ETLINGERA GENUS: PHYTOCHEMICAL SCREENING AND ANTICANCER ACTIVITY

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ABSTRACT
Etlingera is one of the plant genera from the Zingiberaceae family and is widely distributed in Asia to the Pacific Islands, with various species ranging from 150 to 200 species. The Etlingera are commonly used as spices, vegetables, and traditional medicines. Many pharmacological activities have been reported from this genus, including antioxidants, antibacterials, cholesterol-lowering, anticancer, and others. The phytochemical content of Etlingera reported are phenolics, diarylheptanoids, flavonoids, steroids, alkaloids, and terpenoids. Eight species from Etlingera, namely Etlingera elatior, E. pavieana, E. brevilabrum, E. pyramidosphaera, E. megalocheilos, E. coccinea, E. fimbriobracteata, and E. corneri are reported provided anticancer activity. This review article aims to review the phytochemical screening from Etlingera genus, and its anticancer activity

Keywords: Etlingera genus, Phytochemical screening, Anticancer activity

1. INTRODUCTION
Cancer is a disease that causes the second most mortality globally, which an estimated 9.6 million deaths in 2018 (WHO, 2018). Cancer is caused by genetics to lifestyle. In cancer treatments, the use of chemotherapy and radiotherapy agents has a high rate of side effects. One of the efforts that can be conducted is to explore the properties of traditional plants. Plant-derived compounds have a vital role as a source of anticancer agents, such as vinblastine, vincristine, topotecan, irinotecan, paclitaxel, and others (Cragg & Newman, 2005).

Zingiberaceae is an herbaceous plant that has been widely used by the community in traditional medicine. Etlingera is a plant genus from the Zingiberaceae family, which has a large number of species and exciting potential—reported more than 150 to 200 species belonging to the genus Etlingera. This plant is widely distributed from Asia to the Pacific islands (Ardiyani & Poulsen, 2019; Trimanto & Hapsari, 2018). This plant is traditionally used as spices, vegetables, traditional medicine, and many more (Ud-Daula & Basher, 2019).

Plant activity of the genus Etlingera has been widely reported, especially from the Etlingera elatior species. The leaves are used as deodorizing, wound medicine, antioxidant, antibacterial, and tyrosinase inhibitor (Chan et al., 2011). E. elatior flowers are also active as antibacterial,
antifungal, and antioxidant (Chan et al., 2011; Wijekoon et al., 2011). Other species of Etlingera have also been reported to have pharmacological activity, namely the leaves and stems of E. brevilabrum as cholesterol-lowering (Mahdavi, 2014), in addition to other species such as E. fulgens are also useful as antibacterial agents (Chan et al., 2011). Research on the Etlingera genus has focused a lot on biological activity to fight harmful microbes to deadly diseases such as cancer. Phytochemical studies isolated from the Etlingera genus plants have resulted in phenolic compounds, diarylheptanoids, flavonoids, steroids, terpenoids, and alkaloids. This study intends to provide a comprehensive insight into the Etlingera genus regarding phytochemicals and their pharmacological activity as an anticancer.

2. PHYTOCHEMICAL INVESTIGATING

The compounds isolated from the Etlingera genus as a whole exhibited pharmacological activity. About 50 new compounds have been isolated and identified from 8 species of the genus Etlingera, including compounds of the phenolic group, diarylheptanoids, flavonoids, steroids, terpenoids, and alkaloids. Based on our review, it can be argued that phenolics and flavonoids are abundant compounds in this genus.

Phenolic

Phytochemical investigations have shown that phenolic groups are the main compounds in the genus Etlingera. A total of 18 compounds (1-18) have been isolated from this genus. Caffeic acid (Chan et al., 2009) (1), 3-O-cafeoylquinic acid (Chan et al., 2009) (2), 5-O-cafeoylquinic acid (Chan et al., 2009; Ghasemzadeh et al., 2015) (3), 5-O-cafeoylquinic acid methyl ester (Chan et al., 2009) (4), vanillic acid (S. Sahidin et al., 2019) (5) and gallic acid (Ghasemzadeh et al., 2015) (6) were isolated from E. elatior species with antioxidant and antibacterial activity (Brand-Williams et al., 1995; Habsah et al., 2005; Sharma, 2011; Wahyuni et al., 2019).

