JOURNAL OF TAIBAH UNIVERSITY FOR SCIENCE
2024, VOL. 18, NO. 1, 2325677
https://doi.org/10.1080/16583655.2024.2325677

Anticancer agents and genetic identification of *Pterospermum*, an indigenous plant of Sarawak, Malaysia

Anthony Nyangson Steven[a], Khairunnabihah Bachuri[a], Nur Syamim Syahirah Mat Hussin[b] and Nur Izzati Mohd Noh [c]

[a]Faculty of Science, Department of Chemistry, Universiti Teknologi Malaysia, Johor Bahru, Malaysia; [b]Faculty of Applied Sciences, Department of Biology, Universiti Teknologi MARA, Perak, Malaysia; [c]Faculty of Science, Department of Biosciences, Universiti Teknologi Malaysia, Johor Bahru, Malaysia

ABSTRACT

In Malaysia, *Pterospermum* is distributed in the states of Peninsular Malaysia, Sarawak, and Sabah. For centuries, the Bidayuh tribe in Sarawak has been using an indigenous plant to cure patients diagnosed with breast cancer. The plant is called Bayur by the tribe, which is the similar local name known for several *Pterospermum* species in Sarawak, Sabah, and Indonesia. This study was carried out to genetically identify the indigenous plant using the core barcode loci, *rbcL* and *matK*, as well as profiling anticancer agents from this plant. Phylogenetic analyses based on both *rbcL* and *matK* loci did not show clear species resolution for the indigenous plant, however, the analyses showed consistent relationships among congeneric species of *Pterospermum*. Interestingly, UHPLC-QQQ/MS analysis showed the presence of anti-cancer agents in the bark extract, thus supporting the potential of the indigenous plant for breast cancer treatment.

ARTICLE HISTORY
Received 15 November 2022
Revised 17 February 2024
Accepted 27 February 2024

KEYWORDS
Pterospermum; medicinal plant; DNA barcoding; UHPLC-QQQ/MS; anticancer

1. Introduction

Pterospermum Schreb is a genus of trees that are distributed in Malaysia, Brunei, Singapore, Indonesia, the Philippine, India, Sri Lanka, and South China [1]. In Malaysia, this genus is distributed in Peninsular Malaysia and East Malaysia (Sabah and Sarawak). No record is found on the use of this plant by the locals in Peninsular Malaysia, but it could be used as timber. Noteworthy, the locals in Sarawak, a state located in East Malaysia, use the bark of an indigenous plant called Bayur as a traditional medicine for breast cancer treatment. This traditional method of treatment has been passed down through generations and is still being practiced to the present day by the Bidayuh tribe.

Pterospermum was traditionally recognized as belonging to the Sterculiaceae; however, based on recent phylogenetic analyses, *Pterospermum* was placed in the Malvaceae [1]. The traditional order Malvales consists of four families, Malvaceae, Sterculiaceae, Bombacaceae, and Tiliaceae [2]. Whereas the recent phylogenetic analyses based on the morphological, molecular, and biogeographical data recognized 10 families (Bixaceae, Cistaceae, Cytinaceae, Dipterocarpaceae, Malvaceae, Muntingiaceae, Neuradaceae, Sarcolaenaceae, Spherosepalaceae and the Thymelaeaceae) as belonging to the expanded order Malvales [2,3]. This comprehensive reevaluation has resulted in the merger of the traditional families into Malvaceae [4].

Generally, the order Malvales consists of flowering trees and mainly woody tropical species with some temperate species [3,5]. *Pterospermum* species commonly have bark textures ranging from smooth to scaly in the colour of cinnamon, fawn, grey, grey-brown, or light brown [1]. Reportedly, all species produce glabrous, membraneous, and translucent winged seeds, the features that aid dispersion by wind [1], which is believed to be the common method of dispersal by the species found in Sarawak. Hypothetically, pollination of *Pterospermum* flowers could be mediated by a butterfly, bat, or moth, although many *Pterospermum* species produce delicate white flowers [1]. The flowers have sepals, which are longer than petals and covered with a dense indumentum of hairs [1]. Typically, spiny pollen is a defining feature of Malvales that occurs in *Pterospermum* [5].

To date, 34 species of *Pterospermum* have been recognized, 11 of which are distributed in Peninsular Malaysia and East Malaysia (Sabah and Sarawak).

CONTACT Nur Izzati Mohd Noh izzati@utm.my Faculty of Science, Department of Biosciences, Universiti Teknologi Malaysia, 81310 Skudai, Johor Bahru, Malaysia

Supplemental data for this article can be accessed online at https://doi.org/10.1080/16583655.2024.2325677.

Pterospermum are wood-producing plants that can regenerate quickly and well in undisturbed to disturbed forests [6]. In the landscape of Bornean floodplain forests, *Pterospermum javanicum* (*P. javanicum*) was found distributed better in bright microsites, whereas *P. acerifolium* was found distributed in shaded microsites [7]. Some species are endemic to tropical evergreen forests, with the typical soil is nutrient-poor and low water retention, such as *P. kingtungense* in the Limestone Mountain of central Yunnan [8], *P. rubiginosum* in Assam, Karnataka, Tamil Nadu, and Kerala of India and *P. reticulatum* in Western Ghats of India [9].

