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Pharmacological Studies on the Potential Anti-inflammatory Effects of a Natural Product(s)

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I dedicate this thesis to my dear family, without their patience, understanding and support; this thesis would not be existed.

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Abstract

Background: Natural products have played a key role in drug discovery contributing to the development of therapeutic agents, which are currently used in modern medicine. *Dietes bicolor* is an uninvestigated plant that belongs to family Iridaceae and known to be rich in flavonoids.

Aim: The current study aimed at investigating the potential anti-inflammatory activity of *D. bicolor* leaf extract (DBL) and unraveling the underlying mechanisms of such activity. This study aimed also at performing phytochemical analysis of the biologically active fraction as well as standardization of the plant extract using the isolated chemical marker.

Methodology: Total leaf extract and fractions were screened for their anti-inflammatory activity using carrageenan-induced paw edema model. The most potent fraction, which was the butanol fraction (DB-BF), was selected for further investigation in carrageenan-induced rat paw edema and croton oil-induced ear edema models. DB-BF was subjected to further phytochemical analysis utilizing several chromatographic techniques. The major compound was isolated and its structure was elucidated using different spectroscopic techniques. The isolated compound was used as a chemical marker for the HPLC standardization experiment of *D. bicolor* leaf extract.

Results: DB-BF demonstrated a dose-related reduction in rat paw edema and ear edema. DB-BF ameliorated the alterations in the pro-inflammatory mediators (PGE₂, TNF- α , IL-6, IFN- γ , IL-1 α , IL-1 β , MCP-1, RANTES and MIP). Moreover, DB-BF suppressed neutrophils

infiltration, myeloperoxidase activity, and the histopathological changes in the investigated ear tissues in croton-oil ear edema model. Vitexin was isolated and identified from the bioactive fraction which may contribute to its prominent anti-inflammatory activity. The content of vitexin in the dried extract was found to be 67.4 mg/g.

Conclusion: *D. bicolor* leaf extract and its active fraction are promising natural remedies for the management of inflammation. Further studies are encouraged to extrapolate its benefit in clinical settings.

Keywords: *Dietes bicolor*; Iridaceae; anti-inflammatory; carrageenan-induced paw edema; croton oil-induced ear edema; vitexin

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List of abbreviations

AA	Arachidonic Acid
CCL	Chemokine (C-C motif) ligand
COX	Cyclooxygenase
CRP	C Reactive Protein
HOCL	Hypochlorous acid
HPLC-DAD	High-Performance Liquid Chromatography with Diode-Array Detection
ICF	Intracellular Fluids
IL-1 α	Interleukin-1 alpha
IL-1 β	Interleukin-1 beta
IL-6	Interleukin-6
IFN- γ	Interferon gamma
IVF	Intravascular Fluids
MCP-1	Monocyte Chemoattractant Protein-1
MIP	Macrophage Inflammatory Protein
MPO	Myeloperoxidase
M Φ	Macrophage
NK cells	Natural Killer cells
NO	Nitric Oxide
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
PGE2	Prostaglandin E2
RANTES	Regulated upon Activation, Normal T-cell Expressed and Secreted

ROS	Reactive Oxygen Species
TNF- α	Tumor Necrosis Factor-alpha

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Introduction

Inflammation

Inflammation is the immune system's response to infection and injury. It has been implicated in the pathogenesises of arthritis, cancer and stroke, as well as in neurodegenerative and cardiovascular disease. Inflammation is an intrinsically beneficial event that leads to removal of offending factors and restoration of tissue structure and physiological function. Inflammation has been known to humankind for at least a few thousand years, in part because it accompanied two major scourges of the past, wounds and infections, and in part because it is rather conspicuous (Nathan, 2002).

Although references to inflammation can be found in ancient medical texts, apparently the first to define its clinical symptoms was the Roman doctor Cornelius Celsus in the 1st century AD. These symptoms came to be known as the four cardinal signs of inflammation: *rubor et tumor cum calore et dolore* (redness and swelling with heat and pain). Celsus mentioned these signs in his treatise *De medicina*, while describing procedures for treating chest pain, where he became an oft-quoted medical celebrity (Majno, 1975). The physiological basis of the four cardinal signs of inflammation were revealed much later by Augustus Waller (1846) and Julius Cohnheim (1867), who discovered leukocyte emigration from the blood vessels and other vascular changes characteristic of an acute inflammatory response. Analyzing living tissues under the microscope, Cohnheim observed vasodilation, leakage of plasma, and migration of leukocytes out of blood vessels and

into the surrounding tissue (Majno and Joris, 2004). Once the initiating noxious stimulus is removed via phagocytosis, the inflammatory reaction can decrease and resolve. During the resolution of inflammation, granulocytes are eliminated, while macrophages and lymphocytes return to normal pre-inflammatory numbers and phenotypes. The usual outcome of the acute inflammatory program is successful resolution and repair of tissue damage, rather than persistence and dysfunction of the inflammatory response, which can lead to scarring and loss of organ function. It may be anticipated, therefore, that failure of acute inflammation to resolve may predispose to auto-immunity, chronic dysplastic inflammation and excessive tissue damage (Nathan, 2002).

Inflammation is a complex defense mechanism in which leucocytes migrate from the vasculature into damaged tissues to destroy the agents that potentially can cause tissue injury. Acute inflammation is a limited beneficial response, particularly during infectious challenge, whereas chronic inflammation is a persistent phenomenon that can lead to tissue damage. One hallmark of acute inflammation is that initially the leucocyte infiltrate is mostly neutrophilic, but after 24 to 48 hours monocytic cells predominate (Ryan and Majno, 1977; Doherty et al., 1988). In contrast, chronic inflammation is histologically associated with the presence of mononuclear cells, such as macrophages and lymphocytes (Ryan and Majno, 1977; Melnicoff et al., 1989).