Compound (E)-4-methoxyccinnamic acid (Tchai & Nuntawong, 2016a) (7), (E)-((E)-3-(4-methoxyphenyl)allyl) 3-(4-hydroxyphenyl) acrylate (Tchai & Nuntawong, 2016a) (8), 3-(4-methoxyphenyl)prop-2-en-1-ol (Tchai & Nuntawong, 2016a) (9), (2E)-3-(4-methoxyphenyl) prop-2-en-1-ol (E. Srisook et al., 2017) (10), (E)-1-methoxy-4-(3-methoxyprop-1-enyl) benzene (Tchai & Nuntawong, 2016a) (11), elemicin (5-allyl-1,2,3-trimethoxybenzene) (K. Srisook & Srisook, 2019) (12), trans-anethole (Naksang et al., 2020a) (13), trans-methyl isoeugenol (1,2-dimethoxy-4-propenylbenzene) (Naksang et al., 2020b) (14) and methyl chavicol (1-allyl-4-methoxybenzene) (Naksang et al., 2020b) (15) were isolated from E. pavieana with various activities different. Compound (E)-4-methoxyccinnamic acid (7) has activity as an antidiabetic, antihyperglycemic and hepatoprotective (Sharma, 2011). The compound (E)-((E)-3-(4-methoxyphenyl) allyl) 3-(4-hydroxyphenyl) acrylate (8) is active as an antimycobacterium tuberculosis, anticancer (Tchai & Nuntawong, 2016b), and anti-inflammatory (Mankhong et al., 2019; E. Srisook et al., 2017). Compound 3-(4-methoxyphenyl) prop-2-en-1-ol (9) exhibits anticancer (Iawsipo et al., 2018) and antiinflammatory activity (E. Srisook et al., 2017; K. Srisook & Srisook, 2019).

Antinflammation activity (Ilijeva & Buchbauer, 2016; E. Srisook et al., 2017; Yu et al., 2009) was also shown by the compound (2E)-3-(4-methoxyphenyl) prop-2-en-1-ol (10), (E)-1-methoxy-4-(3-methoxyprop-1-enyl) benzene (11) and elemicin (12). Trans-anethole compounds (13) exhibits activity as an antioxidant, antimicrobial, anthelmintic and insecticidal activity, secretolytic, and expectorant effects, the spasmylytic effect on contracted smooth muscles,
antinociceptive, anti-inflammatory, gastroprotective, estrogenic effects, reproductive toxicity, and sedative activity (Marinov & Valcheva-Kuzmanova, 2015). The trans-methyl isoeugenol compound (14) provides a hypotensive and vasorelaxant activity, anxiolytic, and antidepressant activity (Ilijeva & Buchbauer, 2016). Methyl chavicol (15) acts as an anti-inflammatory (Naksang
et al., 2020), anesthetic, antiplatelet, antioxidant, and antimicrobial activity (Ilijeva & Buchbauer, 2016).

Other phenolic compounds that have been isolated are (E)-3- (4-hydroxyphenyl) prop-2-enoic acid (16) obtained from E. Calophrys (Wahyuni et al., 2019), E. Elatior (S. Sahidin et al., 2019), and E. pavieana (E. Srisook et al., 2017) with activities as antibacterial (S. Sahidin et al., 2019; Wahyuni et al., 2019), hepatoprotective (Sharma, 2011), anti-inflammatory (E. Srisook et al., 2017), and antioxidant (Brand-Williams et al., 1995). Other compounds that are known to come from E. calophrys (I. Sahidin et al., 2018) and E. elatior (S. Sahidin et al., 2019), namely p-hydroxybenzoic acid (17) and from E. sphaerocephala (Yahya et al., 2011) namely paeonol (18), have activity as antioxidants (I. Sahidin et al., 2018; Tachai & Nuntawong, 2016a).

