CHAPTER I
INTRODUCTION

1.1. Background

Free radicals such as the hydroxyl radical, superoxide anion, hydrogen peroxide, and peroxynitrite species play a role in cell differentiation, signal transduction, and in the biochemical processes of the immune system. Cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction, and the acceleration of the aging process appear to have an etiological link to active oxygen-induced and free radical-mediated oxidation of biomolecules. Antioxidant agents are chemicals that can protect lipids, proteins, and nucleic acids from oxidative damage caused by reactive oxygen species such as hydroxyl, peroxyl, and hypochlorous radicals. Polyphenol molecules are the most prevalent antioxidants in plants. These polyphenols, the majority of which are flavonoids, are sometimes found in the form of ester and glycosides. As a result, there has been a surge in interest in natural antioxidants, and numerous plants have been reported to have antioxidant activity based on extracted bioactive components (Jantakoon *et al*., 2012; Songsiang *et al*., 2012).

According to the literature, a genus of medicinal plants called *Clausena* in the Rutaceae family has a wide range of secondary metabolites and biological activities. Alkaloids, coumarins, limonoids, and essential oils are among the secondary metabolites found in the *Clausena* genus. Many *Clausena* species have antioxidant, antiplasmodial, antimicrobial, antifungal, anticancer, antinociceptive, antitrichomonal, antinociceptive, antiimmunomodulatory, antidiabetic and antiinflammatory activities (Jantamat *et al*., 2019). According to prior accounts, the common big 14 species in the *Clausena*genus include *C.excavata*, *C.lansium*, *C. harmandiana*, *C. anisata*, *C. heptaphylla*, *C. emarginata*, *C. dunniana*, *C. indica*, *C. wallichii*, *C. anisum-olens*, *C. guillauminii*, *C. vestita*, *C. lenis* and *C. pentaphylla* (Li *et al*., 2017).

Clausena harmandiana is one of the most well-known species of the genus *Clausena*. It is a shrubby vigorous plant and is native to Asia. This plant is widely distributed in a large part of Asia, Indo-China (Cambodia, Laos, Thailand, Vietnam), Malaysia, and Indonesia (East Java, Lesser Sunda Islands; Malaysia– northern states). *C. harmandiana* has been demonstrated to have medicinal properties in the treatment of stomachaches, headaches, and illness, and is regarded as a health-promoting herb. The roots of *C. harmandiana* have been shown to contain carbazole alkaloids and coumarins. Coumarins and carbazole alkaloids extracted from *C. harmandiana* and other species in the genus have been shown to have a variety of biological activities, including antiplasmodial, antimicrobial, antifungal, anticancer, antioxidant, and antiinflammatory properties (Jantakoon *et al*., 2012; Jantamat *et al*., 2019). Furthermore, the twigs of *C. harmandiana* have been shown to contain carbazole alkaloids which have antibacterial activity (Maneerat *et al*., 2012). Coumarins, carbazole alkaloid, and ferulate have been isolated from ethyl acetate extract of *C. harmandiana'*s roots (Thongthoom *et al*., 2010). According to these observations, secondary metabolite compounds and bioactivities have never been presented from the stem bark of the *C. harmandiana* plant. Based on the literature review, the stem bark of *C. harmandiana* was chosen for this research.

The stem bark of *C. harmandiana* was used for this study. *C. harmandiana* was extracted with ethyl acetate to isolate secondary metabolite compounds and was followed by their antioxidant activity. According to several related journals, carbazole alkaloids and coumarins are the most abundant compounds in *C. harmandiana*. (Songsiang et al., 2012) reported carbazole alkaloids and coumarins have antioxidant activity from *C. harmandiana* plant. Therefore, it is possible to test antioxidant activity on *C. harmandiana*. Hopefully, coumarins, carbazole alkaloids, and ferulate compounds can be obtained from ethyl acetate extract of stem bark of *C. harmandiana* may become lead compounds for the development of antioxidant agents.

In this research, extraction was carried out using n-hexane, ethyl acetate, and methanol as solvents, and several fractions were produced. The separation process was carried out using chromatographic methods such as vacuum liquid chromatography and gravity column chromatography. After obtaining a single spot, then it was tested with three different eluent systems and the melting point test with Fisher John's instrument. Spectroscopic techniques such as UV, FTIR, and NMR were used to elucidate the structure of isolated compounds. Furthermore, the isolated secondary metabolite compounds were tested for antioxidant activity by using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

1.2. Problem of the research

1. How is the molecular structure of isolated secondary metabolite compounds from the stem bark of the *C. harmandiana* plant?
2. How is the IC_{50} value of the antioxidant activity of the ethyl acetate extract from the *C. harmandiana* plant as well as the isolated secondary metabolite compounds?

1.3. Objectives of the research

1. To determine the molecular structure of isolated secondary metabolite compounds from the stem bark of the *C. harmandiana* plant.
2. To evaluate the IC_{50} value of the antioxidant activity of the ethyl acetate extract from *C. harmandiana* as well as the isolated secondary metabolite compounds.

1.4. Benefits of the research

The benefit of this study is to provide scientific knowledge regarding secondary metabolite compounds found in the stem bark of the *C. harmandiana* plant, as well as whether these substances have antioxidant activity so that this plant can be used to benefit humans. Furthermore, this study is expected to serve as a source of scientific data and a reference for future research.