Different parts of *Pterospermum* species have been recorded as important in traditional medicine. The bark of *P. reticulatum* was used to treat ulcers, wounds, and inflammation by the locals in India [9]. Whereas, the bark of *P. javanicum* and *P. subpeltatum* was used to treat dysentery, toothache, ulcers, and sprains by the locals in South Sulawesi, Indonesia [10,11]. On the other hand, the roots of *P. javanicum* were used by the people of the Sesaot region in West Nusa Tenggara, Indonesia, as diabetic drugs when mixed with wine and as haemorrhoid medicine when mixed with water [12]. In China, the roots of *P. heterophyllum* were known to be effective in treating rheumatoid arthritis and inflammation [13]. The locals in South Sulawesi, Indonesia, were also reported to have applied the leaves of *P. acerifolium* and *P. diversifolium* externally to reduce itchiness [10,11], whereas, the leaves of *P. canescens* Roxb. can be applied externally to cure headaches [14]. Moreover, the paste of P. canescens Roxb. flowers mixed with rice water and vinegar was used externally to treat migraine. The flowers of *P. acerifolium* have been traditionally used for bleeding piles, haematuria, and ulcers [14]. Whereas, the charred flowers and bark of *P. acerifolium* mixed with the powder of *Mallotus philippinensis* were applied to smallpox eruptions [14].

Ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) has a great advantage for simultaneous analysis of multiple phytochemical compounds in plant extracts. Scopoletin, a potent antibacterial and antifungal agent, as well as alizarin, a promising antitubercular agent, from the root extracts of *Morinda citrifolia* were identified using ultra-high performance liquid chromatography triple-quad mass spectrometry (UHPLC-QQQ/MS) [15]. On the other hand, metabolites for antifungal activity from *Vernonia amygdalina* Delila, an African medicinal plant species were identified using UHPLC coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF/MS) [16]. Moreover, UHPLC-MS methods have been established to understand the metabolic network in *Pogostemon cablin*, an aromatic medicinal herb [17], *Raphanus sativus*, a taproot vegetable [18], and *Oryza sativa* during germination under low temperature [19].

Therefore, in this study, DNA barcoding analysis was conducted to genetically identify an indigenous medicinal plant from Sarawak, Malaysia that resembles the morphological characteristics of *Pterosperum*. Simultaneously, morphological characterization was also conducted for identification of the plant. Furthermore, UHPLC-QQQ/MS analysis was performed to profile the anti-cancer agents present in this plant. Our findings provide scientific information about the congeneric species of Bayur distributed in Sarawak, Malaysia, and elucidate the phytochemical constituents produced by this plant for inhibiting cancer cells.

2. Material and methods

2.1. Plant material

The leaves and bark of the indigenous plant were collected from Tebekang-Mingkos-Tebedu Native Conservative Reserve (NCR), Serian, Sarawak, Malaysia. The samples were transported from the collection site to the laboratory within 24 h after collection. The Sarawak Biodiversity Center (SBC) granted permission for sample collection under Permit SBC-RDP-0014-NIMN.

2.2. DNA barcoding and phylogenetic tree construction

Two coding cpDNA loci, *rbcL* and *matK*, were amplified using the universal primers in order to obtain 600 bp *rbcL* amplicons and 850 bp *matK* amplicons (Apical Scientific Sdn. Bhd.). The primers were chosen based on recommendations by CBOL (the Corsotium for the Barcode of Life). The bidirectional sequence reads from each *rbcL* and *matK* gene were analysed using BLASTn (www.ncbi.nlm.nih.gov) for species identification. The identification was considered correct if the homologous *matK* and *rbcL* sequences showed between 96% and 100% identity percentage with the searched sequences, as well as if they derived from the expected Pterospermum genus and Malvales family. Phylogenetic trees were constructed based on the maximum likelihood (ML) method and neighbour joining (NJ) method. The sequences were aligned and phylogenetic trees were built using the MEGA-X software, with 500 bootstrap repetitions and the Tamura-Nei model as the default option.

2.3. Plant extract preparation

The bark was used to prepare the plant extract for UHPLC-QQQ/MS analysis. The ground sample (0.5 g) was mixed with 5 ml of ultra-pure water and subjected to ultrasonication at 68 Hz for 120 min at room temperature. The sample was centrifuged for 30 min at 5000 rpm. The supernatant was diluted with absolute ethanol to a final concentration of 10% (v/v). The diluted total extract was filtered twice using 0.45 and 0.22 μm PTFE filters. The filtrate was diluted to 10^4 dilution

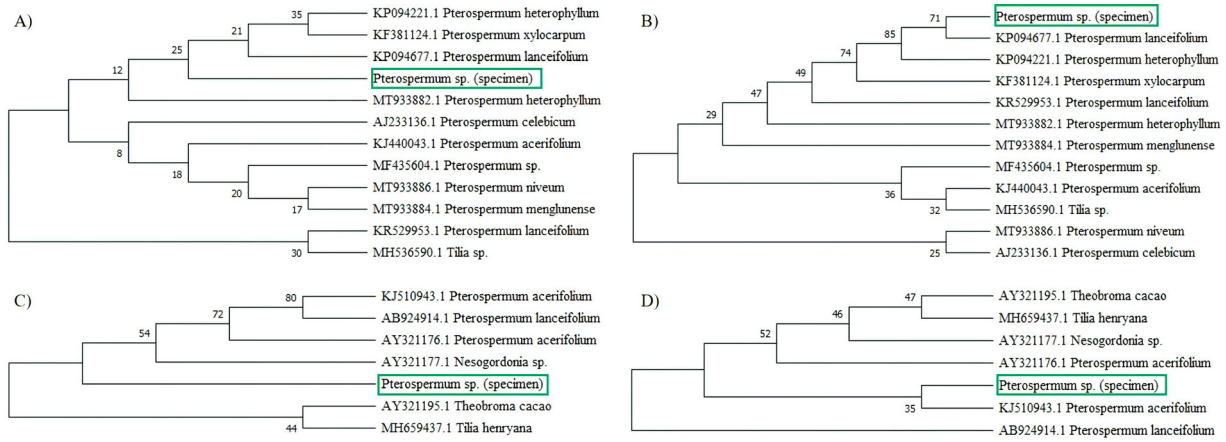

Figure 1. Phylogenetic[Q5] tree based on *rbcL* locus by maximum likelihood (A) and neighbour joining (B); based on *matK* locus by maximum likelihood (C) and neighbour joining (D). The green box represents the plant used in this study.

factor using absolute ethanol and subjected to UHPLC-QQQ/MS analysis.

