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Original Research Article

Phytochemical Profiling of Balinese Alkaloid-Source Plant Purnajiwa (*Kopsia arborea* Blume. and *Euchresta horsfieldii* (Lesch.) Benn.)

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ABSTRACT

Purnajiwa is a popular traditional Balinese plant that grows in several locations in Bali and has been used empirically to treat disease. The latest study confirmed that there were two plants known as Purnajiwa in Bali (*Kopsia arborea* Blume and *Euchresta horsfieldii* (Lesch) Benn.) Nevertheless, both plants are still used for the same purpose. The Kopsia and Euchresta genus are sources of novel and bioactive alkaloidal compounds. This research aimed to identify and compare the phytochemicals of Purnajiwa (*K. arborea* Blume and *Euchresta horsfieldii* (Lesch) Benn) extract collected from three regions in Bali, namely Jimbaran, Mambal, and Bedugul. The phytochemicals of ethanol crude extract of Purnajiwa fruit and leaves were identified using Gas Chromatography-Mass Spectrometry (GC-MS). The findings revealed an assortment of phytochemicals. Aspidospermidin, kopsinine, quebrachamin, and tabersonin were the alkaloids identified in the *K. arborea* fruit. There were more alkaloids in *K. arborea* sample collected from Mambal compared to the Jimbaran sample. In comparison, matrine alkaloids were identified in the fruit and leaves of *E.horsfieldii*. The results show the influence of habitat and geographic location on the phytochemical profiling of medicinal plants. In conclusion, there are varieties of phytochemicals, especially alkaloids in Purnajiwa (*K. arborea* Blume. and *Euchresta horsfieldii* (Lesch.) Benn) collected from three different locations in Bali.

Keywords: Alkaloid, Euchresta, Kopsia, natural product, phytochemical.

Introduction

Plants are natural substances that are frequently utilized in medicine to treat a range of illnesses. In contrast to other naturally occurring materials like microorganisms, minerals, or animals, plants have been used for a very long time and continue to be the primary source of new therapeutic chemicals. Phytochemical substances, particularly secondary metabolites, are critical to the pharmacological activity of therapeutic plants. Numerous plant species produce a variety of phytochemical substances.[1] Variations in the distribution and content of phytochemical substances within the same species of plant can occur as influenced by both geographic location and habitat. Research on traditional plants from different habitats and geographical areas can be a new strategy for exploring medicinal compounds.[1,2]
One of the medicinal plants traditionally used in Indonesia, and Bali in particular, is Purnajiwa. Purnajiwa is known by various regional names in Indonesia, such as purnajiwa, pranajiwa, or pronojiwo.[3] There is not much research on this plant yet. Numerous studies have been conducted to determine the molecular identity of this plant and assess its antioxidant activity.[3–8]

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The latest study confirmed two different taxonomies of Purnajiwa, namely *K. arborea* and *E. horsfieldii*, indicating that two different plants are known as Purnajiwa.[4] Research on *K. arborea* obtained from Denpasar has been conducted to identify the content of its phytochemical compounds, antioxidants and aphrodisiac activities and ascertain its genomic and taxonomic profile.[4–8]
Kopsia arborea Blume is a species in the Kopsia (family Apocynaceae) genus. The genus Kopsia consists of about 30 species distributed in several countries, mainly Asia, China, Australia, and some islands in the Western Pacific. Kopsia is a source of novel and bioactive alkaloids. Kopsia species generally contain indole alkaloids that are very potent and have broad bioactivity.[2,9,10] Alkaloidal compounds in Kopsia typically have unusual skeletons with significant bioactivities.[11] Several studies were conducted to isolate and synthesize indole alkaloids from Kopsia.[10,12–14] *Kopsia arborea* from Yunnan Province, China, was identified to contain three new types of monoterpenoid indole alkaloids, namely kopsiarborines A-C, strychnos, and methyl chanofruticosinate-type monoterpenoid indole alkaloids which are unusual. Andranginine and kopsiyunnanines A-M were also identified in *Kopsia arborea* from Yunnan Province, China.[9,12] *Kopsia arborea* from China was known to contain several alkaloidal compounds, such as nitaphyllin, tenuiphylline, kopsiyunnanine A, and kopsiyunnanine. At the same time, the species from Malaysia was also identified to contain new bisindole alkaloid compounds, namely arbolodinines A-C.[10] *Kopsia arborea* Blume grows in several regions in Indonesia, such as Java, Bali, and Sulawesi. Identification of phytochemical compounds in *Kopsia arborea* Blume obtained from Sulawesi showed the presence of flavonoids, saponins, tannins, alkaloids, and steroids. No further research has been conducted on *Kopsia arborea* Blume species from Indonesia. In Bali, this plant is empirically used to improve sexual function. However, there have been very few studies on the effects or activity of the compound.

