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Short communication

Anti adipogenic activity of *Aegle marmelos* Correa

Aniket Karmase, Sneha Jagtap, Kamlesh K. Bhutani*

Department of Natural Products, NIPER, Sector 67, Mohali, Punjab 160062, India

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ABSTRACT

In continuation of evaluating the anti-obesity effect of *Aegle marmelos*, we have screened the *n*-hexane, dichloro methane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) extracts of the leaves at the concentration of 25, 50, 75 and 100 μg/ml for adipogenesis inhibition in the adipocytes. Nile red staining with the help of fluorometry was used as indicator of the antiobesity activity. The most active DCM extract showed the 33.98 ± 3.55% lipid content at 100 μg/ml and was selected for the further isolation. 14 compounds were isolated from DCM extract of *A. marmelos* leaves. The compounds were screened for the adipogenesis inhibition at 50 and 100 μM concentrations. Out of the 14 compounds, halfordinol, ethyl ether aegeline and esculetin were showing 10.04 ± 0.52, 16.29 ± 0.85 and 25.09 ± 1.31% lipid content respectively at 100 μM. We hereby report the adipogenesis inhibition by *A. marmelos* as one of the pathway for its antiobesity effect.

Introduction

Obesity is the commonest chronic metabolic disorder prevailing with more than one billion overweight adults and 300 million clinically obese worldwide (WHO 2012). Obesity involves complex interactions of genetic, behavioural and environmental factors correlating with economic and social status and lifestyles. Obesity involves abnormal fat absorption, storage and metabolism and there by risk of important co morbidities like coronary artery disease, cerebrovascular disease, cholelithiasis, hypertension, hyperlipidemia, type II diabetes, pulmonary embolism, stroke, gall bladder disease, sleep apnea, gynaecological abnormalities, osteoarthritis, psychiatric illness, malignancy (breast, endometrial, prostate and colon), hyperuricaemia and cancer increases (Kaila and Raman 2008).

Adipose tissue, which is the predominant type of fat storage in humans, serves as a storage depot for excess energy and an important metabolic organ crucial for whole-body insulin sensitivity (Lefterova and Lazar 2009). The reported phytochemicals affects adipose tissue by different approaches like cell growth arrest, apoptosis, lipolysis and decreased lipid accumulation. They also affect major factors involved in adipogenesis, *i.e.* PPARγ, C/EBPα, C/EBPβ, C/EBPδ. Some of these phytoconstituents are tea catechins, genistein, quercetin, rutin, curcumin, resveratrol, berberine, retinoic acid, guggulsterone, ajoene and esculetin (Rayalam et al. 2008).

Aegle marmelos, known as bael in India is a plant of Rutaceae family, and one of the important plants in the Ayurveda. All parts of the plant are used by the Ayurvedic practitioners. Various parts of this plant such as leaves, roots, seeds, bark and fruit possess different activities (Maity et al. 2009). The fruit of the *A. marmelos* is used in beverages. *A. marmelos* extracts are having the antidiabetic property. Oral as well as intraperitoneal administrations of the aqueous extract of bael fruit exhibited hypoglycemic effect against streptozotocin induced diabetic rats (Kamalakkanan et al. 2003; Kamalakkannan and Prince 2003).

Ethanolic extract of bael leaves also inhibited the elevation of serum cholesterol and triglycerides level in Triton WR 1339 treated hyperlipidaemic rat (Vijaya et al. 2009). Oral administration of the aqueous extract of bael fruits and seeds separately at a dose of 250 mg/kg to streptozotocin-induced diabetic rats significantly lowered the serum and tissue lipid profile (Kesari et al. 2006). Aegeline, the major constituent in the *A. marmelos* leaves has shown good antihyperglycemic and antidyslipidemic property and it has reduced plasma triglyceride, total cholesterol and free fatty acids accompanied with increase in high density lipoprotein in dyslipidemic hamster model at the dose of 50 mg/kg body weight (Narender et al. 2007).

To further evaluate antiobesity action, we screened the *A. marmelos* extract and compounds in adipogeneis inhibition assay. In the present study we have tried to correlate the adipogenesis as one of the mechanism for the *in vivo* activities of *A. marmelos* extracts.

Experimental

Plant material, extraction and isolation of compounds

A. marmelos leaves were collected from the National Institute of Pharmaceutical Education and Research (NIPER), Mohali

* Corresponding author. Tel.: +91 172 2232208; fax: +91 172 2232208.
E-mail address: kkbhutani@niper.ac.in (K.K. Bhutani).

