

UNIVERSITI TEKNOLOGI MARA

**MECHANISTIC INSIGHTS INTO
THE NF- κ B/iNOS/HO-1 MEDIATED
IMMUNOMODULATORY EFFECTS
OF *MYRMECODIA PLATYTYREA*
BECC. TUBER EXTRACT AGAINST
OXIDATIVE STRESS AND
INFLAMMATION: INTEGRATIVE
MULTI-OMICS AND *IN SILICO*
CHARACTERISATION**

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ABSTRACT

Myrmecodia platytyrea Becc. is a medicinal plant indigenous to the Southeast Asia region, specifically Indonesia and Malaysia. Its ethnomedicinal uses encompass anticancer, antimicrobial and anti-inflammatory properties, which may be attributed to its significant antioxidant potential. Nevertheless, there is insufficient phytochemical characterisation and an unclear mechanism of action of the ethyl acetate extract of *M. platytyrea* tuber (EAMPT). Hence, the study aimed to consolidate existing research gaps concerning the biological activities of the *Myrmecodia* genus and elucidate the phytochemical composition, antioxidant and anti-inflammatory effects, and mechanisms of EAMPT using an integrative approach. A combined bibliometric and scoping analysis was conducted to delineate current global trends and emerging research hotspots in *Myrmecodia* research. Qualitative and quantitative phytochemical analyses, including metabolomics, were carried out to characterise the phytochemical constituents of EAMPT. *In vitro* studies employing the lipopolysaccharide (LPS)-stimulated RAW 264.7 cell line were conducted to investigate the effects and mechanisms of EAMPT against the biomarkers of oxidative stress and inflammation at both gene and protein levels. Computational tools, including molecular docking and Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) prediction, were applied to evaluate the structure-activity relationship (SAR) of EAMPT's phytoconstituents and target proteins, along with their drug-likeness properties. Global publication on *Myrmecodia*'s biological activity is limited, with merely 45 indexed articles over the last 15 years. Indonesia has contributed the most publications (82.2%), with *M. pendans* being the most studied species (75%). Notably, reports on *M. platytyrea*'s immunomodulatory effects and mechanism, particularly against the crosstalk of oxidative stress and inflammation, are lacking. Phytochemical analyses revealed that EAMPT comprised phenolics, flavonoids, alkaloids, terpenoids and phytosterols. The total phenolic content was 4.15% with 2.8% Trolox equivalent antioxidant capacity. Metabolomic analysis has identified nine terpenoids, eight alkaloids and seven phenolics, with none previously reported in *Myrmecodia* sp. *In vitro*, EAMPT exhibited moderate cytotoxicity ($IC_{50} = 60 \mu\text{g/mL}$) on unstimulated RAW 264.7 cells. Following LPS stimulation, EAMPT suppressed ROS, TNF- α and NO levels while elevating IL-6 levels. Additionally, EAMPT downregulated TNF- α and iNOS gene expressions while upregulating HO-1 and IL-6. EAMPT's inhibitory effect on the iNOS/NO pathway was partially dependent on HO-1 and was modulated through the NF- κ B transcription factors. The findings signify the immunomodulatory mechanism of EAMPT on the NF- κ B/iNOS/NO pathway by regulating HO-1 as the crosstalk signalling molecule. *In silico* studies discovered three prenylated flavonoids – lespedezaflavanone G, euchrenone b1 and artelastofuran, as well as a monoterpene indole alkaloid – vellosimine, as lead phytocompounds exhibiting significant binding affinity to key targets – the iNOS and HO-1. These compounds showed favourable ADMET profiles and drug-likeness properties, especially vellosimine, which adhered to Lipinski's rule of five. Conclusively, this study has established *M. platytyrea* as the source of phytoimmunomodulators for treating inflammation-related diseases, both as an alternative medicine and as a basis for modern drug discovery. Future works on standardising the herbal extract and isolating the lead phytocompounds for in-depth biological evaluations are urgently recommended.

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CHAPTER 1

INTRODUCTION

1.1 Research Background

The World Health Organisation (WHO) has considered chronic inflammatory diseases the most serious threat to human health (Pahwa et al., 2023). Chronic inflammation has significantly contributed to the leading causes of death globally, including cerebrovascular stroke, diabetes, Alzheimer's disease, cancer and renal disease, whereas COVID-19, tuberculosis and pneumonia are the leading infectious causes (World Health Organisation, 2024). Ischaemic heart disease (IHD) is now the leading cause of death worldwide and in Malaysia (Khaw & Yong, 2024; World Health Organisation, 2024). Additionally, these inflammation-related illnesses have caused significant premature death among Malaysian adults (Chan et al., 2022; Khaw et al., 2023), whereas inflammatory ageing, which results in cancer, Alzheimer's disease and osteoarthritis, has caused substantial public health concern among the Malaysian elderly population (Abd Mutalib et al., 2020; Abdullah et al., 2024). The rising morbidity and mortality rates present a serious health concern both locally and abroad, which demands a multilevel strategy from healthcare workers and policymakers in preventing, screening, and managing the diseases while medical researchers continue to discover and develop new drugs that are effective and safe to tackle the evolving inflammatory illnesses.

Inflammation is a physiological response of the body to any stimulus, including infectious or injurious agents, aiming to protect the body from harm and injury. During inflammation, the body senses the stimuli and activates the inflammatory signalling pathways, releasing cytokines, chemokines, prostaglandins, histamines, and other inflammatory mediators into the circulation. This process is crucial to eliminate harmful stimuli and initiate tissue repair and healing. At this stage, acute inflammation is clinically manifested as redness, heat, swelling, pain, and loss of function of the affected body part. The acute inflammatory response typically resolves within hours to days. However, failure of the immune response to stimulate the clearance of neutrophils at the end of an acute reactive phase could result in a chronic inflammatory state (Pahwa