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The genus Euchresta comprises a variety of plants as sources of alkaloids. Several studies have been conducted to determine the various phytochemical compositions identified in Euchresta plants.[15–17] Phytochemical screening of *Euchresta horsfieldii* showed the presence of several phytochemicals, including eugenol, trans-caryophyllene, α-humulene, hexadecenoic acid, 9,12-octadecanoic acid, hexaedioic acid, matrine alkaloid, and 1,2-benzene dicarboxylic acid.[18]
Knowledge of the chemical constituents in a particular botanical source is needed to estimate its biological activity and toxicity. Phytochemical identification is also valuable for the discovery of new bioactive compounds.[19] The fruit and leaves of Purnajiwa have been consumed by the community to obtain certain therapeutic effects and are widely used in various traditional herbal concoctions.[6] The study of Purnajiwa's phytochemical composition has been sparse, particularly concerning alkaloidal phytochemicals. Given that the genera Kopsia and Euchresta generally contain alkaloids, preliminary studies in this area can be conducted to develop applications in traditional medicine worldwide and in Bali specifically. Furthermore, samples were collected from multiple sites in this study to give an overview of how the environment affects a species' phytochemical composition. This comparative study has not been done concerning these two varieties of Purnajiwa. This study aims to identify and compare the phytochemical content, especially the alkaloidal content of ethanol extracts of fruit and leaves of purnajiwa obtained from three locations in Bali, Indonesia, since the phytochemical content is highly influenced by habitat and geographical location.

Materials and Methods

Plant Collection, identification, and preparation
Fruit and leaves of purnajiwa were collected from three locations in Bali (Indonesia). *K. arborea* was collected from Jimbaran (-8.791546, 115.178309) and Mambal (-8.555688, 115.218994), *E. horsfieldii* was collected from Bedugul (-8.241451, 115.161971) (Figure 1) in March to July, 2023. Fruit and leaf samples were taken at the same maturity level identified by morphological similarities. The samples were authenticated by the Laboratory of Genetic Resources and Molecular Biology (voucher no. SDGBM/07/23/034). Plant materials were sorted and washed thoroughly under running water, then oven-dried at 45°C until a constant weight was obtained. The dried material was then pulverized into a homogeneous powder.

Plant Extraction
The powdered material (100 g) was macerated using 96% ethanol solvent (1000 mL, 1:10) for three days. The filtrate was obtained and concentrated using a rotary evaporator to produce a crude extract. The crude extract was used for the identification of phytochemical compounds using GC-MS.

GC-MS Analysis
Identification of phytochemical content was conducted using GC-MS instrument Agilent Technologies 8860 GC system, 5977B GC/MSD (Agilent, USA) equipped with HP-5MS UI column (30 m in length x 0.250 mm in diameter x 0.25 µm in film). GC-MS spectroscopy detection used an electron ionization system (70eV) ranging from 50-300 m/z. The injector temperature used was 250ºC splitless mode. Helium gas was used as a carrier gas with a 1 mL/minute flow rate. The volume of the injected sample is 2 µL. The initial temperature was set at 60º-100ºC with an increase rate of 4ºC/minute, raised to 290ºC with an increase of 10ºC/minute. The appearance of peaks on the chromatogram indicates the presence of chemical compounds in the sample.[20] Phytochemical compounds were then identified by comparing the mass spectra of the detected components with the mass spectral data of the components in the National Institute of Standards and Technology (NIST) library with a similarity index percentage of at least 60%. This study uses the NIST17.L library type using Agilent MassHunter Qualitative Analysis Navigator B.08.00 and GCMS 5977B software.

