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Isolation, Structure Elucidation and Antimicrobial Studies of Aristolochic Acid from *Aristolochia bracteolata* Lam.

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Abstract

Aristolochic acid (AA I) was isolated from the dominant weed *Aristolochia bracteolata* Lam. Its detailed structural elucidation was obtained by sophisticated spectroscopic techniques like UV, IR, ^{1}HNMR, HPLC, LCMS and X-RAY Crystallography. Isolated aristolochic acid was screened against the *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus whentii, Fusarium monoliformae* and *Penicillum imblicatum* for its biological activities. Spectroscopic studies revealed that Aristolochic Acid I (AA I) is the principal constituents of *Aristolochia bracteolata* Lam. Antimicrobial studies led to the significant inhibitory effects against all pathogens.

Keywords: Aristolochic acid, LCMS, XRD, monoclinic, space group, antimicrobial.

Introduction

Aristolochia bracteolata Lam. a dominant weed from arid regions of Baramati belongs to family Aristolochiaceae [1]. As family aristolochiaceae belongs to core family group of eudicots, it exhibits high taxonomical and biochemical flexibility. As it is a rich source if secondary metabolites. The plant is widely used in local medicine for various diseases and shows flexibility in application. Reports of being used as ethnomedicine in Africa, India and Middle east and also quoted in Ayurveda are available [2]. Geographically it is widely spread across Africa, India and Arabian Peninsula. The plants grows 50-740 m above sea level on the banks of rivers, desserts and in sandy eroded soil [3].

The plant reported to show CAM nature of photosynthesis as it bears succulence. [4]. It is known as "worm killer" in earliest reports due to its anthelmintic property [5, 6]. It has long been used in traditional medicine for the treatment of pruritus, intestinal worms, wounds healing and scorpion and snake bites [7, 8]. This *Aristolochia bracteolata* Lam. has excellent phytochemical profile and enthusiastically explored for its pharmacological properties.

It is found that biological properties of *Aristolochia bracteolata* Lam. are exhibiting presence of Aristolochic Acid I (AA I) as dominant secondary metabolite of alkaloid group. Figure 1. one of its principal constituents [9, 10]. Aristolochic acid (AA I) is carcinogenic and causes serious mutations. The FDA has banned the consumption of supplements containing aristolochic acids [11, 12]. Number of studies has been carried out to meticulously cast off this toxic property as a remarkable therapeutic utility to treat cold, headache, pruritus, inflammatory diseases, antitumor activity, insect bites and sexual problems [11, 12, 13]. In our present pursuit of bioactive natural compounds from this indigenous medicinal plant an isolation, detailed studies on chemical investigation and antimicrobial activities were carried out for isolation of Aristolochic Acid I (AA I) from dried roots of *Aristolochia bracteolata* Lam. grown in region of Pune district, Maharashtra, India. This compound is further analyzed for its chemical and biological properties as this can be further used for effective potential drug.

Methodology

Extraction and Isolation

Dried roots of *Aristolochia bracteolata* Lam. (25g) was extracted with 100 ml of methanol under reflux conditions for 3 hrs to give a crude extract, successively extracted with $NaHCO_3$ for conversion of acid into its sodium salt. Then, aqueous solution of sodium salt of Aristolochic acid acidified with concentrated HCl to get isolated carboxylic acid in solid form. The solid obtained (0.758g) was further dried under IR lamp to get the yellow-color solid residue of aristolochic acid.

TLC analysis of standard and isolated Aristolochic acid (AA I), (8-Methoxy-6-nitro-2*H*-phenanthro[3,4-*d*] [1,3] dioxole-5-carboxylic acid showed a single spot in n hexane: ethyl acetate (80:20) mobile phase and Rf value 0.45, no secondary spot was observed in TLC. It indicates that isolated acid is aristolochic acid in its pure form. Aristolochic acid displayed black color on fluorescent silica-gel. It confirms that the isolated compound is aristolochic acid free from any detectable impurities. Melting point recorded was 260^0C.

Experimentation

General: Standard aristolochic acid purchased from the Sigma Aldrich. TLC plate silica gel 60 F_{254} -Merk Germany. Infrared spectrum was obtained on a Bruker ALPHA FTIR spectrometer. Absorptions are reported on the wave number (cm–1) scale, in the range 400–4000 cm–1. The HPLC system was Shimadzu, LC solution equipped with UV detector. Mass spectra of isolated aristolochic acid obtained on Shimadzu LCMS 2020 system using the column Waters X Terra MS C-18 (100x 2.1mm,3.5µ) and mobile phase Methanol: Water (0.1%v/v Trifluro acetic acid). The ^{1}H-NMR and ^{13}CMR spectrum were recorded at 400 MHz using Bruker spectrometer in DMSO – d^6. X-ray diffraction patterns obtained using Philips X-ray diffractometer Model PW 3710.For λ-1.05406 and 2.2897A^0 for Cu-Kα and Cr-Ka radiation respectively.

UV spectrum: (EtOH, λmax, nm): 314;

FT-IR spectrum: (KBr, ν, cm–1) 3353 (OH), 1680 (C=O), 1518 and 1343 (NO2), 1515 (Ar),1630 (Ar);

Mass Spectrum: (EI, 70 eV), *m*/*z* (*I*rel, %): 341 ([M]+, 35), 295 (100) , 280 (45), 150 (30), 113 (15), 99 (15), 87 (15);

^{1}H-NMR spectrum:(400 MHz, DMSOd6, δ, ppm, J/Hz): 8.68 (1H, J = 8.8), 8.56 (1H, s), 7.98 (1H, dd, J = 8.8), 7.90 (1H, s), 7.52 (1H, d, J = 8.8), 6.42 (2H, s).