Diarylheptanoid
A total of 5 diarylheptanoid compounds (19-23) have been isolated from the Etlingera genus. Three compounds were found from E. elatior (Habsah et al., 2005), namely 1,7-bis (4-hydroxyphenyl) -2,4,6-heptatrienone (19), 1,7-bis (4-hydroxyphenyl) -1,4,6- heptatrien-3-one (20) and demethoxycurcumin (21). Meanwhile, two other compounds, 1,7-bis (3,4-dihydroxyphenyl) heptan-3-yl acetate (22) (Daniel-Jambun et al., 2018) and yakuchinone A (23) (Daniel-Jambun et al., 2018), were isolated from E. pubescens and E. calophrys. These five diarylheptanoid compounds have antioxidant activity (Daniel-Jambun et al., 2018; Habsah et al., 2005; I. Sahidin et al., 2018).

![Diarylheptanoid from Etlingera species](image)

Flavonoid
Eighteen (24-41) flavonoid compounds have been reported from the genus Etlingera. Ten of them were found in E. elatior (Chan et al., 2009; Ghasemzadeh et al., 2015; Williams & Harborne, 1977), while the rest were reported in E. littoralis (Chantrapromma et al., 2010; Jeerapong et al., 2010). These compounds are quercetin (24), apigenin (25), kaempferol (26), luteolin (27), myricetin (28), catechin (29), kaempferol 3-glucuronide (30), quercetin 3-glucuronide (31),
isoquercitrin (32), and quercitrin (33) from *E. elatior*. Moreover, compounds pinocembrin (34), pinostrobin (35), 2',6'-dihydroxy-4'-methoxy-dihydrochalcone (36), 2',4',6'-trihydroxydihydrochalcone (37), 2',6',4-trihydroxy-4'-methoxydihydrochalcone (38), adunctin E (39), methylendererin (40) and etlinglittoralin (41) from *E. littoralis*, where the compound etlinglittoralin (41) this is a new compound whose activity is not yet known.

Fig. 4 Flavonoid from *Etlingera* species

Many compounds of the flavonoid class are known to have pharmacological activity. Various activities such as antioxidant, neurological effect, antiviral, anticancer, cardiovascular protection, antibacterial, antifungal, anti-inflammatory, hepatoprotective, antimutagenic, anticarcinogenic, antiproliferative, antiprogression, antiviral, antiparasitic, antiprotozoal, analgesic, antiallergic, antiviral, antiparasitic, antiprotozoal, analgesic, antiallergic, antiplatelet related disorders eye disorders, antidiabetic and anti-obesity (Calderón-Montañé et al., 2011; Chen & Chen, 2013; López-Lázaro, 2009; Maalik et al., 2014; D. Patel et al., 2007; Semwal et al., 2016; Wang et al., 2019) are reported by flavonoids such as quercetin (24), apigenin (25), kaempferol (26), luteolin (27), myricetin (28) and catechin (29). Flavonoid glucosides such as
kaempferol 3-glucuronide (30), quercetin 3-glucuronide (31), isoquercitrin (32), and quercitrin (33) have antioxidant and antidiabetic activity (Ahmed et al., 2019).

The compounds pinocembrin (34) and pinostrobin (35) are reported to have antibacterial, anti-inflammatory, anticancer, antifungal, neuroprotective (Rasul et al., 2013) and antiulcer, anti-inflammatory, antiviral, neuroactive, anti-Alzheimer’s, antimicrobial, antioxidant, antimalarial, antiprotease, anticancer activity, respectively. Antivenom, antidiarrheal, trypanocidal, antileukemic, and anti-aromatase (N. K. Patel et al., 2016).

The chalcone-derived flavonoid compounds also have various activities. Compounds 2',6'-dihydroxy-4'-methoxy-dihydrochalcone (36) were reported to have activity as antiplasmodial (Gutierrez et al., 2015; Portet et al., 2007), anticancer (Gutierrez et al., 2015), antioxidant (Gutierrez et al., 2015; Kartika et al., 2019) and antibacterial (Nowakowska, 2007). The compounds 2',4',6'-trihydroxydihydrochalcone (37) have an antimicrobial effect (Gutierrez et al., 2015). Compounds 2',6',4'-trihydroxy-4'-methoxydihydrochalcone (38) are active as anticancer (Gutierrez et al., 2015) and antioxidant (Kartika et al., 2019). Meanwhile, adunctin E (39) and methyllinderatin (40) have been known to be antibacterial (Dethe & Dherange, 2015) and antiplasmodial (Gutierrez et al., 2015; Portet et al., 2007).

