UNIVERSITI TEKNOLOGI MARA

MASS BASED IDENTIFICATION OF *TETRACERA INDICA* CHEMICAL CONSTITUENTS AND THEIR ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

Tetracera indica (Dilleniaceae) is locally known as mempelas. The plant grows in the forest fringes of Thailand and Peninsular Malaysia. Various parts of *T. indica* are traditionally used for treatment of diseases related to inflammation. However, the phytochemical data on *T. indica* is still lacking. Therefore, this study is focused on the phytochemistry of *T. indica* (stem) and evaluation of their anti-inflammatory activities. The objectives of this study are to develop LCMS/MS^n method to rapidly identify the chemical constituents present in the methanol extract of *T. indica* stem, to isolate and characterize chemical constituents using various chromatographic and spectroscopic techniques in order to verify the LCMS/MS^n method and to evaluate anti-inflammatory related biological activities of crude extract, fractions and chemical constituents of *T. indica*. In this work, 18 compounds mostly of the flavonoid type were detected in the HPLC profile. The methanol crude extract of the stem was analysed using two mass spectrometer systems, a QTrap 4500 and a LTQ-Orbitrap MS. With a systematic strategy combining highly resolved peaks, highly accurate mass measurements and understanding of the diagnostic fragment-based fragmentation patterns, 14 compounds from 18 peaks detected in LTQ-Orbitrap MS system were identified and characterized. They consist of six flavonol methyl ether sulphates, two dihydroflavonol methyl ether sulphates, two flavonol methyl ethers, one flavone methyl ether sulphate, one flavone aglycone, and one triterpene. The sulphated compounds were detected through neutral loss scan analysis of 80 mass unit performed on Qtrap 4500 MS system. With mass error < 2 ppm, LTQ-Orbitrap MS system confirmed identification of the sulphated flavonoids. The nine sulphated flavonoids are quercetin-3-*O*-sulphate **24**, kaempferol-3-*O*-sulphate **10**, quercetin-7-methyl ether-3-*O*-sulphate **79**, kaempferol-7-methyl ether-3-*O*-sulphate **80**, quercetin-7,4-dimethyl ether-3-O-sulphate **81**, kaempferol-7,4-dimethyl ether-3-O-sulphate **84**, taxifolin-3-*O*-sulphate **75,** taxifolin-7-methyl-ether-3-*O*-sulphate **76** and wogonin-7-*O*-sulphate **82**. Isolation, purification and characterization of eight compounds using spectroscopic method were done to complement and verify the mass based identification. 5,7-dihydroxy-8-methoxyflavone **28**, kaempferol-7-methyl ether **14**, kaempferol-7,4-dimethyl ether **7**, kaempferol **6**, quercetin-7-methyl ether **15** and taxifolin-7-methyl ether **85**, kaempferol 4-methyl ether **83** and betulinic acid **39**, were characterized by NMR spectroscopy. Occurrence of the nine sulphated compounds, six of which (**75**, **76**, **79**, **80**, **81** and **82)** are reported for the first time from a *Tetracera* species. Anti-inflammatory activities of *T. indica* were evaluated using xanthine oxidase and lipoxygenase inhibitory assays. The methanolic extract of the stem exhibited significant activity in both assays in a concentration-dependent manner. All fractions except hexane fraction showed strong inhibitory activity against lipoxygenase in the lipoxygenase inhibitory assay. Additionally, ethyl acetate fraction showed potential inhibitory activity against xanthine oxidase and lipoxygenase enzyme. Tested at 100 ppm, 5,7-dihydroxy-8-methoxyflavone **28** and kaempferol **6** isolated from the ethyl acetate fraction inhibited lipoxygenase enzyme with 100% and 90% inhibition respectively. In addition, betulinic acid **39**, kaempferol **6** and 5,7-dihydroxy-8-methoxyflavone **28**, kaempferol **6**, isolated from the same fraction, appeared to be the most active compounds against xanthine oxidase giving the inhibition of 95%, 90% and 80% respectively.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

The use of natural products especially plants based natural products, as medicines is a global phenomenon. The natural chemistry in the healing plants is the basis of this medicinal use by many societies all over the world regardless whether the knowledge is documented in written form or orally passed down from generation to generation. Indigenous people in many parts of the world still rely on traditional medicines from natural resources such as plant parts, animal parts or minerals for the treatment of all sorts of diseases.

Plants form the major component of several traditional medicine systems which have been in existence for centuries and they continue to provide mankind with new remedies. Presently, the list of plants with recognized medicinal properties is relatively long. About 5,800 plants are reported in the Chinese Material Medica, and 2,500 are reported in the Indian Material Medica. Thousands of plants are currently detailed for medicinal uses in other parts of the world and many more are only known to the traditional healers (Pan et al., 2014). The use of herbal medicinal products and supplements has increased tremendously over the past three decades with not less than 80% of people worldwide relying on them for some part of primary healthcare (Ekor, 2014). In 2009, 81% of the Malaysia population used plant-based therapies which included herbal therapy for health problem and 87% are using it for health care (Siti et al., 2009). These statistics indicate that people still trust and believe in the values of medicinal plants. In addition, the inheritance of traditional knowledge from diverse ethnicity and rich natural resources of Malaysia are significant factors shaping the pattern for the usage of herbals in the society (Law et al., 2013).

Generally, these medicinal plants are used as complex mixture such as extracts and essential oils or pure and chemically defined active compounds. However, in general, traditional usage of medicinal plants is not supported by scientific data. Modernization of traditional medicine for sustainable future use requires scientific evidence to support the claims as well as ensuring the quality, safety and efficacy of the