^{13}C NMRspectrum: (400 MHz, DMSOd6, δ, ppm): 146.5 (C-aromatic), 149.3 (C-aromatic), 125.3 (C-aromatic), 122.1 (C-aromatic), 120.1 (C-aromatic), 113.3 (C-aromatic), 138.9 (C-aromatic), 123.1 (C-aromatic), 121.0

(C-aromatic), 146.5 (C-NO_2), 114.8 (C-aromatic), 127.8 (C-aromatic), 109.4 (C-aromatic), 155.9 (C-OCH_3), 101.1 (O-CH_2-O), 169.0 (-COOH), 56.3 (OCH_3),

XSA: Crystalline size found 35nm P21 / C with unit lattice *a* (A^0) = 17.9200, *b* (A^0) = 5.2540, *c* (A^0) = 9.5700 (A^0), α=90.0000(A^0), β=106.2700(A^0), γ = 90.000 (A^0).

Antimicrobial studies: Isolated aristolochic acid was screened for antimicrobial activity through the disc diffusion assay method using 100 µl of suspension of tested microorganisms. The bacteria strains were grown in 50 ml of nutrient broth at 37°C and maintained in nutrient agar slant at 4°C. However, the fungal strains were grown and maintained in Sabouraud dextrose agar (SDA). Filter paper discs (6 mm) were individually impregnated with 10 µl of extract of isolated aristolochic acid and then placed on to the agar plates, which had been previously inoculated with tested microorganism. Further incubated at 37 ^{0}C for 24 hr for bacteria and 48 hr for fungal strains. Antibiotic amphicillin served as a positive control for bacteria and Nystatin for fungi. The antimicrobial activity was evaluated by measuring diameter of the inhibition zone around the disc. All the experiments were performed in triplicate and the average zone of inhibition was reported.

Result and Discussion

UV absorption spectra shown the absorption maximum at wavelength 314 nm. It gives information that both standard and sample have same chromophores with highly conjugated system.FTIR spectrum shown the prominent peaks at 3353 cm-1, peak and 1680 cm-1 suggest presence of hydroxy (-OH) and carbonyl group respectively. This supports the presence of carboxylic group. Further, peaks at 1518 cm-1 and 1343cm-1 accounts for the presence of nitro (-NO2) group. Peaks at 1500 and 1600 cm-1 associated with aromatic rings.

Table 1. Antimicrobial activity of isolated aristolochic acid from *Aristolochia bracteolata* Lam.

Test Organisms	Isolated A. acid (ZOI in mm)	Positive Control (Ampicillin) ZOI (mm)	Antibiotic (Nystatin) ZOI (mm)
Escherichia coli	19	22	-
Pseudomonas aeruginosa	08	11	-
Staphylococcus aureus	15	20	-
Aspergillus whentii	17	-	20
Fusarium monoliformae	13	-	21
Penicillum imblicatum	09	-	11

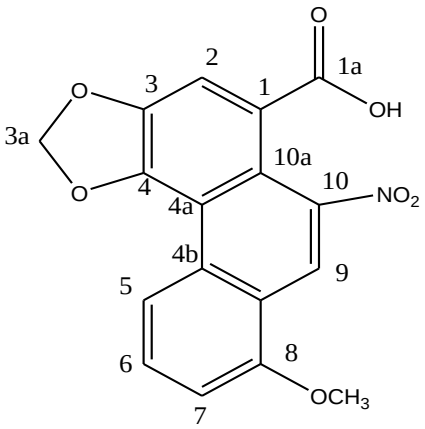

Figure 1: Chemical structure of Aristolochic Acid.

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^{1}HNMR analysis of isolated aristolochic acid shows singlet's at 8.68 ppm 8.56 for hydrogen corresponds to proton ortho to the nitro group and carboxylic group respectively, chemical shifts at 7.52 ppm assigned to the proton ortho to the methoxy group, singlet at 6.42 for two hydrogen confirms the presence of [d][1,3]dioxole ring system. For the HPLC analysis of Aristolochic acid (8-Methoxy-6-nitro-2*H*-phenanthro[3,4-*d*][1,3]dioxole-5-carboxylic acid) system was maintained with the flow rate 1 ml/min with UV detector and (Waters® 2996, USA); C18 250 × 4.6 mm column C18 column. The mobile phase utilized was Methanol: Water: Acetic acid (67:32:1). Peak was detected at retention time 12.66 min as per previous reports in literature which confirms the presence of Aristolochic Acid [13, 14].

Important analysis in identification of aristolochic acid I was determination of mass spectrum. The predominant peak on mass spectra was found at m/z 341.20 which resembles to the molecular ion peak [M+] of Aristolochic acid I (AA I). This approves the major constituent of root extract is aristolochic acid I. Structural properties of aristolochic acid were studied by XS analysis shown that the crystals belong to monoclinic space group. The disc diffusion test studies revealed that isolated aristolochic acid showed growth inhibitory activity against pathogenic bacteria like *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Maximum antibacterial activity was against *E. coli* whereas maximum zone of inhibition was observed in fungal strain *Aspergillus wentii* (Table 1).

Conclusions

From the above studies it is clear that aristolochic acid (I) isolated from *Aristolochia bracteolata* Lam. is exhibiting several unique properties which are worth for further studies. Its structure elucidation was carried out with sophisticated spectroscopic technique and confirmed by previous literature. Anti-microbial activity has shown that isolated aristolochic acid exhibits potent activity against all pathogens. Synthetic drug characterization and microbial studies proves its importance in drug discovery. In conclusion, aristolochic acid it may be effectively used in drug discovery.

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