PRELIMINARY PHYTOCHEMICAL SCREENING OF THE POTENTIAL MEDICINAL PLANTS OF THE MELANAU IN PULAU BRUIT, SARAWAK, MALAYSIA

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ABSTRACT

Potential medicinal plants are widely used by Melanau community in Pulau Bruit, Sarawak, Malaysia and their biological and phytochemical properties have not been thoroughly evaluated. By studying the presence of phytochemical, the uses of it in traditional treatment can be explained scientifically. Preliminary phytochemical screening was performed on ethanolic extracts of leaves of Acanthus ebracteatus, Clinacanthus nutans, Derris trifoliata, Erigeron Canadensis, Gynura procumbens, Lygodium microphyllum, Macaranga pruinosa, Poikilospermum cordifolium and the rhizomes of Boesenbergia pulchella and Etlingera littoralis. The phytochemical compounds were determined using conventional chemical tests. Alkaloids were detected in all tested plant with various cloudiness of precipitation except Macaranga pruinosa. Acanthus ebracteatus contains highest concentration of alkaloid.

Flavonoids were detected in all tested plant with various strength of the color except for Gynura procumbens. Acanthus ebracteatus and Derris trifoliata contains highest concentration of flavonoids. Saponins were detected in Acanthus ebracteatus, Clinacanthus nutans, Derris trifoliata, Erigeron Canadensis, Gynura procumbens and Macaranga pruinosa with various length of froth. Tannins were detected in all samples with either brownish-green or blue-blue black colour appearance. It can be concluded that all tested plants do content promising pharmacology properties based on the presence of various secondary metabolites.

Key words: Melanau, Bruit, Sarawak, medicine, phytochemical

INTRODUCTION

Natural products have been used by native cultures as a source of remedies for thousands of years, dating back to ancient empires in Mesopotamia, Egypt, China, Greece, and Rome (Raju and Rao, 1986). Malaysian medicinal plant researcher has been documented in many publications regarding specific ethnomedical applications, extraction and pharmacological activities of the potential plants. For instance, Clinacanthus nutans is widely known in traditional medicine in Malaysia due to its medicinal properties in treating skin rashes, insect and snake bites, and skin lesions caused by virus. Based on the traditional uses of Gynura procumbens, it seems to possess high therapeutic potential for treatment of various diseases making it a target for pharmacological studies aiming to validate and provide scientific evidence for the traditional claims of its efficacy (Tan et al., 2016). Although a specific plant might have a reported use, other parts of the plant and additional applications of the plant specifically from Kampung Bruit, Sarawak remain uninvestigated, or a plant may not have a recorded ethnomedical use. The current research involved the collection, identification, extraction and phytochemical evaluation of the plant extracts derived primarily from a random

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selection of commonly utilized in traditional medicine of the Melanau community in Pulau Bruit, Sibu, Sarawak, Malaysia. The leaves of *Acanthus ebracteatus*, *Clinacanthus nutans*, *Derris trifoliata*, *Erigeron canadensis*, *Gynura procumbens*, *Lygodium microphyllum*, *Macaranga pruinosa*, *Poikilospermum cordifolium* and the rhizomes of *Boesenbergia pulchella* and *Etingeria littoralis* were chosen.

