CHAPTER 1: INTRODUCTION

1.1. Introduction

Anti-inflammatory drugs have undergone decades long of development, started by the revolutionizing creation of aspirin by Felix Hoffman. Ever since, the mechanism and key molecules implicated in inflammation have been established. Proinflammatory cytokines and molecules such as cyclooxygenases (COXs), TNF-α, and interleukins (ILs) have been deemed as biologically significant for inflammation (Dinarello, 2010). Seeing their efficacy, various anti-inflammatory drugs have been synthesized in order to satisfy the high demands of quick-acting pain-relieving alternatives. Recent drug discoveries are trying to fulfill this demand, especially those derived from natural products which require rapid isolation, purification, and extraction for its bioactive compounds (Thomford et al., 2018).

The stinging tree (*Laportea decumana*) is a plant native to Indonesian soil of the *Urticaceae* family. Its leaves are equipped with long stinging hairs, hence the name: “daun gatal”. Traditionally, these leaves have been utilized as pain remedies. In Papua, locals have been applying the leaves directly onto the areas where pain is perceived. Treatment effectivity is represented by the appearance of numbness, though no scientific evidence is able to support the curative properties of “daun gatal” itself (World Health Organization, 2009). Few other plants in the family have mostly proven other kinds of disease-resolving properties; ethanol extracts of *Laportea aestuans* was reported to possess an extent of antioxidant activity (Simaremare et al., 2018), while extracted roots of *Laportea crenulata* have been found to exhibit concentration-dependent anti-microbial activity against bacteria, such as *Bacillus subtilis* and *Escherichia coli* (Khan et al., 2009). These findings are the foundation of this study.

Furthermore, it has been proven by the aforementioned studies that different methods of extractions may cause the extract to demonstrate significant effect changes, which correlates highly to its active compound constituents. A common extraction method is termed as the maceration method, which involves the rapid soaking of crude material with certain organic solvents, extracting the desired compounds (Bucar, Wube, and Scmid, 2013). Normally, an extraction method such as maceration, uses a preferred solvent to obtain a compound based on its chemical properties, such as

polarity and thermostability (Sasidharan et al., 2011). Hexane is a the least polar solvent to be used in maceration methods (Altemimi et al., 2017). While there is no recorded phytochemical analysis of hexane fraction from *L. decumana* leaves*,* this study will aim to screen only a few groups of phytochemicals that are documented as potent anti-inflammatory agents and later on insinuate their involvement in anti-inflammatory effect exertion. Flavonoids, such as catechin and epigallocatechin gallate (EGCG), are known anti-inflammatory agents with varying chemical structure which was thought to individually contribute to different anti-inflammatory mechanisms. In addition, saponins is another group of compounds known to minimally occur in plants. Dioscin of the saponin family has had its anti-inflammatory activity tested against *in vivo* ear edema in the past, which later on was deduced to exert its effect by the suppression of VCAM-1, ICAM-1, and endothelial lipase expression primarily through the downregulation of NF-kB pathway (Howes, 2018). Other anti-inflammatory mechanisms that originate from phytochemicals generally include: radical scavenging activities, moderate the action of inflammation-related cells (such as mast cells and macrophages), modulation of pro-inflammatory enzymes (such as the COXs), suppression of pro-inflammatory molecules production and hampering the expression of pro-inflammatory genes (Bellik et al., 2012).

To assess anti-inflammatory activity *in vitro*, inhibition of albumin denaturation assay was used. Protein denaturation is thought to be a key inducer of inflammatory diseases such as rheumatoid arthritis. Denatured proteins do not undergo structural changes and do not retain their normal functions, thus accumulation of it causes exaggerated bodily reactions that sometime form inflammations. This denaturation is reversible up to a point of no return, which will be marked with the appearance of disordered species. Known anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) are thought to inhibit the precipitation of these dysfunctional proteins, hence protecting against inflammation (Mizushima and Kobayashi, 1968; Saso et al., 2001). Furthermore, plans to continue assessing anti-inflammatory *in vivo* are viable options should resources be adequate. Optimized *in vivo* assays have been developed in order to measure anti-inflammatory

activity of herbal products. Formalin-induced paw edema on mice and carrageenan-induced granuloma pouch model are prime candidates for future research (Nile and Park, 2012).

1.2. Research Objectives

The primary objective for this study is to explore the possibility of using hexane fraction of *Laportea decumana* leaves as a potent anti-inflammatory agent. Qualitative assessment of this extract is done by thin layer chromatography (TLC), targeting specifically for saponins and flavonoids compounds. This TLC method is paired with mobile phase optimization for respective phytochemical families.

1.3. Research Scope

Methods that would be used in order to pursue the objectives include:

- Sample preparation and maceration
- Thin layer chromatography
- Phytochemical detection and visualization
- Qualitative image analysis
- Albumin denaturation inhibition assay
- Statistical analysis