Free Radical Scavenging Active Components from *Cedrus deodara*

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An activity-directed fractionation and purification process was used to identify the antioxidant components of *Cedrus deodara*. Dried heartwood powder of *C. deodara* was first defatted with petroleum ether and then extracted with chloroform. The chloroform extract showed strong antioxidant activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. This fraction was then subjected to separation and purification using silica gel column chromatography. Three compounds with potent antioxidant activity were isolated in significant yields and identified by spectroscopic methods (^{1}H NMR, ^{13}C NMR, IR, and MS). They were identified as (−)-matairesinol, (−)-nortrachelogenin, and a dibenzylbutyrolactollignan (4,4′,9-trihydroxy-3,3′-dimethoxy-9,9′-epoxylignan). This is the first report of the occurrence of these compounds in *C. deodara*.

Keywords: *Cedrus deodara; free radical; antioxidant activity; (−)-matairesinol; (−)-nortrachelogenin; dibenzylbutyrolactol*

INTRODUCTION

There is considerable recent evidence that free radicals induce oxidative damage to biomolecules and play an important role in cardiovascular disease, aging, cancer, inflammatory diseases, and a variety of other disorders (*1*−*4*). Antioxidants that scavenge free radicals are now known to possess preventive as well as therapeutic potential in free radical mediated disease conditions (*5*−*11*).

These observations have accelerated the search for potential pharmacological antioxidant principles from traditional medicinal plants. Use of *Cedrus deodara* (Roxb.) Loud. (Pinaceae) is recommended in the Ayurvedic system of medicine for treatment of various ailments (*12*). The alcoholic extract of *C. deodara* is known to possess a variety of biological effects, such as anticancer, anti-inflammatory, diuretic, and spasmolytic activities (*13*−*15*). However, a systematic approach of identifying bioactive principles from this plant is lacking. In our effort to identify bioactive principles from Indian medicinal plants, we now report an investigation of the free radical scavenging activities and constituents from *C. deodara*.

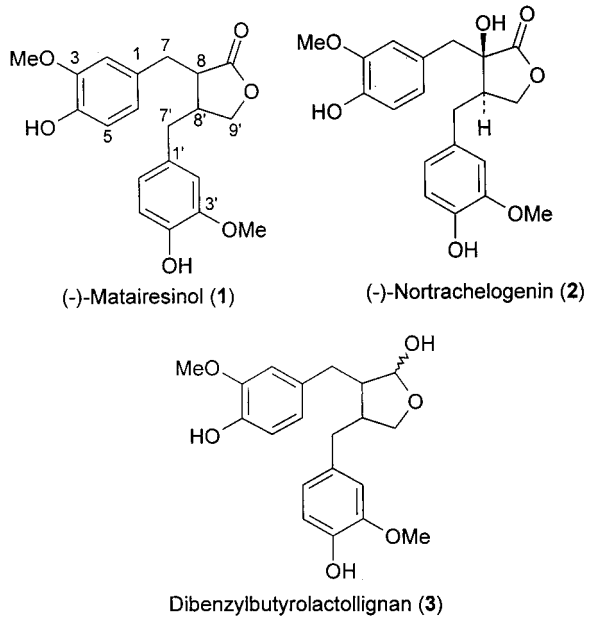

Figure 1. Compounds isolated from *C. deodara*.

MATERIALS AND METHODS

General Procedures. ^{1}H NMR spectra were obtained on a Varian 200 MHz spectrometer (Palo Alto, CA). IR spectra were recorded on Nicolet spectrometer. Mass spectra were obtained on a VG 70-70H micromass Instrument. Specific rotations were recorded on a Jasco DIP-370 digital polarimeter at room temperature.

Plant Material. Dried heartwood of *C. deodara* was purchased from a local store. It was identified by Prof. Manoharachary, Head, Botany Department, Osmania University, Hyderabad, India.

Extraction and Isolation Procedures. The dried heartwood (250 g) was cut into small pieces and made into powder in a powder mill. The powder was extracted in a Soxhlet apparatus with petroleum ether for a period of 24 h and then extracted with chloroform for 24 h. The chloroform extract on evaporation under vacuum yielded 45 g of residue.

The DPPH-active chloroform extract (10 g) was twice chromatographed over a silica gel (60−120 mesh) column and eluted first with chloroform followed by 2% methanol in chloroform to obtain compound **1** (yield = 1.44%). Further elution of the column with 5% methanol in chloroform resulted in compound **2** (yield = 13.50%). Elution with 7% methanol in chloroform yielded dibenzylbutyrolactol lignan **3** (yield = 1.62%). All of the compounds are found to be TLC pure.