Genus *Acanthus* is a species belongs to the family *Acanthaceae*, native to tropical and warm temperate regions with the highest species diversity in the Mediterranean Basin and Asia (Wiersema and Leon, 2016). *Acanthus ebracteatus* normally recognized with its common name as sea holly and holly mangrove. The plant is known in Peninsular Malaysia as Jeruju Hitam (Giesen et al., 2013) and locally known as Geligir (Figure 1.a). *Boesenbergia* is a genus that belongs to the family *Zingiberaceae* (Taweechaisupapong et al., 2010). *Boesenbergia puchella* is locally known as Lapuin (Figure 1.i). *Clinacanthus nutans* belongs to the Acanthaceae family. Commercially in Malaysia, it is known as Belalai Gajah or Sabah Snake Grass (Roosita et al., 2008). It is named as Belalai Gajah or Gading Gajah (Figure 1.b) as it has a slightly curved stem that resembles the curve of an elephant’s trunk. The leaves are characterized as simple, opposite, narrowly elliptic-oblong or lanceolate 2.5-13 cm long, and 0.5–1.5 cm wide with apex acute or acuminate; dentate or subentire margins while the leaf base are cuneate, obtuse rounded or truncate; often oblique (Yahaya et al., 2015). *Derris trifoliata* known as *Derris uliginosa* or *Pongamia uliginosa* and belongs to the family of *Leguminosae* and *Papilionoideae* as the sub-family. It comes with an odd-pinnate compound leaves about 12.5-20 cm long with 3-7 leaflets (Sarkar et al., 2012). It is locally called as Kalipas (Figure 1.c). *Erigeron canadensis* or *Conyza canadensis* is from the family Asteraceae and has about 50 species found in the tropical and warm regions. It is commonly called as Canada fleabane, bitter weed and horseweed (Shakirullah et al., 2011) but locally it is known as Nyaraya (Figure 1.d). *Etingeria littoralis* is from the family of *Zingiberaceae* with the local name, Tepus (Figure 1.j). The Borneo and New Guinea areas have the largest number *Etingeria* species. The genera *Etingeria* have been used commercially in the community of Malaysia (Chan, 2008). *Gynura procumbens* is found in various parts of Southeast Asia (Zhang and Tan, 2000). The plant leaf is commonly consumed and scientifically it has been shown to be safe for consumption (Rosidah et al., 2008). In Malay, *G. procumbens* is called Sambung Nyawa which means “prolongation of life” by locals (Figure 1.e). Genus *Lygodium* is from the family Schizaeaceae and contains around 26 species distributed through the tropical regions in the world (Garrison, 1998). *Lygodium microphyllum* is a small-leaf climbing fern with dark brown long-creeping stems, has wiry rhizomes and twining fronds which can grow up to 30 m long. *L. microphyllum* is widely distributed across tropical Africa, south-east and East Asia and some western pacific islands. In Peninsular Malaysia, it is called as Duit-duit (Hanum and Hamzah, 1999) and in Pulau Bruit, Sibu, vernacular names is Kerikat (Figure 1.f). *Macaranga* is classified under the family of *Euphoiaceae* for more than 300 species with the only genus in the subtribe *Macaranginae* and it is native to tropical Africa, Madagascar, South-East Asia, Australia and the Pacific region (Davies, 1998). It is commonly found in the peninsular part of forests in Malaysia, Kalimantan and Indonesia. *Macaranga pruinosa* referred as Mahang in peninsular Malaysia and specifically called as Benuak (Figure 1.g) by the locals. *Poikilospermum cordifolium* leaf produced small nectar droplets on their upper surface, which may be an important food source for ants. Amino acids regarded as essential for most insects regularly presented (Menzel and Blüthgen, 2010). Local called it as Labat (Figure 1.h) (Kassim et al., 2016).

**MATERIALS AND METHODS**

**Plant Materials**

Approximately 1 kg of the leaves of *A. ebracteatus*, *C. nutans*, *D. trifoliata*, *E. canadensis*, *G. procumbens*, *L. microphyllum*, *M. pruinosa*, *P. cordifolium* and the rhizomes of *B. pulchella* and *E. littoralis* were collected from Pulau Bruit, Sibu, Sarawak (Figure 1).

**Preparation of Ethanol Extract**

Crude ethanolic extracts were prepared by macerating the leaves of *A. ebracteatus*, *C. nutans*, *D. trifoliata*, *E. canadensis*, *G. procumbens*, *L. microphyllum*, *M. pruinosa*, *P. cordifolium* and the rhizomes of *B. pulchella* and *E. littoralis* were collected from Pulau Bruit, Sibu, Sarawak (Figure 1).

**Preliminary Phytochemical Screening**

The crude ethanolic extracts were subjected to preliminary phytochemical screening for detection of alkaloids, flavonoids, tannins and saponins.

**Test for Alkaloids**

Crude extract (0.5 g) was diluted with 10 ml of 10% acetic acid in ethanol, boiled and filtered while hot. 2 ml of diluted ammonia and 5 ml of chloroform
Table 1. Yield of ethanol extract of leaf of A. ebracteatus, C. nutans, D. trifoliata, E. canadensis, G. procumbens, L. microphyllum, M. pruinosa, P. cordifolium and rhizomes of B. pulchella and E. littoralis

