OBAFEMI AWOLOWO UNIVERSITY

EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF THE FRACTIONS OF *Archidium ohioense* (SCHIMP. EX MULL)EXTRACT

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APPROVAL

This research project was supervised by us and approved in accordance with the partial fulfilment for the award of Master of Science (M.Sc.) in Biochemistry of the Obafemi Awolowo University, Ile- Ife, Osun State, Nigeria.

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DEDICATION

I dedicate this research work to God Almighty.

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LIST OF ABBREVIATIONS

6B	Test Tube Fraction from Ethyl Acetate Fraction of *Archidium ohioense*
$6B_2$	Isolate from 6B
COX	Cyclooxygenase
DCM	Dichloromethane
EtOAc	Ethyl Acetate
LOX	Lipooxygenase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Reduced
NO	Nitric Oxide
NSAID	Non-Steroidal Anti-Inflammatory Drug
SAID	Steroidal Anti-Inflammatory Drug
XO	Xanthine Oxidase

ABSTRACT

This study evaluated the anti-inflammatory potential of *Archidium ohioense* (a moss) and elucidated the structure of the compound responsible for this activity. This was with a view to finding the bioactive compound(s) responsible for its anti-inflammatory reactions.

The plant materials were dried and milled into powders. The powdered plant sample (1.5 kg) was extracted with 80% (v/v) methanol for 48 h at room temperature. The filtrate was concentrated *in vacuo* to yield the crude methanol extract. The crude extract was partitioned with n-hexane, dichloromethane, ethylacetate and butanol to afford five different fractions. Each of the fractions obtained was screened for presence of secondary metabolites. Also the membrane stabilization potential, albumin denaturation inhibition, xanthine oxidase and lipooxygenase inhibitory techniques of the fractions were evaluated using standard methods. The structure of the compound responsible for the anti-inflammatory activity from the ethyl acetate fraction (most active) was elucidated via chromatographic and nuclear magnetic resonance spectroscopic techniques.

All the fractions possessed strong anti-inflammatory properties at various concentrations tested. The ethyl acetate fraction (EtOAc) exerted maximum percentage stability of 97.39±0.00 at 0.15 mg/ml, n-hexane fraction (nHF) exerted 96.23±0.00% at 0.25 mg/ml, dichloromethane fraction (DCMF) exerted 91.85±0.00% stability at 0.30 mg/ml,butanol fraction exerted 87.71±0.00% at 0.30 mg/ml and aqueous fractions (with least) percentage membrane stability of 23.93±0.01 at 0.15 mg/ml. The EtOAc, DCM and nH fractions competed favourably with diclofenac (a standard drug) which exerted 86.94±0.00% at 0.30 mg/ml. All the fractions of *A. ohioense* demonstrated anti-denaturation activities on heat-treated bovine serum albumin (BSA)

with the EtOAcfraction having the highest inhibitory activity, followed by DCM. Also, EtOAc fraction demonstrated higher and better percentage inhibitory activities for both lipooxygenase and xanthine oxidase than the DCM fraction at 0.05 mg/ml. The EtOAc fraction had its maximum percentage inhibitions of 75.00±1.67 for lipooxygenase and 62.50±8.84 for xanthine oxidase. The percentage inhibition of lipooxygenase and xanthine oxidase by DCM fraction were 71.67±3.54 and 50.00±0.00 respectively. Ascorbic acid, a well-known inhibitor of lipooxygenase inhibited the enzyme to 70.00±2.36% while allopurinol (a standard drug) had 77.50±1.76% inhibition against xanthine oxidase.