Structure Determination of Isolated Compounds. *(−)-Matairesinol (**1**):* pale yellow powder, mp 119 °C (EtOH); [M$^+$] at *m*/*z* 358 (50), 221 (5), 164 (8), 138 (100), 94 (10), 77 (8); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.53 (4H, m), 2.95 (2H, br), 3.86

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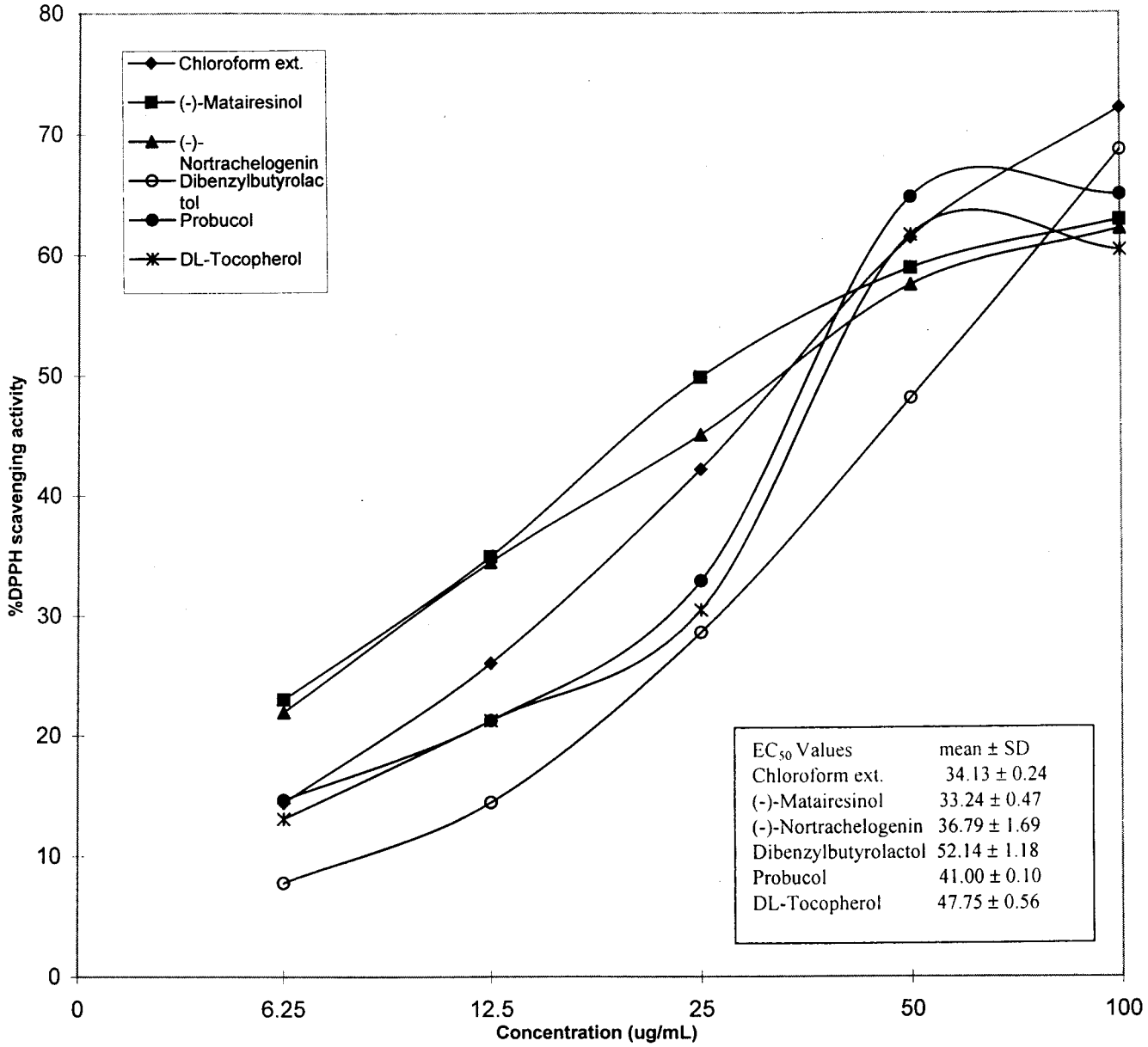

Figure 2. Anti-free-radical activity of *C. deodara* compounds.

(6H, s), 4.20−4.40 (2H, m), 5.5 (2-OH, bs), 6.40−6.80 (6H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 34.48 (C-8), 38.10 (C-8′), 40.90 (C-7), 46.60 (C−7′), 55.70 (2-OMe), 71.30 (OCH_2O), 111.01, 111.53, 114.11, 114.40, 121.21, 121.95, 128.32, 129.45, 129.70, 144.30, 144.43, 146.38 (12 Ar-C), 178.94 (C=O); IR γ_{max} (KBr) cm^{-1} 3560 (−OH), 1765 (lactone carbonyl); $[\alpha]_D$ −37.50 (*c* 0.5, $CHCl_3$).

The data are in agreement with the reported literature values (*16*).

*(−)-Nortrachelogenin (**2**):* amorphous powder; $[M^+]$ at *m/z* 374 (5), 138 (100), 122 (10), 105 (8), 77 (8), 43 (80); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.40−2.55 (2H, m), 2.65−2.80 (2H, m), 3.10−3.20 (1H, d), 3.85 (3H, s), 3.90 (3H, s), 3.95 (2H, m), 5.60 (2H, d), 6.50−6.80 (6H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 31.50 (C-7), 41.90 (C-8), 43.74 (C-7′), 55.94 (d, 2-OMe), 70.26 (OCH_2O), 76.33 (−OH), 111.55, 112.81, 114.35, 114.56, 116.82, 121.42, 123.12, 126.20, 130.35, 144.27, 144.95, 146.59(12 Ar-C), 178.66 (C=O); IR γ_{max} (KBr) cm^{-1} 3560 (−OH), 1760 (lactone carbonyl); $[\alpha]_D$ −30.90 (c 0.44, $CHCl_3$).

