

Note

Artoindonesianin F, a potent tyrosinase inhibitor from the roots of *Artocarpus heterophyllus* Lam

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Tyrosinase inhibition activity-guided fractionation of the ethyl acetate extract of the roots of *Artocarpus Heterophyllus* Lam., yield a stilbene derivative, (E)-4-[(1E)-3-methyl-1-buten-1-yl]-3,5,2',4'-tetrahydroxystilbene (artoindonesianin F). The structure has been established based on physical and spectral data (UV, IR, ¹H, ¹³C NMR and Mass). The compound has shown a potent mushroom tyrosinase inhibition activity when compared with standard commercial compound, kojic acid.

Keywords: *Artocarpus heterophyllus*, artoindonesianin F, tyrosinase inhibition, kojic acid.

In our continuous interest on the search for biologically active molecules for cosmetic use particularly from Indian medicinal plants^{1,3}, the present investigation was carried out on the roots of *Artocarpus heterophyllus* Lam. The genus *Artocarpus* (Moraceae) generally known as breadfruit tree comprises about 50 species, of which nine species are recorded in India. *A. heterophyllus* is a large evergreen tree and cultivated throughout the hotter parts of India. The fruit (jack fruit) from this plant is the most popular fruits in south India and it also called poor mans food⁴. *A. heterophyllus* is a medicinally important plant and has been used in indigenous medicine in the treatment of various diseases. The roots are said to be useful in the skin diseases, asthma and diarrhoea. In Bihar, India the plant is used for toothache, stomach complaints, sores and sterility in women⁴. In Combdia, the wood is considered a nervous sedative, and is administered in convulsions. The pith is taken internally as an abortifacient⁵. The roots are reported to show anti-diarrhoeal property and leaves are useful to control fever, boils and wounds⁶.

Various groups worked on this plant and reported different variety of compounds like: phenolics,

flavonoids, triterpenes, stilbenes, flavonoids, steroids and some volatile compounds from different parts of the plant. These are heterophyllol⁷, cycloheterophyllin⁸, isocycloheterophyllin⁹, norartocarpetin and artocarpesin¹⁰, morin¹¹, artoflavone¹², artocarpesin and norartocarpesin¹³, artocarpanone¹⁴, ten flavonoids: artocarpanoneone A, cycloartocarpanone A, artocarpanone, artocarpetin, norartocarpetin, artocarpin, cyanomaclurin, artocarpetin A, cudraflavone A and cyclohetero-heterophyllin¹⁵, artocarpetin B, heteroartoinin A, kuwanone T, artonin A, artonin B and heterophyllin¹⁶, triterpenoids: cycloartenol, cycloartenone, (24S) and (24R)-9,19-cyclolanost-3-one-24,25-diol¹⁷, some volatile compounds: ethyl 3-methylbutanoate, 3-methylbutanol, isoamyl-acetate, 2-phenylethanol, 3-methyl butyl-3-methylbutanoate, 2-methyl propyl propyl 3-methylbutanoate, pentane 2,3-dione and butanol¹⁸. In this article, we report the isolation of a known stilbene derivative, (E)-4-[(1E)-3-methyl-1-buten-1-yl]-3,5,2',4'-tetrahydroxy-stilbene **1**, which has shown potent tyrosinase inhibition property from the roots of *A. heterophyllus*.

Results and Discussion

The preliminary screening of the ethyl acetate extract of the roots of *A. heterophyllus*, showed positive response in mushroom tyrosinase inhibition assay^{19,20}. The bioassay guided fractionation of the ethyl acetate fractions of the roots of *A. heterophyllus* repeated chromatography with a silica gel and recrystallization with chloroform furnished a stilbene derivative, (E)-4-[(1E)-3-methyl-1-buten-1-yl]-3,5,2',4'-tetrahydroxystilbene **1**. The structure of **1** was elucidated on the basis of UV, IR, ¹H and ¹³C NMR and mass spectral data²⁰.

The melanin biosynthesis activity (mushroom tyrosinase enzyme activity) of artoindonesianin F showed excellent activity (IC₅₀= 0.20 µg/mL) where as the kojic acid, a positive control, showed lesser activity (IC₅₀=1.75 µg/mL) in the standard method^{19,20}. Compound **1** has been previously isolated from the aerial parts of *A. integer*²¹ and showed an anti-malarial activity against *Plasmodium falciparum*. Recently, it has been reported from the stem bark of *A. nobilis*²¹. This is the first time that compound **1** has been isolated from *A. heterophyllus* and has shown a potent tyrosinase inhibition activity.

