayed with Dragendroff's reagent. Fraction-2 obtained fine crystals, further purified by recrystallization from methanol to provide pure compound respectively. Obtained compound was further identified by UV, FTIR, LCMS and ^{1}HNMR analysis and compared with literature data.

Characterization of isolated compound D2

Melting point of obtained fraction was determined by Thiele tube melting point apparatus. A UV/VIS spectrum was obtained in the range of 200-800 nm wavelengths using a 5704SS ELICO spectrophotometer. The Fourier transform infrared (FTIR) spectrum was measured with a KBr discs in wave numbers (cm^{-1}) in the range of 4000-450 cm^{-1} recorded as inverted peaks using Perkin-Elmer RX1 spectrophotometer. Obtained fraction was subjected to mass spectrometry analysis (MS) to determine molecular ions present in isolated compound. Fraction was analyzed and measured in $CDCl_3$ (denaturated chloroform, DMSO) with a Shimadzu MS 2010 A (temperature of 27 °C) using M-nitrobenzyl alcohol (NBA) as matrix. The retention index was determined relative to the retention times in JEOl Model GSx 400 spectrometer. NMR was performed to determine the structure of the compound, ^{1}HNMR was recorded using Bruker AMX 400 NMR spectrometer at 300K in $CDCl_3$ and chemical shift was recorded in δ (ppm) and J in hertz value relative to Tetramethyl saline (TMS) as internal reference at 400.137 MHz.

RESULTS AND DISCUSSION

Identification and characterization of isolated compound D2

In the present study compound D2, obtained as a pale yellow colour crystals (hRf: 86.66) in TLC (ethyl acetate: methanol, 7:3) and melting point was recorded as 136-138 °C. UV spectrum showed UVλ_{max} nm wavelength at 653.5 and absorbance at 0.262 (fig. 1). The FTIR spectrum shows the presence of broad main absorption band of Hydroxyl group (-OH) at 3358 cm^{-1} and additional moderate intense bands 1059 cm^{-1}, 1275 cm^{-1} and 1360 cm^{-1} present between the ranges (600 cm^{-1}-1600 cm^{-1}). Band 2930 cm^{-1} is due to stretching (C-H) and 1652 cm^{-1}shows stretching of C=N group (fig. 2). The Mass spectrum indicated the molecular ion peak at m/z 267 and base ion peak at m/z 191(fig. 3). Its molecular formula was assumed as $C_{17}H_{17}NO_2$ and molecular weight is 267.33. The ^{1}H NMR spectrum of compound D2 showed signal at δ_H 8.20 (1H, dd, J= 4Hz), and multiplet between δ 7.77-7.19 characteristic to aromatic protons, one methoxy protons [δ_H 3.95(3H,S)], two methyl and hydroxyl protons exhibited signals between [δ_H 2.0 (1H, br S)-1.27(3H,S)]. The physico-chemical properties and spectroscopic data indicated that compound D2 as alkaloid and considering the comparative results of spectral studies and structure of D2 was predicted as, 2-(6-methoxyphenanthridin-8-yl) propan-2-ol as shown in fig. 4. These spectroscopic data support the proposed structure D2 (fig. 4, table 1). The structure of compound was established on the basis of spectroscopic studies.

Table 1: ^{1}H NMR Data for isolated compound D2 (400.137 MHz, δ in ppm, *J* in Hz)

Position	1[a]
3	8.20 (1H, dd, J= 4Hz)
4	7.46 (1H, d, J= 6.08 Hz)
5	7.19 (1H, S)
6	7.46 (1H, d, J= 6.08 Hz)
9	7.46 (1H, d, J= 6.08 Hz)
10	7.28 (1H, S)
13	1.27 (3H, S)
14	2.0 (1H, br S)
15	1.2 (3H, S)
16	7.46 (1H, d, J= 6.08 Hz)
20	3.95 (3H, S)

