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DPPH RADICAL SCAVENGING ACTIVITY OF A COMPOUND ISOLATED FROM BARRINGTONIA ACUTANGULA LEAVES

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ABSTRACT

A secondary metabolite was isolated from a medicinal plant, *Barringtonia acutangula*. The structure of compound was identified as 2, 4-dihydroxy-methyl benzoate by using spectroscopic techniques. Compound 2, 4- dihydroxy-methyl benzoate was isolated for the first time from this plant. This compound was screened for DPPH radical scavenging activity, exhibited remarkable antioxidant activity against the DPPH free radical.

KEYWORDS: *Barringtonia acutangula*, antioxidant, DPPH radical scavenging activity.

INTRODUCTION

Barringtonia acutangula (L.) Gaertn. is an evergreen small tree , growing in tropical forests, along sides of rivers and streams, or brackish water areas. Previous studies indicated that triterpenoid derivatives were the main constituents of *B. acutangula*.[1-3] Steroidal compounds such as barringtogenic acid, tangulic and acutangulic acids were found in *B. acutangula* leaves.[4] Three flavan-3-ol derivatives and four flavonoid glycosides were isolated from the bark and leaves of *B. acutangula*.[5,6] This paper deals the isolation and structural elucidation of 2, 4- dihydroxy- methyl benzoate from the leaves of *B. acutangula.* Compound 2, 4-dihydroxy- methyl benzoate isolated for the first time from this plant. The aim of this study was to evaluate the antioxidant activity of the isolated compound by DPPH radical scavenging assay.

MATERIALS AND METHODS

Materials: Infrared spectra were recorded on a Perkin-Elmer FT-IR type 1650 spectrophotometer in -wave number region 200-4000 cm^{-1}. **NMR:** Bruker 500 MHz records the ^{1} H-NMR and ^{13}C-NMR spectra. DPPH (1,1-diphenyl-2-picrylhydrazyl), and L-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analar grade.

Collection and identification of plant material: The plant material was collected from Madurai, Tamil Nadu, India and authenticated by Dr. John Britto, Rapinat Herbarium, St. Joseph's College, Tiruchirappalli. Tamil Nadu.

Extraction and Isolation: The dried leaves of *Barringtonia acutangula* (1.0 kg) were macerated in methanol at room temperature twice, and the methanol extract was evaporated under reduced pressure to give a crude extract (60 g). The crude extract in methanol was partitioned first with n-hexane. The methanol layer was added with water (5% v/v) to increase the polarity and then partitioned with ethyl acetate. The ethyl acetate extract (20 g) was separated by column chromatography on silica gel (70-230 mesh and eluted with petroleum ether: ethyl acetate to ethyl acetate: methanol mixtures (from 9:1 to 1:9, respectively) to afford six major fractions (F1 to F6). These fractions were subjected to repeated column chromatography (silica gel) using different gradient solvent systems to afford 2 compounds. Compound 1 (13.2 mg, petroleum ether: ethyl acetate, 6: 4) and compound 2 (26 mg, Chloroform: Methanol, 9:1). Based on the antioxidant activity, compound 2 (R_f value is 0.63) was further characterized by spectral studies and identified as 2, 4- dihydroxy-methyl benzoate.

DPPH Radical Scavenging: The antioxidant assay of compound 1 against DPPH (2,2-diphenyl-1-picrihidrazil) radical was measured by UV spectrometer at λ 517 nm as described Shimada, et al., (1992).[7] A 2 ml aliquot of DPPH solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations (20, 40, 60 and 80 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. The inhibition percentage (%) of radical scavenging activity was calculated using the following equation.

$$\text{Inhibition (\%)} = (Ac - As) / Ac \times 100$$

where Ac is the absorbance of the control reaction (containing all reagents except the test compound), and As is the absorbance of the test compound.

RESULTS AND DISCUSSION

The present study on the methanolic extract of *B.acutangula* resulted in the isolation and characterization of compound 1. Pale yellow solid, mp 88-96°C. IR ($CHCl_3$) ν_{max}: 3080 (OH), 1680 cm $^{-1}$ (C=C), 1666 cm $^{-1}$ (C=O), 1116 cm $^{-1}$ (C-O), 832 cm $^{-1}$ (C-C), 749 cm $^{-1}$ (C-H). EI-MS m/z (rel. int. %): The molecular ion peak is [M+] 168.7. The other fragments are 152.9, 136.0, 125.0, 107.9 and 84.9. MS m/z: 168.7(C_8 H_8 O_4, calculated 168.14678). Mass spectrum of the isolated compound shown in Fig.1. ^{1} H-NMR (DMSO, 500 MHz): δ 9.25 (s, RCHO), 7.72 (Ar-OH), 3.92 (s, OCH_3). The presence of two singlet aromatic proton signals at δH 6.30 and 6.38 suggest that compound 1 is typical for a methyl benzoate with two substituent.[8] ^{13}C– NMR (DMSO, 500MHz): δ164.40, 164.90 and 170.10 (C=O, acids and esters). The ^{13}C–NMR spectrum indicated that the compound had 8 C atoms that are present in aromatic rings, acids, and esters. Based on the spectroscopy studies the isolated compound is 2, 4-dihydroxy-methyl benzoate.