Fig. 5. Flavonoid from *Etlingera* species (cont.)
Steroid

A total of 6 steroid compounds (42-47) have been isolated from the Etlingera genus. The compounds stigmast-4-en-3-one (42), stigmast-4-ene-3,6-dione (43) and 5α, 8α-epidioxyergosta-6,22-dien-3β-ol (44) were reported from *E* _elatior_ (Habsah et al., 2005), the compound stigmast-4-en-6β-ol-3-one (45) reported from *E. elatior* (Habsah et al., 2005), and *E. calophrys* (I. Sahidin et al., 2018; Wahyuni et al., 2019), the compound stigmasterol (46) reported from *E. calophrys* (I. Sahidin et al., 2018), and sitosterol compounds (47) were reported from *E. alba* (Mantik et al., 2018) and *E. sphaerocephala* (Yahya et al., 2011). The six steroid compounds are reported to be active as anti-tumor agents (Habsah et al., 2005).

![Steroid compounds](image)

**Fig. 6 Steroid from *Etlingera* species**

Terpenoid

Two terpenoid compounds (48 and 49) have been isolated from the Etlingera genus. The compound 16-hydroxylabda-8 (17),11,13-trien-15,16-olide (48) was reported from *E. elatior* that act as antioxidant (Habsah et al., 2005), while compound 8 (17),12-labdadiene -15,16-dial (49) has been reported from *E. sessilanthera* exhibits antimicrobial properties (Daniel-Jambun et al., 2018). Both of these terpenoid compounds are terpenoid compounds derived from labdane.
Alkaloid
One alkaloid compound (50) has been isolated from the genus Etlingera. This compound (E)-3-(4-methoxyphenyl) prop-en-1-amine (50) has been isolated from E. pavieana, and its pharmacological activity is not yet known.

Fig. 7 Terpenoid from Etlingera species

Fig. 8 Alkaloid from Etlingera species

3. ANTICANCER ACTIVITY
Etlingera genus provides much pharmacological activity; one of them is anticancer. Many studies reported the activity, including Ethyl acetate extract of E. elatior rhizome has a strong cytotoxic effect against CEM-SS and MCF-7 cell lines (Chan et al., 2011). The ethanol extract of E. elatior flowers showed anticancer activity against the MDA-MB-231 cell line, MCF-7 cells, and HeLa cells (Zan et al., 2011). Ethyl acetate extract of E. elatior seeds had cytotoxic activity against IC50 P-388 leukemia cells 19.21 mg / mL (Rusanti et al., 2017). The ethanol extract of E. pavieana rhizome has antiproliferative activity against MCF-7 cells (Tachai & Nuntawong, 2016a) and has activity against MDA-MB-231, Hela, HepG2, and C33A cells (Iawsipo et al., 2018). E. punicea extract has cytotoxic activity against MCF-7 cells by MTT assay method to see IC50 (Nagappan, 2019).

Etlingera elatior
Etlingera elatior rhizome provides the anticancer activity. The chloroform and methanol extracts of E. elatior rhizomes provided anti-tumor activity with an inhibition rate of 92.18% and 85.9% against the Raji cell line. In vitro, the ethyl acetate extract of E. elatior gave a cytotoxic effect on CEM-SS cells with IC50 4 mg / mL and MCF-7 with IC50 6.25 mg/ml (Habsah et al., 2005). Besides, previous research conducted by Vairappan et al. (2012) showed that essential oil from the rhizome of E. elatior has potential against the cell lines MCF-7, HeLa, P 388, and HI 60 (Vairappan et al., 2012).
Table 1. Anticancer Activity of Plant Genus *Etlingera*