[a]Recorded in $CDCl_3$

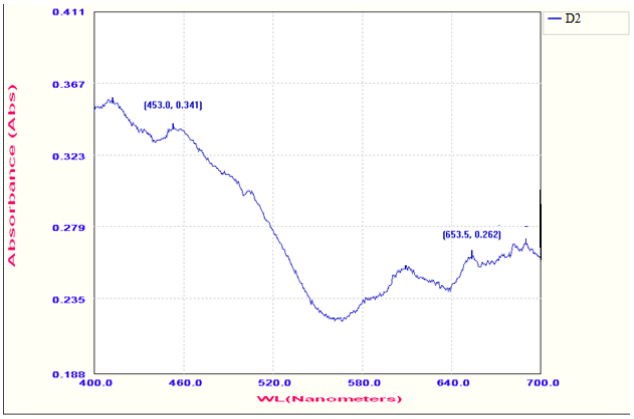

Fig. 1: UV spectrum of isolated compound D2

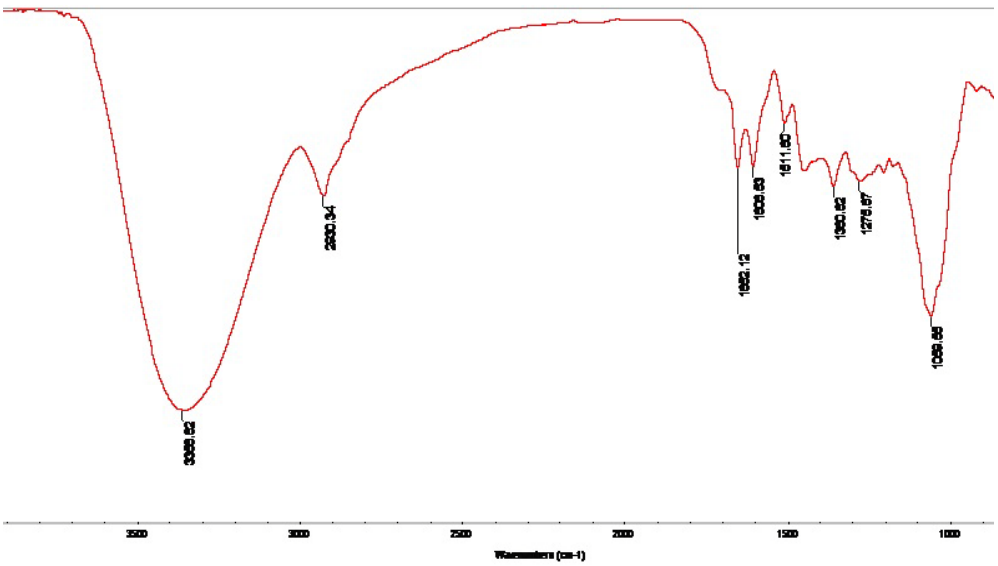

Fig. 2: FTIR spectrum of isolated compound D2

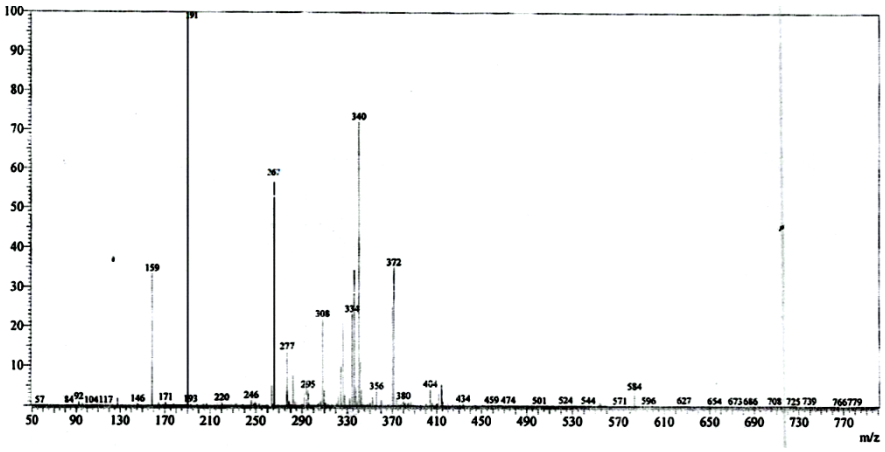

Fig. 3: Mass spectrum of isolated compound D2

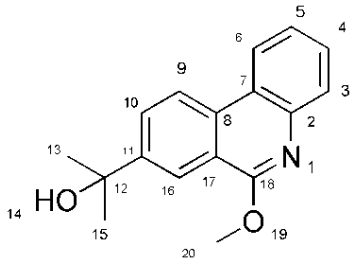

Fig. 4: Structure of isolated compound 2-(6-methoxyphenanthridin-8-yl) propan-2-ol

According to reports, alkaloids are secondary metabolites originally defined as pharmacologically active compounds, primarily composed of nitrogen [31]. Presence of furanoflavones and isolated numerous phytocompounds from *D. indica* seeds have previously reported by many authors [32-35], Pongamol [36-38] and, Furanodiketon [39], Karanjachromene[40]isopongachromene [39], isoponga flavone [41], O-methyl pongaglabol [38], 3,3^1,4^1,7-tetramethoxyflavone [39], and some fatty acids [42], Pongamoside D [15]. The other flavonoid includes Glabrachalcone, isopongachromene, Karangin, Glabrachalcone, Isopongachromene [43]. A new crystalline compound Pongamol isolated from Pongamia oil which crystallizes from alcohol was reported [44]. Pongapin (II) has been isolated from the ethanolic extract of seed of *P. pinnata*. The residue crystallized from methanol gives a colourless needle [33]. Pongol, a new furanoflavone, obtained as shining yellow needles from ethanol [34].

Several researchers reported that the alkaloids, phenols, triterpenoids, glycosides and tannins, have high potential that can be developed as antimicrobial compounds against pathogenic microorganisms [45, 46]. Natural antimicrobial compounds derived from secondary metabolite play a significant role that provide as resistance mechanisms against many microorganisms [47-50]. After reviewing literatures, this is the first time report of occurrence of alkaloid compound from *D. indica* seed oil and to the best of our knowledge; no other studies have been reported. In these viewpoints, there is an increasing demand for effective antimicrobial agents, justifying in searching new antimicrobial alkaloid drugs.

CONCLUSION

In traditional systems of medicines, *D. indica* has been widely used for variety of ailments. The literature survey revealed that *D. indica* is an important medicinal plant and shows the presence of many phytoconstituents responsible for varied medicinal properties. Alkaloid compound D2 with desirable phytochemical, certainly encourages future advanced research and provides a significant basis for the development of pharmaceutical drug.

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AUTHORS CONTRIBUTIONS

Vidyasagar GM designed the experiment and involved in the interpretation of data. Nuzhat Tabassum carried out the experimental work, analysis of data and manuscript preparation. Authors went through the final manuscript.

CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest.

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