2.4. UHPLC-QQQ/MS analysis

The plant extract was analysed using Agilent 6410 Triple Quad LC/MS coupled with Agilent 1290 Infinity Series UHPLC (Agilent Technologies, USA). The chromatographic separation was carried out on an Agilent Eclipse XDB-C18 column (150 mm $\times$ 4.6 mm, 5.0 μm, Agilent, USA). The column temperature was set at 25°C and the injection volume was 1.0 μl. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at a flow rate of 0.5 mL/min. The elution gradient was 0–5 min, 30–100% (B); 5–10 min, 100% (B); 10–11 min, 100–30% (B); 11–30 min, 30% (B). The effluent was directed into a triple quadrupole mass detector operated in a positive ESI mode with parameters set as capillary voltage (VCap), 4000 V; fragmentor voltage, 150 V; skimmer, 65 V; octapole (OCT 1 RF Vpp), 750 V; drying gas temperature, 300°C and pressure of the nebulizer, 45 psi. Data analysis was performed with Agilent Mass Hunter Qualitative Analysis B.05.00 Software (Agilent Technologies, USA).

3. Results and discussion

3.1. Molecular systematics of Pterospermum sp.

High-quality sequences were obtained for both *rbcL* and *matK* using the universal primers. The phylogenetic analysis of both *rbcL* and *matK* resulted in paraphyletic branches between the indigenous plant and other *Pterospermum* species when using the ML method (Figure 1(A and C)). In order to consider the species resolved, the conspecific individuals must be grouped in one monophyletic clad [20]. The separation of the indigenous plant and other *Pterospermum* species in paraphyletic branches thus, it was considered an identification failure for species resolution. Nevertheless, the indigenous plant identified belongs to the Malvales family in the ML tree. In contrast, when utilizing the NJ method, the indigenous plant constructed a monophyletic clad with *P. lanceifolium* (Figure 1(B)) and *P. acerifolium* (Figure 1(D)) based on the *rbcL* and *matK* loci, respectively. Although NJ method meets the requirements for species identification [21,22], the phylogenetic analysis must be validated by bootstrap percentage which is categorized as strong ($>$ 85%), moderate (70–80%), weak (50–69%) and poor ($<$ 50%) [23]. The phylogenetic analysis of both *rbcL* and *matK* failed to show a strong bootstrap percentage in the NJ method, where the bootstrap percentage of the indigenous plant with *P. lanceifolilum* and *P. acerifolium* was only 71% and 35%, respectively. However, the indigenous plant was identified as a member of the *Pterospermum* genus. Noteworthy, the paraphyletic branches of the indigenous plant constructed by the ML method consist of *P. lanceifolilum* and *P. acerifolium*.

In this study, we attempt the first species identification of *Pterospermum* distributed in the tropical forest of Sarawak, Malaysia, based on the primary barcode *rbcL* and *matK* loci for plants [20]. The *rbcL* locus is the most characterized plastid genome region, with wide representation from all major groups [24–26]. Conversely, the evolution of the *matK* locus is considered the fastest in the plastid genome region [23,27–29] with a substitution rate 2–3 times greater than *rbcL* in angiosperm [26]. However, phylogenetic analysis of the indigenous plant based on both *rbcL* and *matK* loci using the ML method did not show any clear species resolution. Contrary, the analysis of *rbcL* and *matK* loci for species identification of the indigenous plant remain equivocal concerning different relationships with *P. lanceifolilum* and *P. acerifolium* constructed in NJ phylogenetic trees. Reportedly, the species resolution ability of *matK* and *rbcL* varied from family to family or genus to genus [20,23]. Furthermore, several studies have shown low species identification success rates

Table 1. Anticancer agents identified in *Pterospermum*.

Compound	Type	Mass	Formula	m/z	RT
SP600125	JNK inhibitor	220.0629	$C_{14}H_8N_2O$	111.0391	0.58
Cytarabine	Pyrimidine nucleoside	243.0848	$C_9H_{13}N_3O_5$	122.5499	0.585
Spisulosine	Sphingoid base	285.304	$C_{18}H_{39}NO$	286.3113	14.878
Xestoaminol C	Sphingoid analogue	229.2411	$C_{14}H_{31}NO$	230.2484	12.327
Phytosphingosine	Sphingoid base	317.2939	$C_{18}H_{39}NO_3$	318.3012	12.346
Dihydroceramide C2	Sphingolipids	343.3098	$C_{20}H_{41}NO_3$	344.317	15.208
Enigmol	Sphingoid base	301.2986	$C_{18}H_{39}NO_2$	302.306	13.485
Encelin	Sesquiterpenoids	244.1099	$C_{15}H_{16}O_3$	245.1171	12.424
Hetisine	Terpenoids	329.1981	$C_{20}H_{27}NO_3$	330.2057	7.9
Burseran	Phenylpropanoids	386.1728	$C_{22}H_{26}O_6$	409.1619	13.125

when utilizing *rbcL* and *matK* for DNA barcoding of tropical tree species from the tropical forest areas in western Hainan Island [21,22], southwest China [30], and India [31], but high identification success rates at the genus and family level. Given that we constructed phylogenetic relationships for the indigenous plant and a number of *Pterospermum* species, our results thus suggest that *matK* and *rbcL* serve well as the barcoding loci to resolve congeneric species of *Pterospermum*. Additionally, the results of genetic identification were crossed check with morphological identification. The herbarium specimens of the indigenous plant (voucher specimen number PID070923-07) exhibited the morphological characteristics consistent with *P. diversifolium*. A thorough taxonomical study reported that *P. diversifolium* is similar to *P. acerifolium* but its stipules are entire (compared to divided in *P. acerifolium*) and quartenary veins on the leaf lower surface are clearly visible (while not visible to faintly visible in *P. acerifolium*) [1]. Contrary, *P. lancefolium* has entire stipules and leaf lower surface with red stellate hairs with short brances (80–100 μM) [1].

