

## *In vitro* glucose uptake by isolated rat hemi-diaphragm study of *Aegle marmelos* Correa root

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### Abstract

The methanol extract of the root of *Aegle marmelos*, a medicinal plant, was fractionated into eight fractions using column chromatography. The antidiabetic activity of all the fractions was studied using the glucose uptake by isolated rat hemi-diaphragm *in vitro* model. Using the bioassay-guided fractionation, two compounds **1** and **2** were isolated by column chromatography and identified as 6-methyl-4-chromanone and skimmianine respectively by NMR and mass spectral methods.

### Introduction

An increasing number of research papers and reviews clearly indicate that medicinal plants exhibit a variety of therapeutic properties and could provide health security to rural people primary health care. Among medicinal plants *Aegle marmelos* Correa (family Rutaceae) appears to be relevant and this plant is available in India, Bangladesh, Burma and Sri Lanka. Its distribution is mainly within the sub-Himalayan forests, in dry hilly places ascending to 4,000 feet. It is called "Shivadume", the tree of Shiva. Since ancient time, its leaves are offered to Shiva and Parvathi. *A. marmelos* has an important place in indigenous systems of medicine. With respect to pharmacology, alcoholic and aqueous extracts of the leaves had similar effects as digoxin in amplitude and contractions of the frog heart and methanolic extracts of roots inhibited the beating rate by approximately 50% of cultured mouse myocardial cells (Kakiuchi et al., 1991). Alcoholic extracts of the roots and fruits showed hypoglycemic and antidiabetic activity (Kamalakkannan et al., 2003;

Karunanayake et al., 1984; Sabu and Kuttan, 2004). With respect to clinical applications, it should be noted that the roots are astringent, bitter and febrifuge. They are useful in diarrhea, dysentery, dyspepsia, stomachalgia (Shoba and Thomas, 2001), cardiopalmus, seminal weakness, vomiting, intermittent fever swellings. The leaves of *A. marmelos* are useful as laxative, febrifuge and expectorant, also in ophthalmia, deafness, inflammations, cataract, diabetes, asthmatic and antifungal complaints (Rana et al., 1997). Also, the effect of these extracts was examined in the regulation of hyperthyroidism (Kar et al., 2002) and for the analgesic activity in mice. The stem extract inhibit *in vitro* proliferation of human tumor cell lines (Lampronti et al., 2003).

Diabetes mellitus is a major disease characterized by derangement in carbohydrate, fat and protein metabolism, affecting nearly 10% of the population. In the recent past many hypoglycemic agents are introduced, still the diabetes and the related complications continue to be a major medical problem



not only in developed countries but also in developing countries. Many Indian medicinal plants are reported to be useful in diabetes. However, search for new antidiabetic drugs continue. Although the leaves and fruits of *A. marmelos* were studied widely for their antidiabetic activity (Narendrakannan et al., 2006; Kesari et al., 2006), the reports on antidiabetic activity of roots are limited. The present study was aimed at investigating the antidiabetic effect of *A. marmelos* and identifying the active principles.

## Materials and Methods

**Plant material:** *A. marmelos* was collected from Kottakkal, Mallapuram district, Kerala during September 2007 and authenticated at the Botany Division, Centre of Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Kerala, where the voucher specimen is maintained.

**Extraction:** About 20 g of air-dried root of the *A. marmelos* was powdered and refluxed with methanol for 8 hours over a water bath. The extract was filtered and concentrated under reduced pressure in a rotary evaporator below 50°C. The concentrated extract was dissolved in 10 mL of methanol and used for the gas chromatography analysis.

**Fractionation using column chromatography:** The air-dried root (500 g) was ground and extracted exhaustively with methanol (3 x 1.0 L) for 48 hours. The extracts were combined and freed of solvent to give 2.65 g of residue. The residue was subjected to column chromatography on 80 g of Si gel and eluted with n-hexane (100 mL) followed with a mixture of n-hexane and ethyl acetate containing increasing amounts of ethyl acetate. Finally the column was eluted with pure ethyl acetate. Seventy-eight fractions (25 mL each) were collected and pooled according to their similarity on analytical TLC plates and dried. Combined fractions 1-9 (FA, 165 mg), 10-21 (FB, 190 mg), 22-27 (FC, 141 mg), 28-35 (FD, 132 mg), 36-47 (FE, 141 mg), 48-60 (FF, 181 mg), 61-72 (FG, 156 mg) and 73-78 (FH, 153 mg) were obtained.

**Isolation of compounds:** The fraction FC (100 mg) was subjected to column chromatography on 20 g of Si gel G and eluted with a mixture of n-hexane: ethyl acetate (8:2). Fractions (5 mL each) were collected and pooled according to their similarity on analytical TLC plates and dried. Combined fractions 7-10 (**1**, 21 mg), 18-22 (**2**, 10 mg) were obtained. They were characterized by NMR and MS spectral data as 6-methyl-4-chromanone and skimmianine respectively.

<sup>1</sup>H NMR (500 MHz) and <sup>13</sup>C NMR (150.9 MHz) spectra were measured on a JEOL JNM LA-500 instrument using acetone-d<sub>6</sub> and CD<sub>3</sub>OD solvents. APCI-MS were

determined on an Agilent (Palo Alto, CA, USA) MSD 1100 single-quadropole spectrometer.