<table>
<thead>
<tr>
<th>No</th>
<th>Species</th>
<th>Part of Plants</th>
<th>Test Model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>E. elatior</em></td>
<td>Rhizome</td>
<td><em>in vitro</em> against Raji cells line</td>
<td>(Habsah et al., 2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>In vitro</em> against MCF-7, HeLa, P 388, and HL 60 cells line</td>
<td>(Habsah et al., 2005; Vairappan et al., 2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flower</td>
<td><em>In vitro</em> against MCF-7, MDA-MB-231, HeLa, HT-29, HepG2, and B16 cells line</td>
<td>((Ghasemzadeh et al., 2015; Krajarng et al., 2017; Zan et al., 2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seed</td>
<td><em>In vitro</em> against P-388 leukemia murine cells</td>
<td>(Rusanti et al., 2017)</td>
</tr>
<tr>
<td>2</td>
<td><em>E. pavieana</em></td>
<td>Rhizome</td>
<td><em>In vitro</em> against MDA-MB-231, HeLa, HepG2, and C33A cells line</td>
<td>(Iawsipo et al., 2018)</td>
</tr>
<tr>
<td>3</td>
<td><em>E. brevilabrum</em></td>
<td>Rhizome</td>
<td><em>In vitro</em> against MCF-7, HeLa, P 388, dan HL 60 cells line</td>
<td>(Vairappan et al., 2012)</td>
</tr>
<tr>
<td>4</td>
<td><em>E. pyramidosphaera</em></td>
<td>Rhizome</td>
<td><em>In vitro</em> against MCF-7 dan P388</td>
<td>(Vairappan et al., 2012)</td>
</tr>
<tr>
<td>5</td>
<td><em>E. megalochelos</em></td>
<td>Rhizome</td>
<td><em>In vitro</em> against MCF-7, HeLa, P388 dan HL 60 cells line</td>
<td>(Vairappan et al., 2012)</td>
</tr>
<tr>
<td>6</td>
<td><em>E. coccinea</em></td>
<td>Rhizome</td>
<td><em>In vitro</em> against MCF-7, P388 dan HL 60 cells line</td>
<td>(Vairappan et al., 2012)</td>
</tr>
<tr>
<td>7</td>
<td><em>E. fimбриобрacteata</em></td>
<td>Leave</td>
<td><em>In vitro</em> against CaSki cell lines</td>
<td>(Shahid-Ud-Daula et al., 2019)</td>
</tr>
<tr>
<td>8</td>
<td><em>E. corneri</em></td>
<td>Rhizome</td>
<td><em>In vitro</em> against MCF-7 cell lines</td>
<td>(Ghazaly et al., 2020)</td>
</tr>
</tbody>
</table>

*E. elatior* flowers have also reported anticancer effects. The aqueous extract of *E. elatior* flowers showed anticancer activity against the breast cancer lines MCF-7 and MDA-MB-231 (Ghasemzadeh et al., 2015). Another study showed that *E. elatior* flowers inhibited the proliferative activity of MDA-MB-231, MCF-7, HeLa, HT-29, HepG2 cell lines with IC₅₀ > 100 µg/ml (Zan et al., 2011). Besides, extracts from *E. elatior* flowers showed decreased cell viability in a dose and time-dependent manner in melanoma B16 cells, causing the frightening apoptotic events with nuclear condensation and exposure phosphatidylserine, and membrane-laden mitochondrial potential. It also affects the caspase-independent *E. elatior* flower extract activity and down-regulation of the ERK and Akt signaling pathways (Krajarng et al., 2017).

Also, ethyl acetate extract from *E. elatior* seeds was reported to have cytotoxic activity against P-388 murine leukemia cells with IC₅₀ 19.210 µg/mL. Lapachol, apigenin, methylated chrysin, 6,2'-dihydroxyflavonone, 3-Hydroxy3,4'-dymethoxyflavone, and 4'-Hydroxy-5,7-dimethoxyflavanone compounds contained in *E. elatior* seeds are thought to provide this anticancer effect (Rusanti et al., 2017).
Etlingera pavieana

Etlingera pavieana rhizome extract provides an antiproliferative effect of MDA-MB-231, HeLa, HepG2, and C33A cells with IC₅₀ values of 160, 182, 190, and 192 mg/mL, respectively. The trans-4-methoxycinnamaldehyde (4-MCA) compound is thought to have anti-cell proliferation and cancer cell colony formation, induce apoptosis, and cell cycle arrest (Iawsipo et al., 2018).

