ISOLATION AND CHARACTERIZATION OF PHYTOCONSTITUENTS FROM STEM OF *Elytranthe* *parasitica*(L.) Danser

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ABSTRACT

Mistletoes have earned a strong profile in treating cancer. *Elytranthe parasitica* (L.) Danser (EP), a hemi-parasitic plant belonging to Mistletoe family or Loranthaceae is known for its potent anti-cancer properties. Exploring the phytochemistry by isolating and characterizing phytoconstituents from unidentified mistletoes such as *Elytranthe parasitica* (L.) Danser would prove useful for cancer therapy. Stem parts of *Elytranthe parasitica* were extracted with methanol and sequentially fractionated with solvents in the increasing order of polarity. The diethyl ether fraction was selected for bioactivity guided fractionation. Fractionation was performed via gel filtration chromatography and analytical TLC to isolate the phytochemical(s). Activity guided fractionation led to the isolation of two phenolic compounds and two phytosterols: a phenolic acid (gallic acid), a flavanone (pinostrobin), a triterpenoid (lupeol) and a phytosterol (β -sitosterol)

Keywords: Elytranthe, Isolation, Gel Permeation, NMR, Sephadex LH-20, Silica

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INTRODUCTION

Traditional plants provide an infinite source of life-saving drugs for most of the world's population.¹ Bioactive extracts and fractions from these plants yield diverse bioactive molecules that play a crucial role in combating diseases.² An exhaustive study of phytochemicals from medicinal plants could help discover prototypes in the drug discovery process.³

Genus *Elytranthe* comprises many plants demonstrating strong anti-cancer activity.⁴ *Elytranthe parasitica* (L.) Danser is an annually growing parasite (Mistletoe family, Loranthaceae). Known as the parasite honeysuckle, it is found commonly on host trees such as the sacred fig, neem, mango, and jackfruit trees along the Western Ghats of India.⁵ Traditionally, it has been used as veterinary medicine and as a leaf paste to eradicate ticks.⁶ *Elytranthe maingayi* and *Elytranthe tubaeiflora*, growing indigenously in Indonesia, have been traditionally used in cancer therapy.⁴ Accumulating evidence has found that mistletoes exhibit high antioxidant and anti-cancer activity.⁷ European mistletoe (*Viscum Album*) comprises cytotoxic compounds: lectins, viscotoxins and flavonoids⁸ and is extensively used as in complementary cancer therapy. A previous study on phytochemical analysis of *Elytranthe parasitica* indicated the presence of phytoconstituents: tannins, flavonoids, phytosterols, saponins, and carbohydrates.⁹ Water and alcoholic extracts of *Elytranthe parasitica* stem parts exhibited moderate free radical scavenging and antioxidant activity¹⁰ and inhibited Ehrlich's Ascites Carcinoma *in vivo*.¹¹ However, most mistletoes species, except *Viscum album* have not been investigated thoroughly. Exploring the phytochemistry by isolating and characterizing from unidentified mistletoes would be valuable; this would provide an insight into the phytochemicals responsible for the bioactivity. In the present study, we endeavored to isolate and characterize phytocompounds from the dried stem of *Elytranthe parasitica* (L.) Danser.

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EXPERIMENTAL

Chemicals

Sephadex LH-20 (#8203705543) was procured from GE Healthcare, Uppsala, Sweden. Silica gel (60-120 mesh), pre-coated HPTLC and TLC silica gel aluminum plates 60F254 (20 cm × 20 cm with 0.2 mm thickness) were procured from Merck Life Sciences Ltd Pvt, Mumbai, India.

Preparation of Reagents

TLC analysis was performed by visualizing the plates in short and long ultraviolet light. The plates were derivatized by dipping in Anisaldehyde sulphuric acid [ANS] reagent and heating [120 °C, 5 min].

Instrumentation

CAMAG HPTLC system (CAMAG Chemie-Erzeugnisse & Adsorptionstechnik AG, Muttenz, Switzerland) consisting of Linomat 5, Scanner 4, UV Cabinet 4, Twin trough chambers, WinCATSV 1.2.3 software. ¹H and ¹³C NMR spectroscopy was performed on Bruker 400 MHz High-Resolution Multinuclear FT-NMR spectrometer (Bruker Corporations, Massachusetts, USA). Chemical shifts were expressed as δ (parts per million). Agilent 6250 Accurate-Mass Q-TOF (Quadrupole Time-of-Flight) MS/MS system joined to an Agilent 1200 HPLC (Agilent Technologies, California, USA) was used to record the ESI-MS spectra. FTIR spectra were recorded on a PerkinElmer System 2000 FT-IR Fourier spectrometer (PerkinElmer, Massachusetts, USA) using KBr pellets.