This study concluded that the ethyl acetate fraction of the plant exhibited strong anti-inflammatory activity. Also the compound responsible for this action was elucidated to be 4, 4-dimethyl cyclohexanone.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Inflammation is a natural process used by the cells to fight any infection and heal injuries, and is often noticed as soreness and swelling (Jaykumar *et al*., 2012; Centre for inflammation Research, 2014). The aetiology of inflammation is varied and includes infection, noxious chemicals and drugs, stress or physical factors, ultra-violet exposure, hypoxia, nutritional imbalance, allergic irritants, geneticand environmental factors(Iwalewa *et al,* 2007). Whatever might be the stimuli, it brings about tissue damage. Cells respond against such injurious agents in order to combat further damages to tissues. The purpose of the response is to localize and eliminate the agent as well as to remove the damaged tissue components so that the body can begin to heal (Britannica, 2012).Such responses of the cells against the injurious agents is called inflammatory response.

When tissuesare first damaged, the blood vessels in such areas constrict momentarily, a process called vaso-constriction. Following the release of histamine, the blood vessels dilate (vasodilation), thereby increasing the blood flow into the area. Furthermore, the walls of the blood vessels become more permeable. Sequel to that, protein-rich fluid, called exudate, is now able to leak into the tissues. The exudate contains clotting factors and antibodies. The leaking out of exudatesand other substances may cause the white blood cells to adhere to the walls of the blood vessels resulting in their accumulation. All of this, is to localize and eliminate the agent as well as to remove the damaged tissue components so that the body can begin to heal (Britannica, 2012).

If the agent causing the tissue damage cannot be eliminated, or if there are some degrees of interferences with the healing process, theoriginally acute inflammatory response may progress to the chronic stage. The chronic inflammatory response then manifests histologically by the presence of lymphocytes and macrophages, resulting in fibrosis and tissue necrosis. If there is a persistent chronic inflammation, it increases the development of the degenerative diseases such as rheumatoid arthritis, atherosclerosis, heart disease, Alzheimer, asthma, acquired immunodeficiency disorder(AIDS), cancer, congestive heart failure (CHF), multiple sclerosis (MS), diabetes, infections (bacteria, fungi, parasites), gout, inflammatory bowel disease (IBD), aging and other neurodegenerative CNS depression, all of which are associated with immunopathological disorder that appears to play a key role in the onset of the condition (O'Byrne and Dalgleish, 2001).

These diseases and disorders have been linked to increased expression of pro-inflammatory mediators which activates inflammatory cells by increasing the expression of pro-inflammatory cytokines, up-regulating genes that produce nuclear factor kappa B (NF-κB), NADPH oxidase, phospholipase A_2,cyclooxygenases 1 and 2 (COX-1 and COX-2), 5-Lipooxygenase (5-LOX), myeloperoxidase, inducible nitrogen oxide synthase (iNOS), increasing oxygen consumption and producing many oxygen-free radicals that can finally lead to certain degenerative diseases (Charles *et al.*, 1999; Locati and Murphy, 1999).

Nitric oxide (NO) is an example of reactive nitrogen species that participates in normal physiological processes such as vasodilation and neurotransmission; however, overexpression may result in disease as observed in inflammation, asthma, cardiovascular disorders and organ transplant rejection (Coleman, 2002).

Therefore, if inflammation is not controlled correctly by the body, it may cause illnesses such as asthma and arthritis. Long term (or chronic) inflammatory disorders are amongst the major killers in the UK, including heart disease, lung and airway disease (e.g. associated with smoking), chronic liver disease (e.g. associated with viral infection and alcohol) and chronic kidney disease. Uncontrolled inflammation also leads to tissue scarring (fibrosis) which can prevent organs such as the lung, kidney and liver from working properly (Centre for inflammation Research, 2014).

Whilst this range of disorders may appear daunting, the fact that they may have a common cause means that by understanding the biology of inflammation, we will be able to design generic approaches to a range of disorders affecting different organs. Methods are being developed to look at the inflammation and scars within the body to allow scientists to treat the problems more effectively without having to take tissue samples from the organs (Centre for inflammation Research, 2014).

1.1: The Study Plant

Archidium ohioense is a tropical moss plant belonging to the family of ***Archidiaceae*** and grows largely on rocky grounds. Its geographical distribution ranges from coastal antarctica to the tundra of the northern hemisphere, and from the