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Local name</th>
<th>Yield (%)</th>
<th>Scientific name</th>
<th>Local name</th>
<th>Yield (%)</th>
</tr>
</thead>
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<tr>
<td>A. ebracteatus</td>
<td>Geligir</td>
<td>5.05</td>
<td>L. microphyllum</td>
<td>Kerikat</td>
<td>25.66</td>
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<tr>
<td>C. nutans</td>
<td>Gading Gajah</td>
<td>13.34</td>
<td>M. pruinosa</td>
<td>Benuak</td>
<td>12.74</td>
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<tr>
<td>D. trifoliata</td>
<td>Kalipas</td>
<td>2.92</td>
<td>P. cordifolium</td>
<td>Labat</td>
<td>18.55</td>
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<tr>
<td>E. canadensis</td>
<td>Nyaraya</td>
<td>6.46</td>
<td>B. pulchella</td>
<td>Lapuin</td>
<td>7.39</td>
</tr>
<tr>
<td>G. procumbens</td>
<td>Sambung Nyawa</td>
<td>7.12</td>
<td>E. littoralis</td>
<td>Tepus</td>
<td>5.35</td>
</tr>
</tbody>
</table>

Fig. 1. Ten potential medicinal plants claimed by Melanau community, Kampung Bruit, Sarawak, Malaysia.

were added to 5 ml of filtrate. The filtrate was shaken gently to extract the alkaloid base. The chloroform layer was extracted with 5% HCL. The filtrate was treated with a few drops of Mayer’s reagent. Formation of white precipitates indicated the presence of alkaloids (Anyasor et al., 2010).

Test for Saponins

Crude extract (1 g) was boiled in 10 ml of distilled water in a water bath and filtered. The filtrate was shaken vigorously (1-2 min) for a stable persistent froth (for at least 15 min) regarded as presence of saponins (Anyasor et al., 2010).

Test for Tannins

Crude extract (0.5 g) was boiled in 20 ml of water and then filtered. A few drops of 2 ml of 10% ferric chloride was added (Nikolova et al., 2011). An intense blue black color was taken as an evidence for the presence of hydrolysable tannins, while brownish green indicated that of condensed tannins (Anyasor et al., 2010).
Percentage of dried yield (%) of each plant were determined as in Table 1 based on ratio 1:10 of dried raw sample to quantity of undenatured ethanol (ml). Phytochemical screening carried out on each plant showed the presence of phytochemical constituents and the results are summarized in Table 2.

Table 2. Phytochemical constituent of ethanol extract of leaf of A. ebracteatus, C. nutans, D. trifoliata, E. canadensis, G. procumbens, L. microphyllum, M. pruinosa, P. cordifolium and rhizomes of B. pulchella and E. littoralis

<table>
<thead>
<tr>
<th>Part</th>
<th>Scientific name</th>
<th>Local name</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Tannins</th>
</tr>
</thead>
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<tr>
<td>Leaf</td>
<td>A. ebracteatus</td>
<td>Geligir</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>C. nutans</td>
<td>Gading Gajah</td>
<td>++</td>
<td>++</td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>D. trifoliata</td>
<td>Kalipas</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. canadensis</td>
<td>Nyaraya</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>G. procumbens</td>
<td>Sambung Nyawa</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L. microphyllum</td>
<td>Kerikat</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>M. pruinosa</td>
<td>Benuak</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P. cordifolium</td>
<td>Labat</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Rhizome</td>
<td>B. pulchella</td>
<td>Lapuin</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>E. littoralis</td>
<td>Tepus</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Note: Flavonoids and Tannins: + weak colour, ++ mild colour, +++ strong colour.
Alkaloids: + negligible amount of precipitation, ++ weak precipitate, +++ strong precipitate.
Saponins: + 1-2cm froth, ++ 2-3cm froth, +++ >3cm froth.

RESULTS AND DISCUSSION

Percentage of dried yield (%) of each plants were determined as in Table 1 based on ratio 1:10 of dried raw sample to quantity of undenatured ethanol (ml). Phytochemical screening carried out on each plant showed the presence of phytochemical constituents and the results are summarized in Table 2.