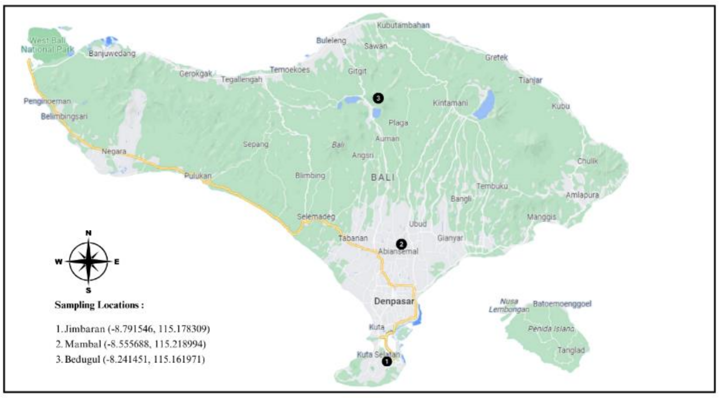

Figure 1: Sampling location of *Kopsia arborea*

Results and Discussion

The compounds identified from the GC-MS analysis of ethanol extracts of purnajiwa leaves and fruits are shown in Tables 1 and 2, Figures 2 and 3. The distribution of each compound varied greatly. Palmitic acid is the only compound identified in purnajiwa fruit from all three locations. Meanwhile, none of the same compounds appeared in the leaves in the three samples. This finding shows the influence of the growing environment on phytochemical content. Both genetic and environmental factors influence plants' secondary metabolites. Although genetic processes essentially supervise these compounds, ecological factors impact the variation of compounds produced. The environment is considered an essential factor affecting the level of gene expression in pathways related to the biosynthesis of secondary metabolites in medicinal plants.[2123] The significant variation in secondary metabolites between populations is expected due to increased habitat heterogeneity on a larger geographical scale.[24]
The environment significantly affects the distribution and composition of phytochemicals in various vegetation, including medicinal and aromatic plants. Temperature and wind patterns affect precipitation, plant architecture, flowering, fruiting, and phytochemical composition.[25] Several studies have shown significant effects of agroclimatic on total phenol content and antioxidant activity. Research on *Aloe vera* from various states in India showed that temperature, precipitation level, fertility, and soil moisture affect the total phenolic content and antioxidant activity.[25] Another study on potatoes (*Solanum tuberosum*) showed environmental influence on ascorbic acid (vitamin C), carotenoid, phenolic, anthocyanin, and antioxidant activity.[26] Similar results were also shown in research on *Pistacia atlantica* Desf, in which ecological factors also affect the flavonoids, tannins, anthocyanins, and antioxidant and antimicrobial activities.[27]
Numerous studies have shown the influence of the environment on the phytochemical content.[28,29] As shown in this study, *K. arborea* taken from two locations in Bali showed variations in the distribution and composition of phytochemical compounds. Also, *K. arborea* taken from Jimbaran and Mambal was investigated in this study. Phytochemical identification of the Jimbaran sample showed the presence of 8 compounds in the fruit extract and 14 compounds in the leaf extract. In comparison, the Mambal sample showed 15 compounds in the fruit extract and 10 compounds in the leaf extract (Figures 2 and 3). The Jimbaran fruit sample contains two alkaloidal compounds, aspidospermidin, and kopsinin, while the Mambal fruit sample contains four alkaloidal compounds, aspidospermidin, kopsinin, quebrachamin, and tabersonin. No alkaloid was identified in either leaf sample. The two locations showed different climate conditions, such as air humidity of around 27% and 24%. They are located at different altitudes, 28 and 600 meters, respectively, above the sea. The differences in phytochemical compositions of the *K. arborea* samples are predicted to be influenced by both geographical and climate conditions, which affect its metabolites biosynthesis. Research conducted on *K. arborea* from Denpasar (Bali) showed the presence of several phytochemical compounds, including phenolics (39.83mg GAE/g), alkaloids (478.81 mg/g), flavonoids (63.42 mg QCE/g) and tannins (327.016 mg TAE/g).

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Phytochemical identification using GC-MS showed the presence of 21 peaks on the chromatogram, dominated by vincadifformin alkaloid, with the highest peak area of 22.36%. The study showed a high level of alkaloid from *K. arborea* as compared to other phytochemicals.[6] Comparatively, previous studies showed variations in alkaloids in *K. arborea* collected from Denpasar and those from Jimbaran and Mambal, which could be due to the influence of geographical conditions.