General Procedure

Isolation of Phytoconstituents

Stem parts of *Elytranthe parasitica* were extracted with methanol and sequentially fractionated with solvents in the increasing order of polarity: petroleum ether, diethyl ether, ethyl acetate, butanol and water. The diethyl ether (EP.DEE) fraction (20 g) was fractionated on a Sephadex LH-20 packed column using methanol via isocratic elution. The column eluents were detected by Thin Layer Chromatography (TLC) and pooled thereafter to acquire six fractions (FR 1, 2, 3, 4, 5 and 6). FR 3 (7 g) was fractionated on Sephadex LH-20 column with 100% methanol to obtain 14 fractions (FR 3A-FR 3N). Compound 1 (3.8 mg) was obtained by fractionating subfraction FR3 G, H (1.90 g) with 50% methanol: water. 1 g of fraction FR1 was fractionated stepwise gradient using solvent combinations petroleum ether: ethyl acetate followed by ethyl acetate: methanol to yield compound 2 (3.5 mg) and compound 3 (3 mg) respectively. Subfraction FR 3E (450 mg) was fractionated on sephadex LH-20 with 100% methanol to afford compound 4 (9 mg).

Detection Method

Characterization of phytoconstituents

Chromatographic isolation of the stem parts of *Elytranthe parasitica* bioactive fraction yielded five phytoconstituents (Compound 1-4, Fig 1). The isolated phytoconstituents were identified and characterized by spectroscopic techniques (¹³C NMR, ¹H NMR, HR-ESI-MS, LC-ESI-MS/MS, FTIR and UV) and comparing their spectral data with previously reported literature.

Spectral Data

Gallic Acid (1)

NMR: (¹H, 400 MHz, DMSO) δ ppm: 11.89 (COOH), 8.8 (H-1), 9.2 (H-2), 6.9 (H-3); NMR: (¹³C, DMSO) δ ppm: [167.71 (C-1, C=O), 145.5 (C-2), 138.1 (C-3), 120.5 (C-4), 108.8 (C-5)].

Lupeol (2)

NMR: (¹H, 400 MHz, DMSO) δ ppm: 4.67 (s, 1H, H-29), 4.56 (s, 1H, H-27), 3.21 (d, 1H, H-3), 2.37 (m, 1H, H-19), 1.91 (m, 1H, H-21), 1.69 (s, 3H, H-30), 1.67 (t, 1H, H-13), 1.61 (s, 3H, H-2A), 1.54 (s, 3H, H-2B), 1.53 (d, 1H, H-11), 1.42 (d, 1H, H-14), 1.33 (q, 1H, H-21), 1.39 (s, 1H, H-21), 1.29 (q, 1H, H-12), 1.28 (s, 1H, H-9), 1.04 (s, 1H, H-23), 1.01 (d, 1H, H-15), 0.98 (s, 3H, H-23), 0.97 (s, 3H, H-27), 0.91 (t, 1H, H-18), 0.84 (s, 3H, H-25), 0.79 (s, 3H, H-28), 0.69 (d, 1H, H-5).

NMR: (^{13}C , DMSO) δ ppm: 150.80, 109.32, 79.02, 77.27, 77.01, 76.76, 55.31, 48.72, 48.32, 48.00.

β -sitosterol (3)

NMR: (^1H , 400 MHz, DMSO) δ ppm: 3.53 (tdd, 1H, H-3), 5.36 (t, 1H, H-5), 0.93 (d, 3H, H-19), 0.84 (t, 3H, H-24), 0.83 (d, 3H, H-26), 0.81 (d, 3H, H-27), 0.68 (s, 3H, H-28), 1.01 (s, 3H, H-29).

Pinostrobin (4)

NMR: (^1H , 400 MHz, DMSO) δ ppm: 5.60 (1H, *dd*, H-2), 3.12 (1H, *dd*, H-3_{ax}), 2.80 (1H, *dd*, H-3_{eq}), 6.04 (1H, *d*, H-6), 6.08 (1H, *d*, H-8), 12.18 (1H, *br s*, 5-OH), 3.76 (3H, *s*, 7-OCH₃), 7.45 (2H, *m*, H-2',6'), 7.57 (3H, *m*, H-3',4',5'),

NMR: (^{13}C , DMSO), δ ppm: 196.8 (C-4), 168.1 (C-7), 164.4 (C-5), 163.8 (C-8a), 140.0 (C-1'), 129.5 (C-3',5'), 129.3 (C-4'), 127.2 (C-2',6'), 103.0 (C-4a), 95.0 (C-8), 94.0 (C-6), 79.2 (C-2), 56.0 (7-OCH₃), 43.6 (C-3).