3.2. Anti-cancer potential of Pterospermum

The UHPLC-QQQ/MS analysis identified 10 anti-cancer agents (Table 1), which are the main interest in this study, present in the bark extract. Compounds were identified by matching their exact (measured) masses of protonated $[M+2H]^{+2}$, $[M+H]^{+}$ or $[M+Na]^{+}$ adduct (Supplementary Figure 1) with m/z. Numerous in vitro and in vivo studies have reported the inhibitory effects of these anti-cancer agents on their target cell types.

The protonated molecular ion $[M+2H]^{+2}$ of SP600125 was observed at m/z 111.0391 (Supplementary Figure 1(A)). An earlier report showed that the detection of $[M+H]^{+}$ ion of SP600125 was obtained at m/z 221 [32]. SP600125 is the first type of c-Jun N-Terminal Kinase (JNK) inhibitors that have been evaluated in clinical phases for controlling cancer cells' progression [33]. Conversely, SP600125 lacks selectivity among the JNK family [33], despite the proficient inhibitory effects of this broad spectrum JNK inhibitor on different cell types, including breast cancer [34], stomach cancer [35], and cholangiocarcinoma [36]. Therefore, co-treatment has been proposed for the pronounced effects of SP600125 on cancer cells; for example, combined treatment with the synthetic C-2 homologous series of Jaspine B derivatives enhances tumor inhibition in bladder cancer cells [37]; SP600125 and indirubin-3-monoxime (I3M) synergize to cause apoptosis in breast cancer cells [38]; and SP600125 increases sensitivity in lung and breast cancer cells during radiotherapy [39].

Furthermore, we detected the protonated molecular ion product spectrum $[M+2H]^{+2}$ of cytarabine at m/z 122.5499 (Supplementary Figure 1(B)). Whereas, a previous study detected the $[M+H]^{+}$ ion of cytarabine at m/z 111.9 using the optimized mobile phase of 0.1% formic acid in water (85%) and 15% acetonitrile at a flow rate of 0.4 mL/min [40]. Cytarabine is a pyrimidine nucleoside analogue that is widely used to treat acute non-lymphocytic leukaemia, lymphocytic leukaemia and chronic myelocytic leukaemia (drugbank.com) (accessed on September 2, 2022). This well-established drug exhibits a mode of action by directly incorporating into DNA to interfere with DNA chain elongation [41]. In addition, the phosphorylated cytarabine (cytarabine triphosphate) can acts as a potent DNA polymerases inhibitor and thus, interferes with DNA chain elongation, DNA synthesis and DNA repair [41]. Cytarabine is a specific drug for actively dividing cells due to its action on ceasing DNA replication, which occurs during an S-phase of a cell cycle [42].

Sphingoid bases and analog were also identified in the bark extract. These long-chain bases act as the precursors for ceramide and sphingolipids. They are cytotoxic for various cancer cell lines. We detected the protonated molecular ion $[M+H]^{+}$ of spisulosine at m/z 286.3113 (Supplementary Figure 1(C)), similar to the spectrum ion of spisulosine detected by Stokvis et al. [43]. Both natural and synthetic spisulosine display very strong cytotoxic properties on lymphoma, lung, colorectal, melanoma, and prostate cancer cell lines [44], but it was discontinued from phase I clinical trials due to an unfavourable risk/benefit balance [45]. Nevertheless, progressive development of the synthetic spisulosine analogues has been done to counter the side effects. Xestoaminol C, the truncated analogue of spisulosine, was identified as a novel reverse transcriptase inhibitor and found less potent than spisulosine [44]. This anti-cancer agent inhibits cell

proliferation in several cancer cell lines, such as glioblastoma [46], leukaemia, and kidney [47]. We detected the protonated molecular ion $[M+H]^+$ of xestoaminol C at m/z 230.2484 (Supplementary Figure 1(D)). In comparison, Dasyam et al. [47] showed the detection of $[M+H]^+$ ion of 3-epi-Xestoaminol C at m/z 256.2271. The molecular formula of 3-epi-Xestoaminol C was reported as $C_{15}H_{30}NO_2$, whereas the molecular formula of xestoaminol C was identified as $C_{14}H_{31}NO$. Moreover, phytosphingosine is a highly bioactive compound and is structurally similar to sphingosine, which induces apoptotic cell death in breast, T-cell lymphoma, and lung cancer cells [21,22,48]. The protonated molecular ion product spectrum $[M+H]^+$ of phytosphingosine was detected at m/z 318.3012 (Supplementary Figure 1(E)). In contrast, the ion spectrum of phytosphingosine in methanolic extract of fruits of *Brucea javanica* was recorded at m/z 317.294 [49]. It is known that dihydroceramide C2, a metabolic sphingolipid intermediate that can be converted into ceramide. Dihydroceramide C2 and ceramide have different saturation of the fatty acyl chain, leading to different biological functions and properties. Ceramide is a highly bioactive compound that is involved in cells' apoptosis, proliferation, and differentiation [48]. Whereas, dihydroceramide can promote autophagy that leads to either cell death or survival [50]. This autophagic character can also inhibit the cell proliferation of cancer cells. Similar to Lim et al. [51], we recorded the protonated molecular ion product spectrum $[M+H]^+$ of dihydroceramide C2 at m/z 344 (Supplementary Figure 1(F)). Reportedly, enigmol exhibits greater potency for anticancer activity than sphingosine [52]. *In vivo* studies showed that enigmol exhibits anticancer activity against prostate cancer cell lines [52,53]. The protonated molecular ion $[M+H]^+$ of enigmol was observed at m/z 302.306 (Supplementary Figure 1(G)). Whereas, the ion spectrum of enigmol in methanolic extract of fruits of *Brucea javanica* was recorded at m/z 301.2979 [49].

