CALLUS CULTURE AS THE METHOD IN PROVIDING ANTIMALARIAL COMPOUNDS OF PIPER GENUS

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Malaria is still a serious cases all over the world especially in tropical country with a predict number 300-500 millions people infected per year. The resistance cases of these anti-malaria substance make a new effort to find the new strategy against malaria. This study aim to summarize the potential Piper genus as the source of potential antimalarial compound and recent research of callus induction in piper plant to obtain metabolites. The study of Piper genus represent the 11 Piper species were chemically studied and assayed against the plasmodium. The potential antimalarial compound isolates from several Piper genus were 20,60-Dihydroxy-40-methoxydihydro-chalcone, 3-Farnesyl-p-hydroxybenzoic acid, Piperine, Chabamide, Benzoic acid derivatives, Guineensine, pellitorine, brachystamide B, sarmentine, and sermentosine, 5,8-Hydroxy-7-methoxyflavone, Preynylated hydroxybenzoic acid, 4-Nerolidylcatechol, Piperitone, Champor, and Viridiflorol (EO). An effort to propagate necessary antiplasmodial resources especially by callus induction has been conducted for 7 Piper species such as Piper betle, P. colubrinum, P. crocatum, P. longum, P. nigrum, P. permucronatum and P. solmsianum to obtain higher content of secondary metabolites with a different plant growth hormone (PGR) supplementation while there are most of Piper genus have not been well studied. It conclude that callus culture could be the promising method to obtain antimalarial secondary metabolites as antimalarial.

Key Words: antimalarial, callus, piper, plant growth hormone (PGR), secondary metabolites

1. INTRODUCTION

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 year¹. Malaria caused by the infecting of Plasmodium falciparum as the protozoan parasite distribute by the mosquitos, which transmitted by mosquito bites³. Several chemical substance used to treat this diseases such as chloroquin, quinine and artemisin and its derivatives has shown the side effect and resistance cases³.

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 resources⁴. However, the utilization of the bioactive compound directly from the nature in a huge amount will threatened 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 quality, and a higher level of content compared to the wild plant⁵. Callus cultures is potential for the sustainable and large-scale production of secondary metabolites in pharmaceuticals. Therefore, in this present study we want to explained the recent research of Piper genus related to the potential antimalarial compound findings and recent callus induction in several Piper plants as potential technique to obtain the secondary metabolites.
metabolites as antimalarial.

2. METHODS

(1) Procedures
All of articles and scientific writing were collected and formulated based on PRISMA (Preffered Reporting Items for Systematic Reviews and Meta-Analysis). Information on Piper genus was gathered via internet using five scientific databases Google Scholar, Pubmed, SciFinder, Scopus and Web of Science.

(2) Data analysis
The data analyzed descriptively and tabulated into the Tables 1 and 2 to summarize the whole data and each important highlights were discussed.

3. RESULTS AND DISCUSSION

(1) Potential Anti-malarial Compound Isolated from Piper Plants
Several research has been conducted to gain the promising substance against Plasmodium spp. There the 11 compound from 12 species of Piper found as potential agents for antimalaria.

From the Table 1 several species of Piper has been identified and bioactive compound obtained as antimalarial agent. The quantity of the compound effectiveness measured by IC50 value which mean the lower the IC50 means the more potent the molecule (<50). These all recent studies about the potential chemical substances showed the great opportunity to develop the technique for secondary metabolites acquisition from Piper genus to be applied for pharmaceutical and medicinal as the future prospective. The effective technique to obtain the compound of interest in large-scale production should be well studied and develop.

(2) Callus Culture of Piper to Obtained Metabolites

Callus is the middle stage of many purpose in secondary metabolites acquisition in many plants including Piper. This method also affected by several factor involved such as plant growth regulator (PGR) and suitable medium. Table 2 shows the data of recent research of callus induction in Piper genus.

There are various suitable condition including plant growth regulator for each plant species of Piper. The different of this concentration and treatment did not happen only in the differ spesies but also the same species. This indicated the different individual has a different needs of growth regulator and condition. This case may related to the role of endogenous hormone in plants. The endogenous hormonal system plays a leading role in the regulation of growth and development of plants. This regulatory system responds sensitively to even slight changes in the plant environment, which is manifested in reorganization of the hormonal status. It is necessary to know that simultaneous analysis of different groups of phytohormones allowed us to reveal a complex pattern of changes in plant hormonal system in response to treatments with exogenous growth regulators and to evaluate their contribution to the control of resistance to stress factors 6).

Plant hormones rarely act alone, and for most processes, at least those that are observed at the organ level, many of these regulators have interacted in order to produce the final effect. Classical plant hormones like auxins, cytokinins, gibberellins, abscisic acid, ethylene and growth regulatory substances with similar biological effects. A better knowledge of the uptake, transport, metabolism, and mode of action of phytohormones and the appearance of chemicals that inhibit synthesis, transport, and action of the native plant hormones has increased our knowledge of the role of these hormones in growth and development 7).

