

COMPARATIVE STUDIES OF PHYTOCHEMICAL SCREENING, ANTIOXIDANT, ANTI-INFLAMMATORY ASSAYS AND LC-MS/MS ANALYSIS OF *EUPHORBIA HIRTA* L. COLLECTED FROM MALAYSIA AND IRAQ

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(Received 12 December 2021, Revised 3 February 2022, Accepted 12 February 2022)

ABSTRACT : *Euphorbia hirta* L., belonging to the Euphorbiaceae family, is widely used in traditional remedies and have different pharmacological applications. Samples of the plant were collected in Malaysia and Iraq. The objectives of this study are to understand how variations in climate and geography might affect the plant's nutrient, secondary metabolite and antioxidant levels. The aerial part of *Euphorbia hirta* plant was simultaneously collected in Malaysia and Iraq. Hot and cold extraction methods involving the use of hexane, chloroform, methanol and water solvents were then undertaken. All extracts were subsequently screened for the presence of phytochemicals and total phenolic and flavonoid contents. In addition, in vitro antioxidant and anti-inflammatory activities were conducted. Finally, the optimum extracts (metabolic extracts) of the two species were subjected to LC-MS/MS analysis. The results showed that the highest phenolic and flavonoid content, antioxidant properties and anti-inflammatory activities were found in the methanolic extract of the Malaysian species obtained using hot methods (336.65±9.46 mg Gallic acid equivalent of dry plant material weight, 63.94±2.81mg (QE)/g dry wt. 80.80±0.53% and 88.81% respectively). An LC-MS/MS analysis of the two species revealed the presence of various phenolic, flavonoids and steroid compounds. In conclusion, differences in agro-climatic conditions, extraction methods and types of extraction solvent used have clear effects on the total phenolic and flavonoid content, and the antioxidant and anti-inflammatory potential of the *E. hirta* plant.

Key words : *Euphorbia hirta*, antioxidant, anti-inflammatory effect, LC-MS/MS.

How to cite : Dania F. Alsaffar, Sura F. Alsaffar, Ahmed Yaseen, Shaida Fariza Sulaiman, Roziahaman Mahmud and Nur Hidayah Kaz Abdul Aziz (2022) Comparative studies of phytochemical screening, antioxidant, anti-inflammatory assays and LC-MS/MS analysis of *Euphorbia hirta* L. collected from Malaysia and Iraq. *Biochem. Cell. Arch.* **22**, 1447-1458. DocID: https://connectjournals.com/03896.2022.22.1447

INTRODUCTION

According to the World Health Organization (WHO), a huge number of people currently use medicinal plants to promote improvements in healthcare (Ekor, 2014 and Schwartz *et al*, 2016). Globally, four out of five people depend on plants used in primary health care such as *Panax ginseng*, *Ginkgo biloba* and *Chamomilla recutita* (Ghosh *et al*, 2019; Alsaffar and Al-Kaissy, 2009). Furthermore, one in four of the remedial drugs are extracted directly from or constitute derivatives of plants (Brower, 2008). Therefore, medicinal plants should be studied appropriately to promote a more comprehensive understanding of their botanical attributes in addition to establishing their chemical properties, safety, and efficacy (Pranabesh Ghosh, 2019). *Euphorbia hirta*

L., a member of the Euphorbiaceae family has spread extensively throughout the temperate or tropical regions of India, Asia, Australia and Africa. It is also native to Central America, where it is commonly known as asthma weed and milk weed, often being found in rubbish dumps and at the roadside (Sinhababu and Banerjee, 2016; Asha, 2014; Linfang *et al*, 2012). In Malaysia, its commonly used Malay names include ara tanah, gelang and keremak susu; while in Iraq its Arabic common name comprises labeinah, em elhaleeb and euphorbia (Rajeh *et al*, 2010 and Asha, 2014).

Pharmacological studies of *E. hirta* have revealed its antioxidant, antimicrobial, antiepileptic, sedative anxiolytic, anti-inflammatory, analgesic, antipyretic, anti-asthmatic, anti-histaminic, anti-diabetic, anti-cancer,

qualities, have the potential to prevent and manage various human diseases such as cancer, diabetes, cataracts, neurodegenerative disorders, cardiovascular conditions and liver disease (Alsaffar *et al.*, 2017). In this study, the results of DDPH assay showed minor contrasts in the antioxidant activity between the two species and significant differences between various types of solvent extracts. Several previous studies have indicated that phenol, which is the main antioxidant component, and the total contents were directly proportional to their antioxidant activity. Another sizeable body of research has established that the quality and quantity of the phenolic and flavonoid compounds are strongly correlated with the antioxidant activities of plants (Jing *et al.*, 2015).

Several researchers have stated that *E. hirta* extract produces various pharmacological effects one of them being that of an anti-inflammatory agent. The *in vitro* egg albumin method provides an inexpensive alternative method of assaying the anti-inflammatory activity of herbal medicine using a denaturation technique. Inflammatory activity can be impeded through inhibition of protein denaturation, a method which could be confirmed by further studies (Sarveswaran *et al.*, 2017). Numerous recent studies have shown that the anti-inflammatory activity of many plants contributes significantly to the production of polyphenols and flavonoids (Okoli and Akah, 2004). Hence, in this study, the presence of phenol and flavonoids in the methanol extract of *E. hirta* in the two species may have contributed to its anti-inflammatory activity. In the LC-MS/MS chromatogram of the Malaysian *E. hirta* species the major two compounds were kaempferol-3-O-glucoside (astragalins) and Quercetin-3'-glucoside (isoquercetin) with respective retention times of 5.4669 minute and 3.9079 minutes. This was also reported in a previous study of *E. hirta* methanolic extract of the Malaysian species (Abu Bakar *et al.*, 2020). In the case of Iraqi *E. hirta* methanolic extract, the kaempferol-3-O-glucoside (astragalins) with (RT = 5.4861 minutes) and 1,3,5(10)-estratrien-17-one 3,7,8-triol (RT=14.6633 minutes) were the major compounds obtained. This result suggests that the antioxidant and anti-inflammatory potency of *E. hirta* methanolic extracts in the two species results mainly from the contribution of phenolic compounds, tannins and flavonoids (Abu Bakar *et al.*, 2020).

CONCLUSION

This study demonstrated that the levels of secondary metabolites in *E. hirta* plants differ in the Malaysian and Iraqi species. The former contain higher levels of phenolic and flavonoid compounds than the latter. It can, therefore, be concluded that agro-climatic locations, in combination

with geography, have significant effects on the *E. hirta* plant phytoconstituents and their antioxidant potential. In addition, the present study clearly demonstrated that the solvent type and extraction methods have a fundamental effect on the level of antioxidant compounds in the *E. hirta* areal parts. Finally, it can be concluded that *E. hirta* has demonstrated the existence of strong correlations between antioxidant and anti-inflammatory activities and their phenolic and flavonoid contents. Further investigation of the secondary metabolites by LC-MS/MS analysis of *E. hirta* methanolic extracts revealed the presence of phenolic, flavonoid and steroid compounds. Hence, *E. hirta* could represent a natural source of polyphenol compounds which can be used in pharmaceuticals for the treatment of a range of diseases. This research has raised numerous questions which require further investigation.

ACKNOWLEDGEMENT

We would like to thank Universiti Sains Malaysia for funding this project through the Incentive Bridging Grant (304.PFARMASI.6316549).

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