Terpenoids such as encelin and hetisin, are the largest group of bioactive compounds produced by plants. Extensive studies have reported the anticancer actions of terpenoids [54]. A pharmacological study reported that encelin showed strong antitumour activity on hepatocytes L02, hepatoma cell SMMC-772 and ovarian neoplasm cell HO-8910 [55]. However, encelin is widely used as an antidiabetic drug. The protonated molecular ion $[M+H]^+$ of encelin was observed at m/z 245.1171 (Supplementary Figure 1(H)), similar to the ion spectrum detected by Patil et al. [56]. On the other hand, hetisine belongs to a large group of diterpenoid alkaloids, which have been reported to possess various pharmacological activities including antiarrhythmic, antitumour, insecticidal, antifungal, antiviral, and control of peripheral vasculature [57]. In this study, the protonated molecular ion $[M+H]^+$ of hetisin was detected at m/z 330.2057 (Supplementary Figure 1(I)), similar to the ion spectrum detected by Zhang et al. [58].

Moreover, we detected the protonated molecular ion $[M+Na]^+$ of burseran at m/z 409.1619 (Supplementary Figure 1(J)). In comparison, a previous study reported the detection of $[M+H]^+$ ion of burseran was obtained at m/z 387.1808 [59]. Burseran is a lignan that is naturally produced by plants. This phenolic compound was first isolated from *Bursera microphylla* and revealed to have antitumour activity against epidermoid carcinoma of the nasopharynx [60].

Additionally, in our study, 2′,4′,6′-Trihydroxychalcone ($C_{15}H_{12}O_4$), 5,2′,6′-Trihydroxy-7-methoxyflavone ($C_{16}H_{12}O_6$), and Apigenin 7-(4″-Z-p-coumarylglucoside) ($C_{30}H_{26}O_{12}$) were also identified from the bark extract. Chalcones possess antiproliferative activity by interfering with the assembly of microtubules during mitosis and cell replication. Subsequently, this strong antioxidant compound can block the cell cycle and induce apoptosis of cancer cells [61]. On the contrary, flavones prevent the progression of the carcinogenesis via modulation of signal transduction pathways that result in inhibition of cell cycle, angiogenesis, and metastasis, as well as promotion of oxidative stress and autophagy [62]. Flavones are found to be a potent anticancer agent against various cancer cell lines, including breast cancer, colorectal cancer, prostate cancer, lung cancer, and melanoma. Moreover, apigenin which belongs to the flavones class, is found to have the ability to arrest the G2/M phase of the cell cycle in melanoma cells, induce apoptosis in myeloid leukaemia cells, and promote autophagy in breast cancer cells [63].

Previous studies have reported the anticancer properties displayed by different *Pterospermum* species. A recent study showed that the leaf extract of *P. lanceifolium* Roxb. can induce apoptotic cell death in hepatic cancer cell lines (HepG2) by stimulating reactive oxygen species (ROS) generation that leads to mitochondrial membrane potential alteration and modulating chromatin condensation [64]. In the same study, the hepatocellular carcinoma in N-nitrosodiethylamine (NDEA)-induced hepatocarcinoma rats was reversed when the rats were administered with the leaf extract. It was found that the extract can restore the level of antioxidant enzyme (GSH, GST, catalase, SOD, and GPX) and the expression of pro-apoptotic (p53, caspase-3, caspase-9, and Bax) and anti-apoptotic (Bcl-2) genes in the rats. Moreover, the bark extract of *P. acerifolium* (L.) wild has also been identified to have the ability to induce apoptotic cell death via mitochondrial ROS-mediated alteration in lung cancer (A549) and pancreatic cancer (PANC-1) cell lines [65]. The bark extract displays cytotoxic and anti-proliferative activities against both the cancer cell lines. Conversely, a novel luteolin analogue and a kaempferol analogue isolated from the flower of P. acerifolium possessed osteogenic effects

by stimulating osteoblast differentiation [66]. Additionaly, the betulonic acid extracted from *P. truncatolobatum* Gagnep. exhibited moderate cytotoxicity against HepG2 and KB cell lines [67].

Altogether, the scientific evidences on anticancer effects reported by previous studies support the traditional folk use of *Pterospermum* in Sarawak, Malaysia, for breast cancer treatment.

4. Conclusion

Our findings revealed that both *matK* and *rbcL* barcode loci can be used as a complementary tool for congeneric species of *Pterospermum*; however, the effectiveness of these barcode regions should be examined adequately for species resolution. Therefore, we suggest using different *rbcL* and *matK* primer combinations as well as analysing the full sequences for *matK* and *rbcL*, which could result in a higher power of species resolution ability of *rbcL* and *matK* for the indigenous plant, Bayur, from Sarawak, Malaysia. In addition, we also managed to identify 10 anticancer agents in the bark extract of the indigenous plant, of which four (SP600125, cytarabine, spisulosine, and encelin) are established drugs used to treat cancer patients or diabetics. Therefore, the profiling of anticancer agents based on the UHPLC-QQQ/MS analysis suggests the feasible anticancer effects of the *Pterospermum* identified in this study.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported by the Research University Grant vote number Q.J130000.3854.18J80 and Q.J130000.2626.11J59 awarded to A.N.S and co-researcher N.I.M.N. We thank Sarawak Biodiversity Center for granted the permit to conduct the study.

ORCID

Nur Izzati Mohd Noh http://orcid.org/0000-0003-0925-8414

References

[1] Ganesan SK, Middleton DJ, Wilkie P. A revision of *Pterospermum* (Malvaceae: Dombeyoideae) in Malesia. Edinb J Botany. 2020;77(2):161–241. doi:10.1017/S0960428619000337.