Some factors may affected the succeed of Piper plant callus culture method mainly the suitable plant growth hormone (PGR) concentration occur in cells and external supplementation. The callus formed depends on the balanced ratio of plant growth hormone, in general auxin and cytokinin. We could not expect the endogenous hormone occur in plant cells, so variation of PGR concentration needed in specific interval. As an example various concentration of 2,4-D (0.0; 0.5;1.0; 1.5; 2.0; and 2.5 mg/L) to induced P. betle callus 35). This PGR supplementation also impacted to the secondary metabolites accumulation in cells. The optimum condition of PGR seems to stimulate the secondary metabolites production 9).

There is considerable interest in callus culture of Piper as a means of producing therapeutic compounds especially as antimalaria. Several Piper plant has been utilized callus culture technique to obtain metabolites for antimalaria, such as P. longum and P. nigrum callus cultured to obtain piperine 9,10). However, the effort to culture the other potential Piper plant for antimalarial compound production still very lacking such as P. hostmannianum (20,60-Dihydroxy-40-methoxy dihydro-chalcone), P. tricuspe (3-Farnesyl-p-hydroxybenzoic acid), P. chaba (Chabamide), P.
Callus culture systems represent a potential renewable source of valuable antimalarial compounds which cannot be produced by microbial cells or chemical synthesis. The principle advantage of this technology is that it may provide continuous, reliable source of antimalarial compound and could be used for the large-scale culture of plant cells from which these metabolites can be extracted. HPLC known as the effective method to analyzed the amount of specific compound needed after extraction and each HPLC results the increasing of natural products after application of callus culture. This increasing profitable for medicinal purposes, even more the low product yields and supply concerns of plant product harvestation has renewed interest in large-scale plant cell culture technology in the future.

### 4. CONCLUSION

The recent study of crude extract of several Piper genus represent the 11 Piper species were chemically studied and assayed against the plasmodium or protozoa. Callus culture could be the promising method for antimalarial secondary metabolites acquisition and proved to increase the content of metabolites, which has been proved for 7 Piper species.

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| Table 1 | Anti-malarial of Isolated Metabolites from Piper Plants |
|---|---|---|---|
| **Active Metabolite** | **Piper species** | **IC_{50}** | **Ref** |
| 20,60-Dihydroxy-40-methoxyhydro-chalcone | *P. hostmannianum* | 12.7 µg/ml | 11) |
| 3-Famesyl-p-hydroxybenzoic acid | *P. tricuspe* | 29.78 µg/ml | 12) |
| Piperine | *P. chaba* | 59 µg/ml | 13) |
| Chabamine | *P. chaba* | 2.7 µg/ml | 14) |
| Benzoic acid derivatives | *P. acutifolium* | (Not reported) | 15) |
| Benzoic acid derivatives | *P. glabratum* | (Not reported) | 15) |
| Guineensine, pellitorine, brachystamide B, sarmentine, and sermentosine | *P. sarmentosum* | 6.5 – 18.9 µg/ml | 14) |
| 5,8-Hydroxy-7-methoxyflavone | *P. piedecuestanum* | 7.3 µg/ml | 16) |
| Prenylated hydroxybenzoic acid | *P. heterophyllum* | 7.0 µM | 17) |
| Piperine | *P. longum* | 34 µM | 18) |
| Piperine | *P. nigrum* | 12.5 µg/ml | 19) |
| 4-Nerolidylcatechol | *P. peltatum* | 0.67 µg/ml | 20) |
| Piperitone, Champor, Viridiflorol (EO) | *P. aduncum* | 1.3 µg/ml | 21) |

| Table 2 | Callus Induction in Piper to obtained Metabolites |
|---|---|---|---|---|
| **Piper species** | **PGR Conc.and Media** | **Explant** | **Metabolites** | **Callus morph.** | **Ref.** |
| *P. betle* | 1.5 mg/L 2,4-D (MS) | Leaf | Flavonoid, Terpenoid, octadecanoic acid | Friable texture and yellowish white color | 22) |
| *P. betle* | 1.0 and 1.5 mg/L kinetin (MS) | Leaf | (Not reported) | White, Greenish color | 23) |
| *P. colubrinum* | 2.4 µM BA and 0.46 µM kinetin (MS) | Leaf | (Not reported) | White | 24) |
| *P. crocatum* | 0.5 mg/L 2,4-D (MS) | Leaf | Euganol | Compact, crumbs, white translucent yellowishgreen | 25) |
| *P. longum* | 1 mg/l BAP and 0.5 mg/l kinetin (MS) | Nodus | (Not reported) | Greenish white | 26) |
| *P. longum* | 0.5 mg/l BAP, 1.0 mg/l kinetin, 0.5 - 2.0 mg/l 2,4-D (MS) | Leaf | Piperine | (Not reported) | 9) |
| *P. longum* | 3.0-4.0 mg/ 12,4-D (MS) | Leaf | (Not reported) | Hand and compact yellow colored | 27) |
| *P. nigrum* | 0.5 or 1.5 mg/l BA and 1.0 mg/l NAA (MS) | Leaf | (Not reported) | 1-diphenyl-2-picryl-hydrazy | 28) |
| *P. nigrum* | 4.0 mg/l TDZ and BA 1.5 mg/l (MS) | Leaf | Piperine | (Not reported) | 29) |
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