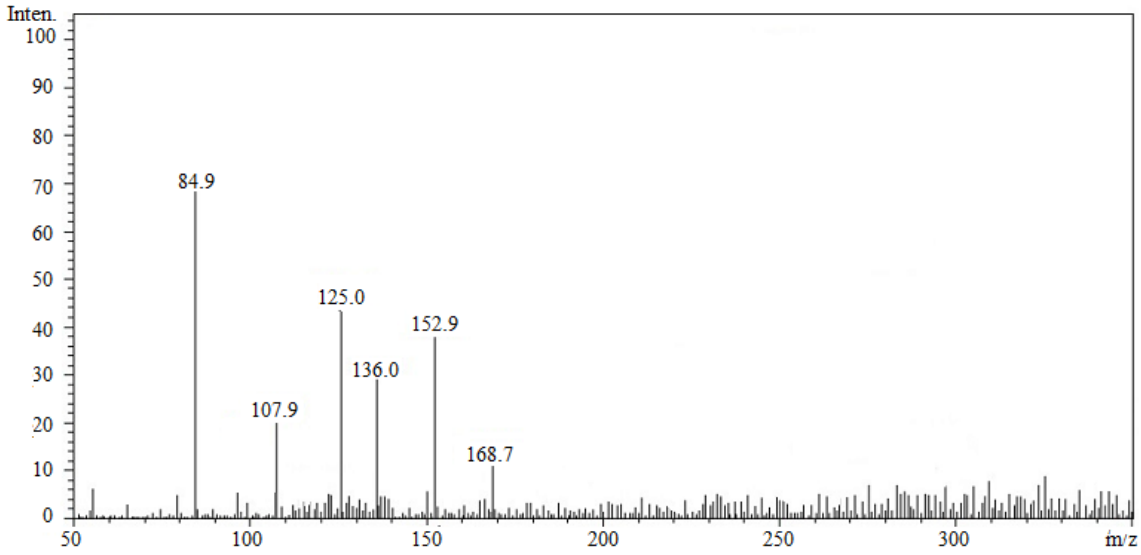

Fig 1: Mass spectrum of isolated compound.

Antioxidant activity assay: The antioxidant activity of the isolated compound was evaluated using a DPPH radical scavenging assay. DPPH$^{\bullet}$ is one of the free radicals widely used for testing preliminary radical scavenging activity of the compounds. DPPH$^{\bullet}$ assay, which is based on the ability of DPPH$^{\bullet}$, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action.[9] The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely used to measure the antioxidant activity.[10] The result of free radical scavenging activity of isolated compound at different concentrations are shown in Fig.2 . From results it is evident that free radical scavenging activity of the compound was concentration dependent. Ascorbic acid, used as a standard, showed stronger antioxidant activity than that of isolated compound. The isolated compound showed an IC_{50} value of 102.19 µg/ml lower than that of standard ascorbic acid (26.84 µg/ml). A lower IC_{50} means better radical scavenging activity.

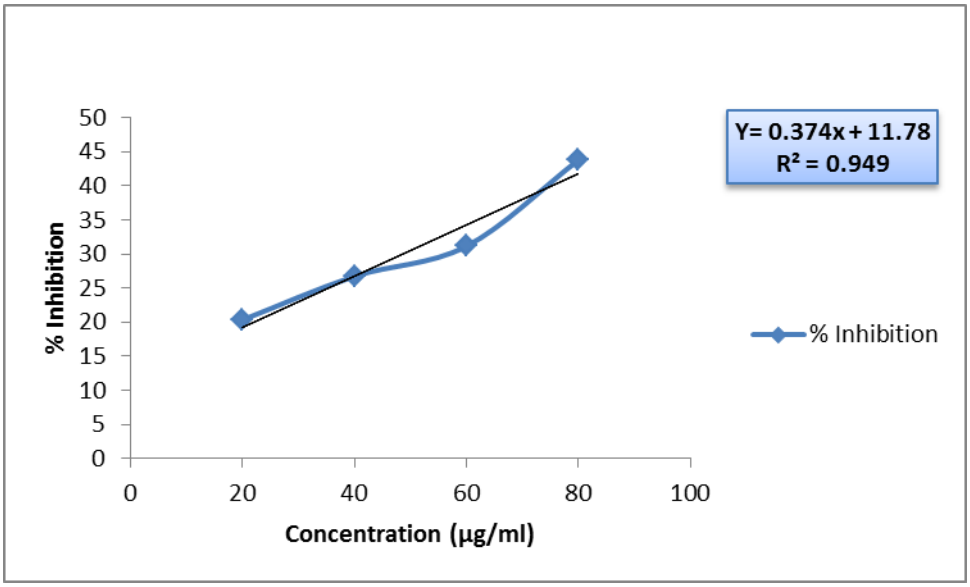

Fig. 2: DPPH radical scavenging activity of isolated compound.

CONCLUSION

The isolated compound was characterized by spectral analysis, identified as 2, 4-dihydroxy- methyl benzoate. The DPPH radical scavenging results of 2, 4-dihydroxy-methyl benzoate exhibited remarkable antioxidant potential. In the future, the isolation and purification of phyto compounds from *Barringtonia acutangula* may be useful in the preparation of novel drugs for the treatment of many ailments.

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