[2] Ibrahim Z, Hassan S, ElAzab H, et al. Cladistic analysis of some taxa in Malvaceae s.l. "Core Malvales" based on anatomical characteristics. Egypt J Exp Biol (Botany). 2018;1:87–105.

[3] Alverson WS, Whitlock BA, Nyffeler R, et al. Phylogeny of the core Malvales: evidence from Ndhf sequence data 1. Am J Botany. 1999;86(10):1474–1486. doi:10.2307/2656928.

[4] Cvetkovic T, Areces-Berazain F, Hinsinger DD, et al. Phylogenomics resolves deep subfamilial relationships in Malvaceae s.l. Genes Genomes Genet. 2021;11:7.

[5] Judd WS, Manchester SR. Circumscription of Malvaceae (Malvales) as determined by a preliminary cladistic analysis of morphological, anatomical, palynological, and chemical characters. Brittonia. 1997;49(3):384–405. doi:10.2307/2807839.

[6] Andila PS, Warseno T, Wibawa IPAH, et al. Ethnobotanical and phytochemical study of bayur (*Pterospermum javanicum* jungh.) on sasak tribe around Mount Rinjani National Park, West Lombok as a conservation effort. J Trop Biodivers Biotechnol. 2021;6:1. doi:10.22146/jtbb.61008.

[7] Yamada T, Ngakan OP, Suzuki E. Habitat differences between two congeneric canopy trees, *Pterospermum javanicum* and *Pterospermum diversifolium* (Sterculiaceae) in an Indonesian floodplain forest. Tropics. 2007;16:165–169. doi:10.3759/tropics.16.165.

[8] Ren Y, Zhu Y, Wang Q, et al. Transcriptome of *Pterospermum kingtungense* provides implications on the mechanism underlying its rapid vegetative growth and limestone adaption. Sci Rep. 2017;7(1):3198. doi:10.1038/s41598-017-03433-1.

[9] Jacob J, Sreejith K. Antioxidant and anti-inflammatory properties of *Pterospermum rubiginosum* heyne ex wight and arn and *Pterospermum reticulatum* wight and arn (Sterculiaceae): an in vitro comparative study. Asian J Pharm Clin Res. 2019: 272–275. doi:10.22159/ajpcr.2019.v12i2.28137.

[10] Salempa P, Noor A, Harlim T, et al. The antibacterial properties of bayur tissues' extract (*Pterospermum subpeltatum* C.B. Rob). J Teknol (Sci Eng). 2014;69(5):87–89.

[11] Salempa P. Fitosteroid dari fraksi kloroform kayu akar Bayur (*Pterospermum subpeltum* C.B. Rob). J Chem. 2012;13(2):47–50.

[12] Hidayat S. The use by local communities of plants from Sesaot protected forest, West Nusa Tenggara, Indonesia. Biodiversitas. 2017;18(1):238–247. doi:10.13057/biodiv/d180130.

[13] Yang L, Liu R, Fan A, et al. Chemical composition of *Pterospermum heterophyllum* root and its anti-arthritis effect on adjuvant-induced arthritis in rats via modulation of inflammatory responses. Front Pharmacol. 2020;11:1814.

[14] Khare C. Pterospermum canescens Roxb. In: Khare C, editor. Indian Medicinal Plants. New York: Springer; 2007. p. 526.

[15] Sam-ang P, Phanumartwiwath A, Liana D, et al. UHPLC-QQQ-MS and RP-HPLC detection of bioactive alizarin and scopoletin metabolites from *Morinda citrifolia* root extracts and their antitubercular, antibacterial, and antioxidant activities. ACS Omega. 2023;8(32):29615–29624. doi:10.1021/acsomega.3c03656.

[16] Mkhonto C, Makananise V, Sagbo IJ, et al. UPLC–QTOF/MS tentative identification of phytochemicals from *Vernonia amygdalina* Delile acetone and ethanol leaf extracts. J Med Plants Econ Dev. 2023;7(1):a181.

[17] Wang X, Zhong L, Zou X, et al. GC-MS and UHPLC-QTOFMS-assisted identification of the differential metabolites and metabolic pathways in key tissues of *Pogostemon cablin*. Front Plant Sci. 2023;14:1–13.

[18] Yan C, Huang Y, Zhang S, et al. Dynamic profiling of intact glucosinolates in radish by combining UHPLC-HRMS/MS and UHPLC-QqQ-MS/MS. Front Plant Sci. 2023;14:1–12.

[19] Yang M, Yang J, Su L, et al. Metabolic profile analysis and identification of key metabolites during rice seed germination under low temperature stress. Plant Sci. 2019;289:110282. doi:10.1016/j.plantsci.2019.110282.