RESULTS AND DISCUSSION

Characterization of Isolated Compounds

Compound 1 was isolated as white crystals (12 mg); its melting point was observed at 260°C. We observed a downfield signal due to the carboxylic acid group at 11.89; signals due to hydroxy groups at H-3, 4 and 5 were observed at 9.15, 8.84 and 6.96 respectively in the ^1H NMR spectrum. In the positive mode HRMS spectra of compound 1, molecular ion peak was observed at 171.02; hence molecular mass was deduced as 170. Other major peaks were detected at 145, 193 and 610. By comparing the above spectral information with earlier literature ¹², compound 1 was predicted to have a molecular formula of $\text{C}_7\text{H}_6\text{O}_5$ and was identified as gallic acid.

Fig.-1: Isolated Compounds from Stem Parts of *Elytranthe parasitica* (L.) Danser (1) Gallic acid (2) Lupeol (3) β -sitosterol (4) Pinostrobin

Compound 2 was soluble in petroleum ether and was isolated as white crystals (3 mg); its melting point was observed at 215°C. When spotted on the TLC plate, compound 2 produced a purple color spot when

sprayed with ANS reagent. Proton NMR spectroscopy indicated the occurrence of an isopropenyl moiety: vinylic proton at 4.56, 4.67 and methyl proton at 1.65. The ESI-MS (negative mode) spectrum of compound 2 gave two prominent peaks at 425.3 (M^+ 1 peak) and 851.2 ($2M^+$ 1); the molecular mass of compound 2 was deduced as 426.

Fig.-2: ¹H NMR Spectrum of Compound 1

Fig.-3: ¹³C NMR Spectrum of Compound 1

The infrared spectrum exhibited the following peaks: 1638 indicated the occurrence of conjugated carbonyl group, 3311 was due to the hydroxyl stretching, 1035 implied the occurrence of C-O. From the

spectral data, the molecular formula was found to be $C_{30}H_{50}O$. The data indicated the presence of pentacyclic triterpenoid, lupeol.¹³

Fig.-4: HRMS Spectrum of Compound 1

Fig.-5: 1H NMR Spectrum of Compound 2

The melting point of compound 3 (3.8 mg) was observed at $135^\circ C$. A signal at δ H 3.12 ppm was characterized as an oxymethine proton at H-3, a triplet signal at δ H 5.38 was characterized as an olefinic proton (H-6). Methyl protons were detected as singlet protons at δ H 0.65 (H-18) and 0.83 (H-19) respectively. In the negative ESI-MS mode, major peaks were observed at 413.26(M $^-$), 369, 364, 347 and 2285

408. The molecular mass was deduced as 414. The FTIR spectra presented the following peaks: 1705 denoted the occurrence of carbonyl group, 3439 was due to the hydroxyl stretching, 1058 inferred the occurrence of C-O. Based on the spectral features, compound 3 was predicted to have the molecular formula $C_{29}H_{50}O$ and was identified as the phytosterol, β -sitosterol.¹⁴

Fig.-6: ^{13}C NMR Spectrum of Compound 2

Fig.-7: ESI-MS Spectrum of Compound 2

The melting point of Compound 4 (3 mg) was observed at 96-98°C. Proton NMR spectroscopy revealed a doublet of a doublet signal at δ H 5.60 (H-2). A singlet at δ 13.15 was identified as the hydroxy group at H-5. A multiplet signal accounting for protons at position H-3',4',5' was observed at δ H 7.57. Two protons at positions H-6 and H-8 were detected as doublets at 6.04 and 6.08 respectively. A multiplet at δ H 7.45 accounted for H-2',6'. A singlet signal at 3.76 was observed, suggesting the presence of a methoxy group at H-7. The ^{13}C -NMR spectrum showed 15 carbon atoms. On comparing with previously reported literature, compound 4 was identified as the flavonone, pinostrobin. In the ESI-MS spectrum (positive mode) of compound 4, the molecular ion peak was detected at 271. Additional peaks were

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Fig.-8: ^1H NMR Spectrum of Compound 3

Fig.-9: ESI-MS Spectrum of Compound 3

Previous study has quantified gallic acid in *Elytranthe parasitica* bioactive fractions via High-Performance Thin Layer Chromatography¹⁶; research reported in the present study validates their findings. Two flavonoids have been previously reported in the plant, pinocembrin-7-O-D-glucoside and chrysin.¹⁷

Fig.-10: ^1H NMR Spectrum of Compound 4 Fig.-11: ^{13}C NMR Spectrum of Compound 4

CONCLUSION

We isolated four phytoconstituents: gallic acid, pinostrobin, lupeol and β -sitosterol from the stem parts of *Elytranthe parasitica* (L.) Danser. All constituents have been isolated and reported for the first time from genus *Elytranthe* and *Elytranthe parasitica* (L.) Danser.

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