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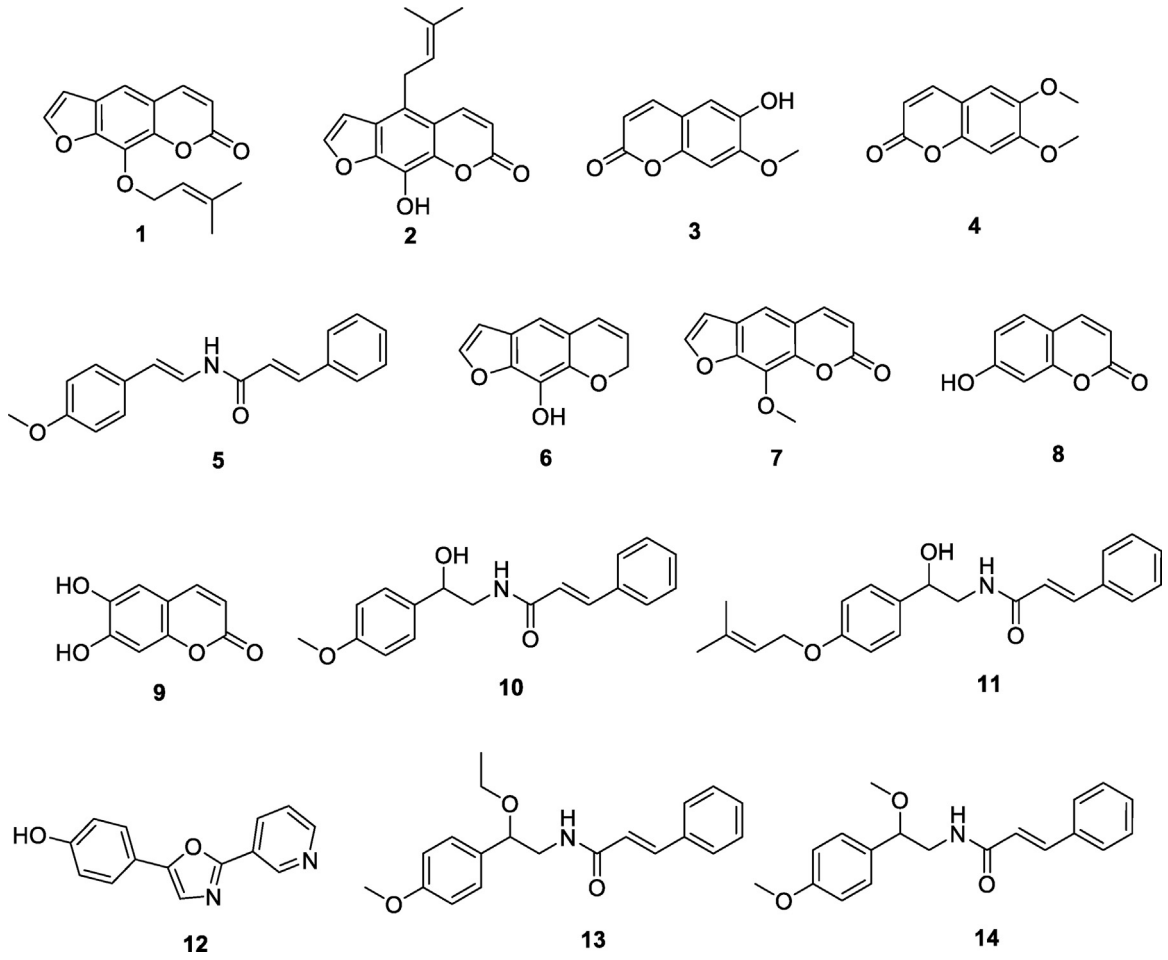

Fig. 1. Isolated compounds from the DCM extract of *A. marmelos* leaves.

campus, India. Leaves were authenticated by qualified botanist in the department of Natural Products and a voucher specimen (Herbarium No. NIP-H-174) is available in the herbarium of department of Natural Products, NIPER.

Plant material was dried in an oven at 40 °C and ground to a coarse powder. The powdered material was sequentially extracted with *n*-hexane, DCM, EtOAc, and methanol. The extracts were dried on a rotary evaporator and stored under refrigeration conditions.

14 compounds were isolated from DCM extract of *A. marmelos* leaves. The extraction and isolation procedure along with HPLC analysis is recently reported by us for the lipolysis activity of the extracts and compounds. The isolated compounds were identified as **1** imperatorin, **2** iso-imperatorin, **3** iso scopoletin, **4** scoparone, **5** anhydroaegeline, **6** xanthotoxol, **7** xanthotoxin, **8** umbelliferone, **9** esculetin, **10** aegeline, **11** marmeline, **12** halfordinol, **13** ethyl ether aegeline, **14** methyl ether aegeline (Fig. 1; Karmase et al. 2013).

Cell culture and quantification of lipid content

3T3-L1 preadipocytes (NCCS, Pune, India) were cultured in DMEM with 4.5 g/l glucose, 10% calf serum, 10,000 U penicillin and 10 mg streptomycin per ml (Himedia, Mumbai, India) in 96 well plates until they reached 100% confluency. For differentiation, 2 day post-confluent cells were incubated for 48 h in DMEM with 10% FBS, antibiotics, 0.5 mM isobutylmethylxanthine (IBMX) and 1 μM dexamethasone (Sigma–Aldrich, St. Louis, MO, USA). Cells were then incubated with same media, except with 10 μg/ml insulin (Sigma–Aldrich, St. Louis, MO, USA) substituted for IBMX and dexamethasone for next 48 h. Cells were then cultured for 3 days in DMEM with 10% FBS and antibiotics. To examine the effect on differentiation, extracts/compound in not more than 1% DMSO carrier were added to the differentiation medium up to day 7. Lipid content was quantified using a commercially available AdipoRed Assay Reagent (Lonza, Verviers, Belgium) according to the manufacturer's instructions. Quercetin was used as a positive standard (Yang et al. 2008).

MTT cell viability assay

To examine the effect of extracts on cell viability during differentiation process, the extracts in not more than 1% DMSO carrier were added to the differentiation medium up to day 7. On day 7, cell viability was determined using MTT assay.

Statistical analysis

Statistical analysis was carried out by using commercially available software SigmaStat 3.5. Values are expressed as mean ± SEM. For multiple comparisons, one way ANOVA was used followed by Tukey and/Dunnett's test. p value < 0.05 was considered to be significant.

Results and discussion

Effect of A. marmelos leaves extracts and isolated compounds from DCM extract on adipogenesis

Obesity correlates different conditions like increased white adipose tissue mass caused by adipocyte hypertrophy and/or