[20] Sikdar Biotechnology Centre, S., Tiwari S, Vijay Thakur V, et al. An in silico approach for evaluation of rbcL and matK loci for DNA barcoding of Fabaceae family. Int J Chem Stud. 2018;6(6):2446–2451.
[21] Kang HM, Son HS, Cui YH, et al. Phytosphingosine exhibits an anti-epithelial–mesenchymal transition function by the inhibition of EGFR signaling in human breast cancer cells. Oncotarget. 2017;8(44):77794. doi:10.18632/oncotarget.20783.
[22] Kang Y, Deng Z, Zang R, et al. DNA barcoding analysis and phylogenetic relationships of tree species in tropical cloud forests. Sci Rep. 2017;7(1):12564. doi:10.1038/s41598-017-13057-0.
[23] Ho VT, Tran TKP, Vu TTT, et al. Comparison of matK and rbcL DNA barcodes for genetic classification of jewel orchid accessions in Vietnam. J Genet Eng Biotechnol. 2021;19:1. doi:10.1186/s43141-020-00094-y.
[24] Setsuko S, Yoshimura K, Ueno S, et al. A DNA barcode database for the woody plants of Japan. Mol Ecol Resour. 2023;23(4):855–871. doi:10.1111/1755-0998.13748.
[25] Amandita FY, Rembold K, Vornam B, et al. DNA barcoding of flowering plants in Sumatra, Indonesia. Ecol Evol. 2019;9(4):1858–1868. doi:10.1002/ece3.4875.
[26] Newmaster SG, Fazekas AJ, Ragupathy S. DNA barcoding in land plants: evaluation of rbcL in a multigene tiered approach. Can J Botany. 2006;84(3):335–341. doi:10.1139/b06-047.
[27] Bieniek W, Mizianty M, Szklarczyk M. Sequence variation at the three chloroplast loci (matK, rbcL, trnH-psbA) in the Triticeae tribe (Poaceae): comments on the relationships and utility in DNA barcoding of selected species. Plant Syst Evol. 2015;301(4):1275–1286. doi:10.1007/s00606-014-1138-1.
[28] Hollingsworth PM, Forrest LL, Spouge, JL, et al. A DNA barcode for land plants. Proc Natl Acad Sci USA. 2009;106(31):12794–12797. doi:10.1073/pnas.0905845106.
[29] Nyffeler R, Bayer C, Alverson WS, et al. Phylogenetic analysis of the *Malvadendrina clade* (Malvaceae s.l.) based on plastid DNA sequences. Organ Diver Evol. 2005;5(2):109–123. doi:10.1016/j.ode.2004.08.001.
[30] Huang XC, Ci XQ, Conran JG, et al. Application of DNA barcodes in Asian tropical trees - a case study from Xishuangbanna Nature Reserve, Southwest China. PLoS One. 2015;10:6.
[31] Tripathi AM, Tyagi A, Kumar A, et al. The internal transcribed spacer (ITS) region and trnhH-psbA are suitable candidate loci for DNA barcoding of tropical tree species of India. PLoS One. 2013;8(2):e105914. doi:10.1371/journal.pone.0057934.
[32] Dvorak Z, Vrzal R, Henklova P, et al. JNK inhibitor SP600125 is a partial agonist of human aryl hydrocarbon receptor and induces CYP1A1 and CYP1A2 genes in primary human hepatocytes. Biochem Pharmacol. 2008;75(2):580–588. doi:10.1016/j.bcp.2007.09.013.
[33] Messoussi A, Mence Feneyrolles C, Lie Bros A, et al. Recent progress in the design, study, and development of c-Jun N-terminal kinase inhibitors as anticancer agents. Chem Biol. 2014;21:1433–1443. doi:10.1016/j.chembiol.2014.09.007.
[34] Huang YC, Pan W, Li H, et al. c-Jun NH2-terminal kinase suppression significantly inhibits the growth of transplanted breast tumors in mice. J Int Med Res. 2020;48:6.
[35] Kim JH, Kim TH, Kang HS, et al. SP600125, an inhibitor of Jnk pathway, reduces viability of relatively resistant cancer cells to doxorubicin. Biochem Biophys Res Commun. 2009;387(3):450–455. doi:10.1016/j.bbrc.2009.07.036.
[36] Lin Y, Zhang B, Liang H, et al. JNK inhibitor SP600125 enhances TGF-β-induced apoptosis of RBE human cholangiocarcinoma cells in a Smad-dependent manner. Mol Med Rep. 2013;8(6):1623–1629. doi:10.3892/mmr.2013.1711.
[37] Yu H, Wu CL, Wang X, et al. SP600125 enhances C-2-induced cell death by the switch from autophagy to apoptosis in bladder cancer cells. J Exp Clin Cancer Res. 2019;38(1):1–13. doi:10.1186/s13046-018-1018-6.
[38] Kim M-Y, Jo E-H, Kim Y-C, et al. Indirubin-3-monoxime prevents tumorigenesis in breast cancer through inhibition of JNK1 activity. Biomed Sci Lett. 2021;27(3):134–141. doi:10.15616/BSL.2021.27.3.134.
[39] Li CH, Lim SH, Ryu HH, et al. Enhancement of radiosensitivity by inhibition of c-Jun N-terminal kinase activity in a Lewis lung carcinoma-bearing subcutaneous tumor mouse model. Oncol Rep. 2016;36(6):3397–3404. doi:10.3892/or.2016.5204.
[40] Alnasser A, Hefnawy M, Alanazi M, et al. Applicable pharmacokinetic study: development and validation of bioanalytical LC-MS/MS method for the simultaneous quantification of cytarabine and glasdegib used for the treatment of acute myeloid leukemia. Arab J Chem. 2023;16(10):105117. doi:10.1016/j.arabjc.2023.105117.
[41] di Francia R, Crisci S, de Monaco A, et al. Response and toxicity to cytarabine therapy in leukemia and lymphoma: from dose puzzle to pharmacogenomic biomarkers. Cancers. 2021;13(5):1–39. doi:10.3390/cancers13050966.
[42] Faruqi A, Tadi P. Cytarabine. XPharm: comprehensive pharmacology reference. 2021. p. 1–5.
[43] Stokvis E, Nan-Offeringa L, Rosing H, et al. Quantitative analysis of ES-285, an investigational marine anticancer drug, in human, mouse, rat, and dog plasma using coupled liquid chromatography and tandem mass spectrometry. J Mass Spectrom. 2003;38:548–554. doi:10.1002/jms.469.
[44] Martinková M, Gonda J, Jacková D. Simple marine 1-deoxysphingoid bases: biological activity and syntheses. Tetrahedron: Asymm. 2016;27(24):1187–1212. doi:10.1016/j.tetasy.2016.10.009.
[45] Schöffski P, Dumez H, Ruijter R, et al. Spisulosine (ES-285) given as a weekly three-hour intravenous infusion: results of a phase I dose-escalating study in patients with advanced solid malignancies. Cancer Chemother Pharmacol. 2011;68(6):1397–1403. doi:10.1007/s00280-011-1612-1.
[46] Chen BS, Yang LH, Ye JL, et al. Diastereoselective synthesis and bioactivity of long-chain anti-2-amino-3-alkanols. Eur J Med Chem. 2011;46(11):5480–5486. doi:10.1016/j.ejmech.2011.09.010.
[47] Dasyam N, Munkacsi AB, Fadzilah NH, et al. Identification and bioactivity of 3- epi -xestoaminol C isolated from the New Zealand brown alga Xiphophora chondrophylla. J Nat Prod. 2014;77(6):1519–1523. doi:10.1021/np500171z.
[48] Park M-T, Kang JA, Choi J-A, et al. Phytosphingosine induces apoptotic cell death via Caspase 8 activation and Bax translocation in human cancer cells. Clin Cancer Res. 2003;9:878–885.
[49] Bagheri E, Hajiaghaalipour F, Nyamathullaa S, et al. Ethanolic extract of *Brucea javanica* inhibit proliferation of HCT-116 colon cancer cells via caspase activation. RSC Adv. 2018;8:681. doi:10.1039/C7RA09618F.
[50] Lachkar F, Ferre P, Foufelle F, et al. Dihydroceramides: their emerging physiological roles and functions in cancer and metabolic diseases. Am J Physiol - Endocrinol

