Isolation of antioxidative compounds from *Micromelum minutum* guided by preparative thin layer chromatography-2,2-diphenyl-1-picrylhydrazyl (PTLC-DPPH) bioautography method

Nur Kartinee Kassim*, Pei Cee Lim, Amin Ismail, Khalijah Awang

*Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

**Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*Corresponding author.

**E-mail addresses:** kartinee@upm.edu.my (N.K. Kassim), limpeicee@yahoo.com (P.C. Lim), aminis@upm.edu.my (A. Ismail), khalijah@um.edu.my (K. Awang).

**ARTICLE INFO**

**Keywords:**

*Micromelum minutum*  
Rutaceae  
Antioxidants  
Preparative thin layer chromatography-DPPH bioautography  
Coumarin

**ABSTRACT**

The application of preparative thin layer chromatography-2,2-diphenyl-1-picrylhydrazyl (PTLC-DPPH) bioautography technique successfully isolated a lignan sesamin (1), two prenylated coumarins (2 and 3) and a marmesin glycosides (4) from *Micromelum minutum* methanol bark extract. Compounds 2 and 3 were identified as new compounds whereas 1 and 4 were first isolated from Micromelum genus. Structural identification of all compounds were done by detailed spectroscopic analyses and comparison with literature data. Antioxidant capacities of extract, active fraction and compounds were measured based on DPPH free radical scavenging activity, oxygen radical absorbance capacity (ORAC) and β-carotene bleaching. The DPPH activity of methanol extract and its fraction present the IC50 values of 54.3 and 168.9 µg/mL meanwhile the β-carotene bleaching results were 55.19% and 5.75% respectively. The ORAC measurements of *M. minutum* extract, compounds 2 and 4 showed potent antioxidant activity with the values of 5123, 5539 and 4031 µmol TE/g respectively.

1. Introduction

The genus *Micromelum* belongs to Rutaceae family. It contains about 11 species which are distributed in tropical Asia and subtropical regions (Luo et al., 2012) where only two species occur in Peninsular Malaysia, *M. minutum* and *M. hirsutum*. In Peninsular Malaysia, they can be found naturally in the hilly parts of the northern half and north-west area. *M. minutum* (synonymous with *M. puhescens* Blume) or its common name Lime berry is also known as cemumar, chememar, cherek, Kematu or Mentanen amongst the local people. The leaves are eaten raw by the Malay or taken as ‘ulam’ as it is believed to be good for leucorrhrea and possess aphrodisiac property (Noraida, 2005). Previous phytochemical investigation on *Micromelum* species, reported that the plant is abundant with coumarins in which some of isolated compounds exhibited significant biological activities. For examples, 6- and 8-prenylated coumarins exhibited both *in vitro* and *in vivo* antitumor activity (Kamperdick, Van, Sung, & Adam, 1999; Luo, Qi, Yin, Xiao, & Zhang, 2009). Marrangatin and minumicrolin showed significant anti-tumoral activities. Coumarin derivatives including 3″,4″-dihydrocapnolactone, 6-hydroxy-3″,4″-dihydrocapnolactone-2″,3″-diol, 8,4″-dihydroxy-3″,4″-dihydrocapnolactone-2″,3″-diol, 8-methoxycapnolactone along with two triterpenes and stigmasterol were isolated from *M. minutum* (Ito, Otsuka, Ruanprungsi, & Furukawa, 2000; Rahmani et al., 2003). Mahanine, a bioactive carbazole alkaloid possessed antioxidative activities, inhibited topoisomerase I and IIb, antimutagenicity against heterocyclic amines; antimicrobial activity against gram positive bacteria; and anti-inflammatory effect (Mandal et al., 2017; Nooron, Athipornchai, Suksamrarn, & Chiabchalard, 2017). In this study, the antioxidant capacities of *M. minutum* were assessed. Antioxidants were reported to possess health promoting effects. However, only one antioxidant activity test is insufficient to show the potency of the test samples. Therefore, three antioxidant capacities tests were conducted namely ORAC, DPPH and β-carotene bleaching assays.

2. Materials and methods

2.1. Chemicals and reagents

Column chromatography was performed using silica gel Merck Kieselgel PF254 Art. No. 1.007749.1000, 60 Art. No. 9385.1000 and No. 1.07734.1000. The DPPH, β-carotene, linoleic acid, BHT, ascorbic acid, α-tocopherol, Folin–Ciocalteau, gallic acid 2,2′-Azobis(2-amidino-