Isolation of secondary products from *Ipomoea digitata* – a medicinally important plant

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Abstract

The paper reports the isolation and characterization of β-sitosterol, t-cinnamic acid (undecyl (E)-3-(4-hydroxyphenyl)-2-propenoate), an unknown coumarin and a lignan type resin glycoside from the tuberous roots of *Ipomoea digitata*. The structures of the compounds were elucidated on the basis of extensive chemical and spectroscopic data. Importantly, one of the compounds exhibited significant antibacterial activity against *Pseudomonas aeruginosa* and *E. coli*. The resin glycosides are known as purgative ingredients and hence have medicinal value. However, the exact chemical and biological activity of the resin glycoside isolated in this study is yet to be ascertained.

Key words: *Ipomoea digitata*, Convolvulaceae, Undcyl (E)-3-(4-hydroxyphenyl)-2-propenoate, Coumarin, Resin glycoside

Introduction

*Ipomoea digitata* is a wide-spread, paleotropic, perennial herb belonging to the family Convolvulaceae. It is commonly known as wild yam or Vidari Kanda in Telugu. The tuberous roots are used as tonic, aphrodisiac, cholagogue, demulcent, diuretic, emmenagogue, galactogogue and rejuvenant. Aqueous infusions of the roots are used in Indian traditional medicine for treating epileptic seizures and as antioxidative in Ayurvedic medicine. Hundred grams of Dabur Chavanprash contains 1.195 g of *I. digitata* root powder. Studies on some other species of this genus revealed the presence of Ipangulins, the first pyrollizidine alkaloids, ergoline alkaloids, stoleniferins and resin glycosides (1-6). Recent reports revealed the presence of resin glycosides in the leaves and stems of *I. digitata* (7). The aerial parts are also used as folk medicine in Japan (8). Chemical constituents of the root of this plant (scoperon, β-sitosterol, taraxerol) were reported earlier by Rao et al. (9). The pharmacological activity of the fraction D (10) of this plant extract has drawn our attention to the chemical and biological study of this plant. One of our efforts to discover the structurally diverse and biologically significant metabolites from *I. digitata* has led to the isolation of two known (named as compounds 1, 2) and two unknown compounds (compounds 3 and 4) with spectral and chemical data. We also report, antibiotic activity of compound 3 on gram-negative bacteria *Pseudomonas* and *E. coli*.

Materials and Methods

Fresh tuberous roots of *Ipomoea digitata* were collected from Nallamala forests of Andhra Pradesh, India. The plant specimen was deposited in the herbarium of the Department of Botany,
Osmania University, Hyderabad, India. Three kilograms of fresh tuberous roots were cut into small pieces and dried under shade. The dried tuberous roots were pulverized by using a mixer and extracted in hexane by dipping it for 3 days, followed by re-extraction in methanol. The hexane extract was filtered and concentrated under reduced pressure. The methanol extract gave 83 g of residue after concentration under reduced pressure. With the aim of isolating polar compounds, the dried root powder (1 kg) was taken freshly and extracted in water for 3 days. Hexane extract was subjected to silica gel column chromatography, eluted with a solvent system of hexane/ethyl acetate to separate the compounds from the mixture. The fraction (10 g) eluted by hexane and ethyl acetate (4:96) resulted in the isolation of a pure compound (compound 1) as determined by thin layer chromatography (TLC). This compound was further analyzed by nuclear magnetic resonance (1H NMR) and mass spectrometry (MS) for identification. The fraction eluted by hexane : ethyl acetate (95:5) was repeatedly subjected to silica gel purification which afforded 65 mg of the compound. The white powder was successively subjected to 1H NMR, MS, infra red (IR) and 13C NMR analysis for structure determination (compound 2). The fraction (28 g) of methanolic extract, eluted with chloroform: ethyl acetate (80:20), gave compound 3. Upon extracting this in hexane and benzene (50:50) for 12 h, yellow coloured needles were obtained. The compound was then subjected to 1H NMR, MS, IR and 13C NMR. The dried water extract was concentrated and extracted again in chloroform (2 x 500 ml) to remove low polar compounds. The remaining residue dissolved in methanol consisted of a single compound that was confirmed by TLC. The methanolic extract (1.5 g) was dried and concentrated to give a semi-solid sticky material, which was separated by column chromatography on silica gel (100:200 mesh). This was separated with chloroform : methanol (50:50) solvent system in the column and designated as compound 4.

The IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. The 1H NMR spectra were recorded at 400 MHz (Varian make). The 13C NMR spectra were performed in CDCl3 at 75.5 MHz for compounds 1 and 2 but 13C NMR of compound 3 was noticed in dimethyl sulphoxide (DMSO) at 75.5 MHz. Electron ionization-MS were obtained at 70 ev and fast atom bombardment (FAB)–MS using Argon (6 kv) as the FAB gas. Silica gel (60-120 mesh and 100-200 mesh, Merck, Darmstadt, Germany) was employed for column chromatography. TLC was carried out on precoated kiesel gel 60 F254 (0.25 thick, Merck, Darmstadt, Germany) plates, with hexane-ethylacetate and chloroform–ethylacetate as solvent systems. Coloured spots were visualized by exposure to iodine vapours followed by spraying with 10% sulfuric acid solution. Dragendorff’s reagent was used for the detection of alkaloids. High performance liquid chromatography (HPLC, Eclipse XDB-C-18 column, 5 µm, 4.6 x150 mm, 25%-100% methanol in water over 8 min followed by 100% methanol to 11 min, 1 ml/min, 30 °C) was carried out in combination with TLC. Methanol, chloroform, ethyl acetate and hexane which were used for extraction of compounds were purchased from the Finar chemicals, Hyderabad. Methanol-sulfuric acid, Dragendorff reagent, ethanol-sulfuric acid were used as reagents to detect the compounds that are separated on the chromatogram. Silica coated glass plates for TLC were purchased from Merck. All other reagents and chemicals used were of analytical grade.

Antibacterial activity was tested against Gram negative bacteria like Pseudomonas and E.coli by single disc method (11). Test bacterial
strains were cultured in sterilized Luria Bertani (LB) broth (pH 5.7) for 16-18 h at 37 °C on a rotary shaker. LB media with agar as gelling agent was prepared and sterilized at 121 °C for 15-20 minutes in an autoclave. Plating was carried out under aseptic conditions. A loop full of liquid broth of bacterial culture was spread over solidified LB media. Sterilized discs (Whatmann filter paper) were placed at equal distances. The four compounds each of 5 mg were weighed and dissolved in 5 ml of DMSO. Dissolved compounds (3 µl) were placed on the discs and plates were wrapped tightly with parafilm and incubated at 37 °C for 24 h.

**Results and Discussion**

Extraction of tuberous root powder of *Ipomoea digitata* resulted in the isolation of four compounds. The first compound is a smooth, whitish pink, amorphous powder. The structure (Fig. 1) of this compound (β-sitosterol) was determined based on the spectral data like $^1$H NMR and Mass (Fig. 2) and also correlated with the previously available data (12). Compound 2, isolated for the first time from *I. digitata*, is a white powder, with a melting point of 80 °C, chemical formula of C$_{20}$H$_{20}$O$_3$ and is identified as undecyl-(E)-3-(4-hydroxyphenyl)-2-propenoate (trans-cinnamic acid). Its structure and $^{13}$C NMR spectrum are shown in figures 3 and 4 respectively. The third compound appeared as a pale yellow coloured needles with a melting point of 205 °C and identified as 5-hydroxy-7-methoxy coumarin based on its spectroscopic data. Its structure and $^1$H NMR spectrum are shown in figures 5 and 6. It exhibited the activity under ultraviolet light. Compound 4 is soluble only in methanol and water, and is a brown coloured, sticky, semi solid, resin type glycoside. The signals in $^1$H NMR (Fig. 7) and Mass spectra allowed us to conclude that this compound is a resin glycoside. The $^1$H NMR and Mass spectral signals of compound 4 did not show any correlation with the available spectral data (7), indicating that this compound is different from the compounds already isolated from the leaves and stems of *I. digitata* earlier. However, the exact physical nature and chemical structure of the glycoside is yet to be determined.
None of the above compounds showed any antioxidant activity though extracts of this plant are used in Ayurveda as an antioxidant. However, coumarin, when tested against *Pseudomonas aeruginosa* and *E. coli*, inhibited the growth of these bacteria but not others. This is the first time that bacterial activity has been found for the coumarin isolated from this plant. Hence, *I. digitata* can serve as a good source for the isolation of this bioactive compound. The inhibition zone (14 mm) of bacterial growth was observed around the disc containing the compound. Many workers reported major effects like antinociceptive, antifungal and anticancer activities for the compounds of *Ipomoea* genus (13-15). The resin glycosides, originated from the Convolvulaceous plants, are well known as the purgative ingredients in some traditional medicines. They are divided into ether-soluble resin glycosides “jalapin” and ether-insoluble resin glycoside “convolvulin” (16). Resin glycosides have also recently been isolated and characterized from the leaves and stems of *I. digitata* (7). They named it as digitatajalapin I since it was isolated from jalapin fraction. The resin glycoside isolated in the present study is ether-soluble type. Therefore, it could be a “jalapin” related glycoside. However, the biological effects of the resin...
glycoside isolated in this study needs to be determined, which is underway.

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**References**


