I. INTRODUCTION

1.1 Background

Malaria is one of serious disease in the world which estimated cases in 2018 reached 219 million with 435 thousand deaths globally and predicted 300-500 millions of people will be infected per year (WHO, 2018). Indonesia is one of the country as the endemic of malaria in the world. Based on Riskesdas (2013) the highest prevalence rates are found in eastern Indonesia, namely in West Papua (10.6%), Papua (10.1%) and East Nusa Tenggara (4.4%).While, in West Sumatera, malaria cases showed a fluctuative number in every year, which in 2009 is estimated the highest cases reacehed 1.357 patients positive from 7.207 blood samples (Dinas Kesehatan Sumbar, 2011). This advanced number should be well managed and solve to avoid the increasing of the cases.

Malaria caused by the infecting of *Plasmodium falciparum* as the protozoan parasite distribute by the mosquitos,which transmitted by mosquito bites (TUSOM, 2020). Several chemical substance used to treat this diseasesuch as chloroquin, quinine and artemisin and its derivatives has shown the side effect and resistence cases (Cammarck, 2011). Some other compound also has been investigated as antimalaria such as cassiarine A isolated from the leaves of *Cassia siamea* and also piperidine alkaloids isolated from *Senna spectabilis* (Ekasari *et.al*, 2009; Pivatto *et.al*, 2014). According to that condition, an effort to find the effective compound against malaria need to develop (Cammarck, 2011).

Piper genus is one of potential candidate as the new source of antimalarial compound. The data shows that the biological activities of the secondary metabolites in Piper genus interestingly provide many of bioactive compound as medicinal

resources. As an example,analysis of phytochemical activity in *Piper aduncum* showed that the plant consist mainly of alkaloids and anhraquinones and an acid which is contain of anti-plasmodial and cytotoxic activity which effective against *P. falciparum* infected Red Blood Cell (RBC) (Vanegas, 2012). This plant effect against *P. falciparum* and the other protozoas also reported in Cuba by using the essential oil containing the substance which indicate that this plant could be a promising antimalarial agent (Monzote et al., 2017). Besides that, the crude methanol extract of *P. betle* leaves (50–400 mg/kg) was also investigated for its antimalarial activity against *Plasmodium berghei* (NK65). The phytochemical and antioxidant potentials of the crude extract were evaluated to elucidate the possibilities of its antimalarial effects (Adhroey *et.al*, 2011). Other recent research of crude ethanol extract of *P. guineense* investigated antiplasmodial and analgesic effects in mice. The antiplasmodial efficacy of the extract was assesed on its ability to reduce parasitemia and writhing, respectively, in mice (Kabiru, 2015).In a previous study, the fruit of *P. chaba* Hunt. was proved to exhibit promising antimalarial activity against the asexual stage of 3D7 (chloroquine-sensitive) and K1 (chloroquine-resistant) strains of *P. falciparum*. This study investigated the antimalarial activity of piperine substance, the major isolated constituent of *P. chaba* Hunt. fruits against both *P. falciparum* clones (Thiengsusuk *et.al*, 2018). These studies revealed that Piper genus could be a promising resources to overcome malaria.

However, the utilization of the bioactive compound directly from the nature in a huge amount will threated the species existency. Plant tissue culture especially callus culture, can be a promising secondary metabolites production maintaining the species in the nature,also continuously with more consistent and controlled quality,

and a higher level of content compared to the wild plant (Ariati *et.al*, 2012;Sulichantini, 2015). Callus is non-diferrentiated meristematic cells which reveal only small vacuoles and lack chloroplast for photosynthesis, among other features (Efferth, 2019).Callus culture has the advantage that it can be engineered and directed to form complete organs and plants depending on the stimulus provided such as growth hormone and media composition (Rivai & Hendra, 2015). This techniques have been applied to produce various classes of chemical compounds from diverse plant species through empirical determination of ideal culture conditions and other methods (Benjamin *et.al*, 2019). Some evidence showed in callus inductionof*P. longum*using growth hormone combination recognized an increasing of alkaloid contain from wild plant extract (18,86 µg/ml) compared with callus extract of plant (34,38 µg/ml) (Siddique et al., 2019). Another research explained the phenylpropanoid content in leaves from *B. cordata* compared to that of *B. cordata* in vitro cultures found that the callus produced higherphenylpropanoid concentrations than the wild plant leaves (Zuninga et.al, 2009). TheHPLC analysis also revealed that the content ofcryptotanshinone in the callus cultured of *S. miltiorrhiza* on theMS basal medium supplemented with plant growth hormone was significantlyhigher than the marketed crude drug.Maximum yield of cryptotanshinone (4.59 mg/g) was observed in the calluscultured on MS basal medium supplementedwith 0.2 mg/L BA for sixty days (Wu et.al, 2003). The other evidence showed the substitution of KIN by 1 mg/L in culture medium combined with 0.5 mg/LNAA increased anthocyanin accumulation to 3.6 folds in *C.sinaica*. A striking increase in anthocyanin concentration reached 157.98 µg/g (11.79 folds) when culture medium contained 2mg/L BA and 1mg/L NAA (Maharik *et.al*, 2009).

Another recent antimalarial compound production also showed the utilization of callus culture technique to obtain the substance needed. As the example, the artemisinin and its derivatives, have been produced in callus as well as suspension culture, as it is naturally very low in the natural plant improved artemisinin production has also been extensively studied. Tahir et al (2016) showed that an ideal plant growth hormone combination for callus formation in*Artemisia annua* was 0.5µM/l of both BAP and NAA, similar to results obtained by Yuliani et al (2018) although similar results were obtained by other workers with higher concentrations of BAP and different auxins. Purnamaningsih (2011) also reported the callus induction method to obtain artemisine from *Artemisia annua* with combination of NAA and BAP. Another important antimalarial is quinine. One source of quinine, *Cinchona ledgeriana,* has been grown as callus culture and showed an increasing of the quinine alkaloids production (Pratiwi *et.al*, 2018). Sumaryono & Imron (2005) also reported the in vitro technology of *Cinchonaledgeriana* Moens to obtain quinine by callus culture using BAP combination. Another *Cinchona* species such as *C. pubescens* and *C. puccirubra* also reported the utilization of callus culture to obtain the necessary anti-malarial compound (Verpoorte et.al, 1985). The secondary metabolites of *Cassia siamaea* named cassiarine A known as antimalarial compound also has been cultured by callus induction to obtain the substances by using the hormone combination and precursor (Fauziaturrahmi, 2020).

Callus cultures also potential for the sustainable and large-scale production of secondary metabolites in pharmaceuticals. As an example, the use of in vitro techniques by induction of callus in the production of secondary metabolites on an industrial scale has been proven in tread plants (*Catharanthus roseus*) as anti-cancer,

anti-diabetic and anti-diarrhea (Naeem, et al., 2017). This cases illustrated that the callus culture can be applied for sustainably production of plant secondary metabolites especially antimalarial compound. Therefore, in this present study we want to explained the recent research of Piper genus related to the potential antimalarial compund findings and recent callus induction in several Piper plants as potential technique to obtain the secondary metabolites as antimalarial.

1.2 Problem Formulation

The problem formulation of this study are:

1. How is the recent research related to the potential Piper genus as the source of potential antimalarial compound?
2. How is the recent research of callus culture in piper plant to obtain metabolite?

1.3 Objectives

1. To explain the recent research related to the potential Piper genus as the source of potential antimalarial compound.
2. To explain the recent research of callus culture in piper plant to obtain metabolite.