Etlingera brevilabrum

The essential oil from E. brevilabrum shows cytotoxic activity in the form of inhibiting cell proliferation against MCF-7, HeLa, P 388, and HL 60 cells with IC₅₀ 15 ± 1.5, 15 ± 0.5, 5 ± 0.1, and 5 ± 0.05 µg/mL, respectively. The contents of α-Fenchol (4.4%), Borneol (3.6%), α-Terpineol (2.1%), δ-Selinene (6.2%), Methyl isoeugenol (19.2%), Thujospene (6.5%), β-Farnesene (10.7%), Caryophyllene (2.1%), β-Bisabolene (2.6%), and Elemicin (35.6%) are thought to have an inhibitory effect on cell proliferation against the above cancer cell lines (Vairappan et al., 2012).

Etlingera pyramidosphaera

The essential oil from the rhizomes of E. pyramidosphaera showed inhibitory activity of cell proliferation against MCF-7 and P388 cells with IC₅₀ values of 7.5 ± 0.5 µg/mL and 5 ± 1.5 µg/mL, respectively. The content is in the form of 2-Methyl-1-undecanol (2.3%), Lauricaldehyde (11.2%), 1-Dodecanol (28.0%), Lauryl acetate (30.0%), 1-Tetradecanol (20.2%), and Tetradecanol acetate (7.4%) are thought to exert a proliferation inhibiting effect on cancer cells (Vairappan et al., 2012).

Etlingera megalochelios

Essential oil from the rhizomes of E. megalochelios showed inhibitory activity of cell proliferation with an IC₅₀ value of 30 ± 0.8 µg/mL against MCF-7 cells, > 100 µg/mL against HeLa cells, 20 ± 0.5 µg/mL against P388 cells, and 20 ± 0.5 µg/mL against HL 60 cells. The essential oil content contained in the rhizome of E. megalochelios is thought to have this cytotoxic effect. The oil content is Verbenol (3.1%), Camphor (4.4%), Terpineol oxide (13.0%), α-Gurjunene (3.3%), Azulene (3.0%), Aromadendrene (8.9%), Aromadendrene oxide (24.8%), Caryophyllene (4.3%), 4,4-Dimethylheptanedioic acid (7.1%), Spathulenol (5.6%), Caryophyllene oxide (5.7%), and 1-Naphthalenepentanoic acid (7.4%) (Vairappan et al., 2012).

Etlingera coccinea

The essential oil derived from the rhizome of E. coccinea demonstrated cytotoxic activity against the MCF-7, P388, and HL 60 cell lines with an IC₅₀ value > 100 µg/mL, whereas HeLa cells exhibited an IC₅₀ value of 80 ± 4.5 µg/mL. The content of essential oils such as 3-Decyn-2-ol (1.3%), 1-Decanol (1.9%), 2-Methyl-1-undecanol (34.1%), 1-Dodecanol (46.2%), Lauryl acetate (5.6%), 1,3-Dicyclohexylpropene (1.6%), 1-Tetradecanol (3.9%), and Tetracosane (1.3%) are thought to have antioxidant effects that influence cytotoxic effects on the above cell lines (Vairappan et al., 2012).

Etlingera fimbriobracteata
Crude extract methanol leaves of *E. fimbriobracteata* has cytotoxic activity against cervical cancer cells (CaSki) using the MTT assay method. This activity is in a dose-dependent manner, with the IC$_{50}$ of this extract being 106.21 µg/mL at 24 h, 80.22 µg/mL at 48 h, and 62.19 µg/mL at 72 h of incubation, respectively. The antioxidant content related to their polyphenolic content is thought to have an antiproliferative effect on CaSki cells (Shahid-Ud-Daula et al., 2019).

**Etlingera corneri**

Crude extract methanol of *E. corneri* rhizome showed activity on the growth and proliferation of MCF-7 cells at a concentration of 15.6 to 2000 µg/mL using the MTT method (Ghazaly et al., 2020).

4. CONCLUSION

According to the data collected, plants from the Etlingera genus have many chemical constituents, including phenolics, diarylheptanoids, flavonoids, steroids, terpenoids, and alkaloids. Some of these compounds provide anticancer activity in various cell lines in vitro. They are derived from data collected from 8 Etlingera species, namely *E. elatior*, *E. pavieana*, *E. brevilabrum*, *E. pyramidosphaera*, *E. megalochenis*, *E. coccinea*, *E. fimbriobracteata*, and *E. corneri*.

5. REFERENCE