Metabol. 2021;320(1):E122–E130. doi:10.1152/ajpendo.00330.2020.

[51] Lim J, Kumar AP, Kim C, et al. Study of complexes of C2- and C6-dihydroceramides with transition metal ions using electrospray ionization tandem mass spectrometry (ESI-MS/MS). Bull Korean Chem Soc. 2009;30(2):397–401. doi:10.5012/bkcs.2009.30.2.397.

[52] Symolon H, Bushnev A, Peng Q, et al. Enigmol: a novel sphingolipid analogue with anticancer activity against cancer cell lines and in vivo models for intestinal and prostate cancer. Mol Cancer Ther. 2011;10(4):648–657. doi:10.1158/1535-7163.MCT-10-0754.

[53] Miller EJ, Mays SG, Baillie MT, et al. Discovery of a fluorinated enigmol analog with enhanced in vivo pharmacokinetic and anti-tumor properties. ACS Med Chem Lett. 2016;7(5):537–542. doi:10.1021/acsmedchemlett.6b00113.

[54] Huang M, Lu JJ, Huang MQ, et al. Terpenoids: natural products for cancer therapy. Expert Opin Investig Drugs. 2012;21(12):1801–1818. doi:10.1517/13543784.2012.727395.

[55] Yang ZD, Gao K, Jia ZJ. Eudesmane derivatives and other constituents from *Saussurea parviflora*. Phytochemistry. 2003;62(8):1195–1199. doi:10.1016/S0031-9422(02)00758-6.

[56] Patil SV, Mane RP, Mane SD, et al. Chemical composition of the essential oil from seeds of *Pinda concanensis:* an endemic plant from Western Ghats of India. Int J Pharm Sci Rev Res. 2016;41(1):49–51.

[57] Yin T, Zhang H, Zhang W, et al. Chemistry and biological activities of hetisine-type diterpenoid alkaloids. RSC Adv. 2021;11(57):36023–36033. doi:10.1039/D1RA07173D.

[58] Zhang N, Song Y, Song Q, et al. Qualitative and quantitative assessments of Aconiti lateralis Radix Praeparata using high-performance liquid chromatography coupled with diode array detection and hybrid ion trap–time-of-flight mass spectrometry. J Chromatogr Sci. 2016;54(6):888–901. doi:10.1093/chromsci/bmv245.

[59] Hajjar D, Kremb S, Sioud S, et al. Anti-cancer agents in Saudi Arabian herbals revealed by automated high-content imaging. PLoS One. 2017;12(6):e0177316. doi:10.1371/journal.pone.0177316.

[60] Marcotullio MC, Curini M, Becerra JX. An ethnopharmacological, phytochemical and pharmacological review on Lignans from Mexican Bursera spp. Mol: J Synth Chem Nat Prod Chem. 2018;23(8):1976.

[61] Constantinescu T, Lungu CN, Jazvinš´cak M, et al. Anticancer activity of natural and synthetic chalcones. Int J Mol Sci. 2021;22(21):11306. doi:10.3390/ijms222111306.

[62] Khan AU, Dagur HS, Khan M, et al. Therapeutic role of flavonoids and flavones in cancer prevention: Current trends and future perspectives. Eur J Med Chem Rep. 2021;3:100010. doi:10.1016/j.ejmcr.2021.100010.

[63] Yan X, Qi M, Li P, et al. Apigenin in cancer therapy: anticancer effects and mechanisms of action. Cell Biosci. 2017;7(1):1–16. doi:10.1186/s13578-016-0129-z.

[64] Pal LC, Prateeksha LC, Singh BN, et al. Phenolics-enriched fraction of *Pterospermum lanceifolium* Roxb. efficiently reverses the hepatocellular carcinoma in NDEA-induced HCC rats. Nutr Cancer. 2022;74(3):1106–1121. doi:10.1080/01635581.2021.1922716.

[65] Tripathi SK, Biswal BK. *Pterospermum acerifolium* (L.) wild bark extract induces anticarcinogenic effect in human cancer cells through mitochondrial-mediated ROS generation. Mol Biol Rep. 2018;45(6):2283–2294. doi:10.1007/s11033-018-4390-6.

[66] Dixit P, Khan MP, Swarnkar G, et al. Osteogenic constituents from *Pterospermum acerifolium* Willd flowers. Bioorg Med Chem Lett. 2011;21(15):4617–4621. doi:10.1016/j.bmcl.2011.05.087.

[67] Thi Khanh Linh L, Thu Uyen N, Huu Dien P, et al. Cytotoxic constituents from Vietnamese *Pterospermum truncatolobatum* Gagnep. Indian J Nat Prod Res. 2022;13(1):32–35.