**Compound 1:** APCI-MS (negative mode) m/z 161 [M-H]<sup>-</sup>; NMR: δ (CDCl<sub>3</sub>) 2.35 (3H, s, methyl protons), 3.01 (2H, d, CH<sub>2</sub>CO), 4.21 (2H, d, OCH<sub>2</sub>), 6.78 (1H, d, aromatic) and 7.32 (2H, m, aromatic). <sup>13</sup>C-NMR data; δ 66.9 (C-2), 37.8 (C-3), 196.8 (C-4), 129.2 (C-5), 128.7 (C-6), 134.10 (C-7), 114.2 (C-8), 154.2 (C-9) and 123.5 (C-10).

**Compound 2:** APCI-MS (positive mode) m/z 260 [M+H]<sup>+</sup>; NMR: δ (CDCl<sub>3</sub>) 3.68 (9H, s, OCH<sub>3</sub>), and aromatic protons at δ 6.4 (1H, d), 7.35(3H, m). <sup>13</sup>C-NMR data; δ 149.8 (C-3), 141.8 (C-4), 118.3 (C-5), 116.9 (C-6), 154.1 (C-7), 104.2 (C-8), 146.2 (C-9) and 163.5 (C-10), 103.4 (C-11), 157.4(C-12) and 140.6 (C-13).

**Gas chromatography analysis:** An Agilent 6890N model GC gas chromatograph was used for the analysis. The analysis was performed as described elsewhere (Lampronti et al., 2003).

**Animals:** Colony bred, healthy Wister albino rats of either sex weighing 200-220 g were taken for the study. The animals were fed on standard laboratory diet with water *ad libitum* and housed at room temperature. The rats were kept of fasting overnight with free access to water during the experiment in the same ambience. The animals were divided into four groups of six animals each.

**Glucose uptake by isolated rat hemi-diaphragm:** Glucose uptake by rat hemi-diaphragm was estimated by the methods described elsewhere (Walaas and Walaas, 1952; Chattopadhyay et al., 1992) with some modifications. Four sets containing six numbers of graduated test tubes (n=6) for each fraction (Fr. **1-8**) were taken. Group I served as a control which contained 2 mL of Tyrode solution with 2% glucose, Group II contained 2 mL Tyrode solution with 2% glucose and regular insulin (Nova Nardisk) 0.62 mL of 0.4 units per mL solution. Group III contained 2 mL Tyrode solution with 2% glucose and 1.38 mL of *A. marmelos* fraction and the Group IV contained 2 mL Tyrode solution with 2% glucose and regular insulin 0.62 mL of 0.4 units per mL solution and 1.38 mL of *A. marmelos* fraction.

The volumes of all the test tubes were made up to 4 mL with distilled water to match the volume of the test tubes of group IV. Albino rats were tested overnight and killed by decapitation. The diaphragms were dissected out quickly with minimal trauma and divided into two halves. Two diaphragms from the same animal were not used for the same set of experiment. Six numbers of diaphragms were used for each group (Fr **1-8**). The hemi-diaphragms were placed in test tubes and incubated for 30 min at 37°C in an atmosphere of 100% oxygen with shaking at 140 cycles/min. Glucose uptake per gram of tissue was



4.21 (2H, d, -OCH<sub>2</sub>), 6.78 (1H, d, aromatic) and 7.32 (2H, m, aromatic) and identified as 6-methyl-4-chromanone. <sup>13</sup>C-NMR data confirms the structure of **1**; δ 66.9 (C-2), 37.8 (C-3), 196.8 (C-4), 129.2 (C-5), 128.7 (C-6), 134.10 (C-7), 114.2 (C-8), 154.2 (C-9) and 123.5 (C-10). Compound **2** had a molecular formulae C<sub>14</sub> H<sub>13</sub> O<sub>4</sub> N evident from its APCI-MS (positive mode) spectrum by exhibiting a peak at m/z 260 [M+H]<sup>+</sup>. Its NMR spectrum showed peaks at δ 3.68 (9H, s, three -OCH<sub>3</sub>), and aromatic protons at δ 6.4 (1H, d), 7.35 (3H, m) and the <sup>13</sup>C-NMR data confirms the structure of **2** as skimmianine; δ 149.8 (C-3), 141.8 (C-4), 118.3 (C-5), 116.9 (C-6), 154.1 (C-7), 104.2 (C-8), 146.2 (C-9) and 163.5 (C-10), 103.4 (C-11), 157.4 (C-12) and 140.6 (C-13). Already skimmianine, was found to have a significant inhibitory effect on spontaneous motor activity, exploratory behavior, cataleptogenic activity, conditioned avoidance response and long-term isolation-induced fighting of animals. A mild antimethamphetamine activity was also observed. Its neuroleptic activity was less potent than that of chlorpromazine (Cheng, 2003), it has inhibitory activity against the enzyme adenine phosphoribosyltransferase (APRT) from Leishmania, a tropical parasite causing endemic disease in poor countries (Napolitano et al., 2008), function as antagonists of 5-hydroxytryptamine receptors in animals (Cheng et al., 1994), and 6-methyl-4-chromanone was earlier isolated from stem extract of *A. marmelos* and found to inhibit the *in vitro* proliferation of human tumor cell lines, including the leukemic K562, T-lymphoid Jurkat, B-lymphoid Raji, erythroleukemic HEL, melanoma Colo38, and breast cancer MCF7 and MDA-MB-231 cell lines (Lampronti et al., 2003). Our results suggest that 6-methyl-4-chromanone **1** and skimmianine **2** may be responsible for the *in vitro* antidiabetic effect.

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