Preliminary phytochemical screening of leaf of D. trifoliata showed the presence alkaloids, flavonoids, saponins and tannins. Leaf of L. microphyllum contained alkaloids, flavonoids and tannins. Melanau community of Kampung Bruit claimed both plants are the remedy for fever (Kassim et al., 2016). As according to Orwa (2009), the decoction of roots of D. trifoliata is used against fever. Meanwhile for L. microphyllum, its leaf decoction is used in bath to treat fever (Hanum and Hamzah, 1999). Active compounds detected might be responsible to the anti-pyretic claimed by community. According to Ahmad et al. (2017), predominant presence of alkaloids and flavonoids might be responsible for antipyretic properties. According to Vasundra Devi and Divya Priya (2013), the presence of flavonoids and saponins in the extract may be involved in inhibition of prostaglandin synthesis. Preliminary phytochemical screening of A. ebracteatus leaf indicates alkaloids, flavonoids, saponins and tannins. Meanwhile, screening of M. pruinosa leaf showed the presence of flavonoids, saponins and tannins. As for G. procumbens leaf, preliminary phytochemical screening indicated alkaloids, saponins and tannins. A. ebracteatus, M. pruinosa and G. procumbens claimed as an antihypertensive remedy by Melanau community (Kassim et al., 2016). A. ebracteatus is one of the traditional herbal medicines with antihypertensive properties widely used in Thailand (Vuttipong et al., 2014). According to Tan et al. (2016), G. procumbens leaf possesses anti-hypertensive and cardioprotective activity. Antihypertensive effect of the plants might be due to the presence of flavonoids. According to Perez-Vizcaino et al. (2009), the blood pressure-lowering effect of flavonoid quercetin could be an important mechanism contributing to the reduced risk of myocardial infarction and stroke. Flavonoids are reported to lower LDL-C and increase HDL-C concentrations in hypercholesterolemic animals (Daniel et al., 2003). Saponins have shown to inhibit pancreatic lipase activity in high fat diet fed mice leading to greater fat excretion due to reduced intestinal absorption of dietary fats (Han et al., 2002). Dyslipidemia that cause endothelial dysfunction may lead to hypertension (Halperin et al., 2006). According to Liu et al. (2003), tannins may inhibit angiotensin converting enzyme which contribute in hypotensive effect. According to Dangi et al. (2002), the total alkaloidal salts were found to have a negative inotropic effect on the frog heart which indicated the antihypertensive properties. P. cordifolium claimed to have anti-inflammatory properties (Kassim et al., 2016). E. littoralis usually consumed by Malaysian women during ailment, illness and confinement (Chan et al., 2008), and it was claimed as remedy to reduce heat during confinement (Kassim et al., 2016). Such effect produced after using E. littoralis may be due to the cooling effect; one of the anti-inflammatory mechanisms. Any of the observed phytochemical constituents present in both plants (alkaloids, flavonoids and tannins) may be responsible for the anti-inflammatory activities. Inflammation is important in wound healing process. Excessive inflammation, however, limits wound healing (Menke et al., 2007). Both E. canadensis and B.
pulchella believed to help in treating wound (Kassim et al., 2016). The blossoming parts of E. canadensis have been used mainly as a row material in folk medicine (Holm et al., 1997). The wound-healing property of both plants may be attributed to the phytoconstituents present in the plant (Table 2), and the quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents. Flavonoids and tannins are known for their significant role in wound healing process (Shivhare et al., 2010). These compounds have vulnerary, astringent and draining properties (Afaq et al., 2005) but also antibacterial and immunostimulant activities (Pousset, 1989). Flavonoids have been documented to possess potent antioxidant and free radical scavenging effect, which is believed to be one of the most important components of wound healing (Devipriya et al., 1999). Flavonoids and tannins have been shown to be important for wound healing due to their antioxidant, anti-inflammatory and antibacterial activities (Mulisa et al., 2015). According to Porras-Reyes et al. (1993), alkaloid taspine promotes early phases of wound healing. According to Metodiewa and Koska (2000), the plants contain high concentrations of saponins has antioxidant activity which further contribute to anti-inflammatory process. C. nutans believed to help in treating cancers as claimed by local community (Kassim et al., 2016). Recently, the extracts from leaves of C. nutans have been used extensively as primary sources of complementary and alternative healthcare or as economical in-house regimens for Malaysian cancer patients (P’ng et al., 2012). Patients have claimed that they have recovered from cancer after consuming C. nutans leaves over a certain period of time. Flavonoids responsible for the mechanism of anti-cancer (Shim et al., 2013) and the flavonoids found in C. nutans might contribute in similar property (Table 2).

CONCLUSION

Preliminary screening of phytochemical findings performed on ten species of potential medicinal plants claimed by Melanau community is valuable and worth to further research. The finding discovered from the initial study can enhanced the present research literature on medicinal plant found in Sarawak. Advanced studies including isolation, purification and characterization of active constituents should be conducted to obtain their chemical composition. Studies on the molecular mechanisms and cellular actions underlying the plants are required to discover more therapeutic properties contained in various claimed medicinal plant used by the community of Sarawak.

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