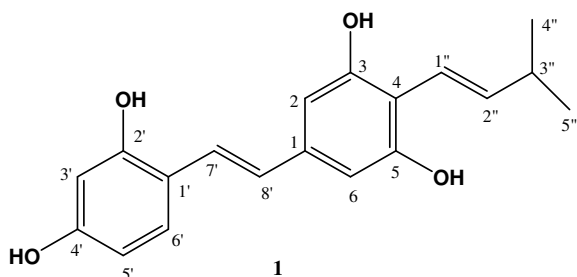


Table I — Comparison of tyrosinase inhibition values

Compound	Tyrosinase inhibition
Kojic acid	IC ₅₀ = 1.75 μg/mL
Artoindonesianin F	IC ₅₀ = 0.20 μg/mL

Experimental Section

Melting points reported are uncorrected. The 400 MHz NMR spectra were recorded on a Bruker AMX 400 in DMSO with TMS an internal standard. The ¹³C NMR spectra were recorded at 100 MHz in CD₃OD. IR spectra were recorded on a Shimadzu IR prestige 21; UV spectra were recorded on Shimadzu UV spectrophotometer; ESI mass spectrum on a Jeol SX 102/DA 6000 mass spectrometer. TLC was performed on pre-coated silica gel 60 F₂₅₄ plates (Merck) and the spots were visualized by exposure to iodine vapour or spraying with 5% sulphuric acid in methanol followed by heating the plate at 110°C for 5 min. The substrate, L-tyrosine, buffer and enzyme mushroom tyrosinase () were purchased from the M/s. Sigma-Aldrich, USA

Plant material

The roots of *A. heterophyllum* were collected from Panrutti, Tamil Nadu, India in June 2006. A voucher specimen was deposited in M/s. CavinKare Research Centre, Chennai, India.

Extraction and Isolation

The air-dried roots of *A. heterophyllum* (360.7 g) were cut into small pieces and crushed, subjected to an extraction with ethyl acetate (2L × 3) at room temperature for 24 hr. The combined extract was concentrated under reduced pressure to get ethyl acetate extract (8.8 g). Part of the extract (8.0 g) was chromatographed on silica gel column (Acme) eluting with chloroform, chloroform:methanol (99:1, 98:2 and 95:5), collected eleven fractions, each fraction volume is 150 mL. Based on the TLC, homogeneous

fractions were combined, divided into four major fractions and weight of fractions are Fr.1 (1.3 g), Fr.2 (2.3g), Fr.3 (0.7g) and Fr.4 (2.10 g). All four fractions were tested for bioactivity and Fr. 3 showed a potent activity. The fraction showed one major spot along with some minor impurities like colouring pigments and the fraction was re-crystallized from chloroform to get compound **1** (170 mg).

Compound **1**: Pale yellow powder; mp: 205°C; IR: 1610 (aromatic), 1030, 910 cm⁻¹; UV(nm) : 245, 312, 345; ¹H NMR (DMSO-*d*₆): δ 1.02 (6H, d, *J* = 6.7Hz, H-4'', H-5''), 2.35 (1H, m, H-3''), 6.22 (1H, dd, *J* = 2.3, 8.4Hz, H-5'), 6.31 (1H, d, *J* = 2.3Hz, H-3'), 6.44 (2H, s, H-2, H-6), 6.48 (1H, d, *J* = 16.2Hz, H-1''), 6.55 (1H, dd, *J* = 16.2, 6.2Hz, H-2''), 6.69 (1H, d, *J* = 16.4 Hz, H-8'), 7.12 (1H, d, *J* = 16.4 Hz, H-7'), 7.35 (1H, d, *J* = 8.5Hz, H-6'); ¹³C NMR (CD₃OD): δ 23.4 (C-4'', C-5''), 34.4 (C-3''), 103.5 (C-3'), 105.7(C-2, C-6), 108.3 (C-5'), 112.6(C-4), 117.9(C-1), 119.5 (C-1'), 124.2(C-7'), 126.2 (C-8'), 128.2 (C-6'), 138.9 (C-1''), 141.2 (C-2''), 157.3 (C-3, C-5, C-2') 159.2 (C-4'). ESIMS (rel.int.): *m/z* 313 (M+H⁺, 50), 257(100).

Tyrosinase inhibition assay

Tyrosinase inhibition activity was determined by dopachrome method using L-tyrosine as substrate^{19,20}. Fresh solutions of L-tyrosine (3 mM), buffer (pH 6.8, 50 mM) and a stock solution of 500 units/mL of mushroom tyrosinase were prepared. The reaction-mixture constitutes 235 μL of L-tyrosine, 365 μL of buffer and 90 μL of enzyme and 10 μL of inhibitor. The assay mixture was incubated at 37°C for 30 min. The dopachrome was measured spectrophotometrically at 475 nm and % of inhibition of was calculated. The results of the standard compound, kojic acid and artoindonesianin F were given in the **Table I**.

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