The data are in agreement with the reported literature values (*16*, *17*).

*4,4′,9-Trihydroxy-3,3′-dimethoxy-9,9′-epoxylignan (**3**):* amorphous powder; $[M^+]$ at *m/z* 360; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.94−2.18 (2H, m), 2.37−2.80 (3H, m), 3.55 (1H, m), 3.90 (6H, bs), 4.15 (2H, m), 5.25 (1H, m), 5.50 (2-OH, bs), 6.42−6.81 (6H, m);^{13}C NMR (50 MHz, $CDCl_3$) δ 38.35 (C-7′), 39.27 (C-7), 45.76 (C-8′), 52.93 (C-8), 55.73 (−OMe), 55.88 (−OMe), 72.36 (C-9′), 103.49 (C-9), 111.06 (C-2′), 111.21 (C-2), 114.14 (C-5′), 114.24 (C-5), 121.24 (C-6′), 121.58 (C-6), 131.47 (C-1′), 132.31 (C-1), 143.84 (C-4′), 144.00 (C-4), 146.39 (C-3′), 146.50 (C-3); IR γ_{max} (KBr) cm^{-1} 3450, 1765, 1712, 1657; $[\alpha]_D$ −41.54 (*c* 1.00, $CHCl_3$).

The data are in agreement with the reported literature values (*18*).

Determination of the Free Radical Scavenging Effect on DPPH Radicals. This method for evaluation of free radical scavenging activity was adapted from that of Yamaguchi et al. (*19*). In brief, compounds dissolved in DMSO were reconstituted in Tris-HCl buffer (pH 7.4) to a volume of 1 mL and mixed with 1 mL of DPPH solution in ethanol (final concentration of DPPH = 250 μM). The mixture was shaken vigorously and incubated in the dark for 30 min. The absorbance at 517 nm was measured spectrophotometrically by a UV−vis 4053 kinetics spectrophotometer (Ultrospec LKB Biochrom, Cambridge, U.K.). Each sample was measured in triplicate, and mean values at different concentrations are plotted in Figure 2. The determination of EC_{50} values was obtained by extrapolation from regression analysis. Comparison of degree of significance was analyzed by applying Student's *t* test. Except

for dibenzylbutyrolactol, (−)-matairesinol, (−)-nortrachelogenin, and chloroform extracts were shown to be significantly ($p < 0.01$) more potent than reference compounds probucol and DL-tocopherol (Figure 2).

RESULTS AND DISCUSSION

The chloroform extract of *C. deodara* showed potent free radical scavenging activity on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The DPPH radical has been widely used to test the free radical scavenging ability of various natural products (*20*-*22*) and has been accepted as a model compound for free radicals originating in lipids (*23*, *24*). This DPPH scavenging activity directed fractionation and isolation experiment confirmed the presence of free radical scavenging compounds in the chloroform extract of *C. deodara*. The active chloroform extract was subjected to chromatography on a silica gel column, eluted first with 2% methanol in chloroform and then with 5% methanol in chloroform to obtain (−)-matairesinol (**1**) and (−)-nortrachelogenin (**2**), respectively. Subsequent elution of the column with 7% methanol in chloroform afforded the dibenzylbutyrolactol (**3**) (Figure 1) as a mixture of epimers. This is the first report of the presence of these three antioxidant principles in *C. deodara*. Their structures were confirmed by spectroscopic evidence and by comparing the data with the literature values. Free radical scavenging ability and EC_{50} values of these compounds are shown in Figure 2.

The present paper establishes that the three compounds (−)-matairesinol (**1**), (−)-nortrachelogenin (**2**), and dibenzylbutyrolactol (**3**) are present in *C. deodara* in significant quantities and may be the major antioxidant principles of medicinal importance. Lignans are known to possess a variety of biological activities, namely, antitumor, antimitotic, antiviral, antihepatotoxic, antistress, and cardiovascular activities (*25*). (−)-Matairesinol has been shown to possess antihypertensive activity through inhibition of cAMP phosphodiesterase (*26*), and trachelogenin is a Ca^{2+} antagonist (*27*). Nortrachelogenin has been reported to act as a sedative (*27*).

The presence of two phenolic groups or substituted hydroxyl groups are essential for phosphodiesterase inhibiting activity of lignans (*26*) and is an important criterion for free radical scavenging activity. A broad spectrum of activities of antioxidants (*28*) and lignans (*29*, *30*) have been reviewed recently. The presence in significant amount of free radical scavenging principles in *C. deodara* may therefore explain the frequent use of this medicinal plant in Indian medicinal preparations prescribed in a variety of disease conditions.

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