



Effect of extraction procedure on the yield and biological activities of hydroxychavicol from *Piper betle* L. leaves

Norhisam Zamakshshari^a, Idris A. Ahmed^{a,*}, Muhammad N.A. Nasharuddin^a,
Najihah Mohd Hashim^{a,b,**}, Mohammad R. Mustafa^c, Rozana Othman^{a,b,**},
Mohamed I. Noordin^a

^a Centre for Natural Products Research and Drug Discovery (CENAR), Universiti Malaya, 50603, Kuala Lumpur, Malaysia

^b Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universiti Malaya, 50603, Kuala Lumpur, Malaysia

^c Department of Pharmacology, Faculty of Medicine, Universiti Malaya, 50603, Kuala Lumpur, Malaysia

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ABSTRACT

Piper betle Linn is one of the most common ethnomedicinal plants with its extract being popularly used in the modern product to enhance functionality. However, extraction methods always lead to differences in biological activities. The objectives of this study were to evaluate the effects of the extraction methods on the yield and biological activities of hydroxychavicol from *P. betle* L. extracts and to determine the correlation between the hydroxychavicol content and biological activities of *P. betle* L. extracts such as antioxidant, antimicrobial, and anticancer properties. The purity of the hydroxychavicol and its concentration (quantitative) in the crude extracts were also evaluated using a reverse-phase HPLC while GC-MS was employed to determine other components (qualitative). The results showed that only certain extraction procedures gave high yields of hydroxychavicol as well as remarkable biological activities. The chloroform extract following boiling with water (M2) gave the highest percentage of hydroxychavicol content based on the HPLC analysis. M2 and pure hydroxychavicol actively inhibited all the five cancer cell lines studied except A549. M2 showed more effective inhibition activity against MCF 7 with an IC₅₀ of 1.74 µg/mL. M2 extract also showed strong antibacterial activity against all the bacteria strains as well as a strong antifungal activity against *Candida albicans*. There was, however, a weak correlation between the hydroxychavicol content and the biological activities of *P. betle* L. extracts. In conclusion, extraction procedures greatly affect the yield and biological activities of hydroxychavicol from *P. betle* L. The designation of a single compound such as hydroxychavicol as a bioactive chemical marker compound in the *P. betle* L. extracts, however, is not enough to determine the biological activities of the extract.

1. Introduction

Herbal and natural raw materials have gained unprecedented attention in cosmetics, food additives, medicinal formulations, fragrances, and nutrition owing to the complex mixtures of several compounds in their matrices exhibiting synergetic and additive properties (Kharbach et al., 2020). *Piper betle* L. is one of the dicotyledonous plant species that grow heavily in Southeast Asia. In Malaysia, it is locally known as *sirih*. This species belongs to the Piperaceae family. It is a climber species and mainly cultivated for its leaves (Choudhary and Kale, 2002). This species is known in traditional folk medicine for oral care. In modern medicine, owing to its non-toxic properties both in vitro

and in vivo, it is being intensively studied and found to have many strong biological activities such as antioxidant, anticancer, antibacterial, antifungal as well as anti-fertility, hepatoprotective, immunomodulatory, anti-allergic, gastro-protective, and wound healing (Dasgupta and De, 2004; Ma et al., 2013; Yadav et al., 2014; Venkadeswaran et al., 2016). To date, this plant has been known as a high-value herbal plant by the Malaysian government and worths further studies. Many health care products have been formulated with *P. betle* L. extract as one of the main ingredients (Ali et al., 2018). *P. betle* L. is rich with phenolic compounds from the class of phenylpropanoid (Rimando et al., 1986). One of the major and active compounds found in this species is hydroxychavicol. This compound has been reported to possess strong

* Corresponding author.

** Corresponding authors at: Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universiti Malaya, 50603, Kuala Lumpur, Malaysia.

E-mail addresses: [IDRISAHMED@UM.EDU.MY](mailto: IDRISAHMED@UM.EDU.MY) (I.A. Ahmed), [NAJIHAHMH@UM.EDU.MY](mailto: NAJIHAHMH@UM.EDU.MY) (N. Mohd Hashim), [ROZANAOTHMAN@UM.EDU.MY](mailto: ROZANAOTHMAN@UM.EDU.MY) (R. Othman).

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