Several studies have shown the presence of alkaloidal compounds in many species in the Kopsia genus. Identification of *Kopsia hainanensis* showed the presence of 18 alkaloids distributed in various classes: sarpagine, eburnane, aspidofractinine, vincadine, aquammiline, corynanthean, ajmalicine, and aspidospermidine[30]. Likewise, the identification of phytochemical compounds in several species of Kopsia including *K. arborea, K. dasyrachis, K. deverrei, K. favida, K. fruticosa, K. grandifolia, K. griffithii, K. hainanensis, K. jasminfora, K. lancibracteolata, K. lapidilecta, K. larutensis, K. macrophylla, K. officinalis, K. pauciflora, K. profunda, K. singapurensis, K. teoi*, and *K. terengganensis* showed the presence of about 61 monoterpene alkaloids such as aspidofractiines, chanofructicosinates, aspidospermins, danuphylolines, eburnamines, aquammilines, sarpagines, aspidophylines, strychnos, stemmadine, mersinine, pauciflorines, sjutanthines, rhazinilams, lundurines, aspidospermas, catharinensis, leuconoxines, pericines, alstonines, quebrachamines, arbophyllinines, arboflorines, andrasinines, corynantheines, carboline, arbophyllidine mersicarpine, azepane-fused tetrahydro-b-carboline, andranginine.[2]

In the present study, some *K. arborea* samples also contained different alkaloids in varying concentrations. Various types of alkaloids were identified in both the fruit and leaf samples. Aspidospermidin, kopsinine, quebrachamin, and tabersonin were identified in the *K. arborea* fruit. In comparison, matrine was identified in the fruit and leaves of *E.horsfieldii*. The molecular structures of the five identified alkaloids are shown in Figure 4.

Aspidospermidine and kopsinine were identified in the *K. arborea* from Jimbaran and Mambal, while matrine in the *E. horsfieldii* from Bedugul. Meanwhile, quebrachamin and tabersonin were identified in *K. arborea* from Mambal. Kopsinin was the highest alkaloid compound identified in the Jimbaran sample, with a peak area of 5.3%. A similar alkaloid appeared in the Jimbaran sample, although in a small concentration (5.3%), along with another alkaloid, aspidospermidin (0.28%). The dominant compounds identified in the Jimbaran sample were the terpenoid aristoline (8.61%), 9-octadecanoid acid (8.6%), and palmitic acid (6.12%). Matrine alkaloid compounds appeared in the chromatogram of purnajiwa from Bedugul with a peak area of 2.35%. However, the same sample also showed the presence of nitrogen-containing compounds identified as Pyrazino[2,3-c]pyrimidino with a very high concentration of 29.67%. The dominant compounds other than Pyrazino[2,3-c]pyrimidino identified in Bedugul purnajiwa were pentadecanoic acid (12.59%), D-fructose (12.54%), and the sulfur-containing compound Thiopene (12.39%).

Table 1: Chemical composition of *K. arbore* fruit collected from Jimbaran, Mambal, and Bedugul (Bali)

Compositions	Type of Compound	Bedugul		Mambal		Jimbaran	
		Peak area	RT	Peak area	RT	Peak area	RT
Alpha cubebene	Terpenoid	0.51	11.079	-	-	-	-
Aristolin	Terpenoid	-	-	-	-	8.61	14.04
11-Octadecanoic acid	Fatty acid	-	-	0.46	10.342	-	-
9-Octadecanoic acid	Fatty acid	-	-	-	-	8.6	10.568
Cis-9-Hexadecanoic acid	Fatty acid	-	-	0.5	9.459	0.27	9.457
Eicosanoic acid	Fatty acid	-	-	0.36	12.221	-	-
Isophthalic acid	Fatty acid	7.17	24.594	-	-	-	-
Linoleic acid	Fatty acid	3.27	19.911	-	-	-	-
Oleic acid	Fatty acid	-	-	9.38	10.539	-	-
Palmitic acid	Fatty acid	0.49	18.025	5.19	9.559	6.12	9.548
Pentadecanoic acid	Fatty acid	12.59	14.976	0.37	9.029	-	-
Tetradecanoic acid	Fatty acid	-	-	0.92	7.8493	-	-
Aspidospermidin	Alkaloid	-	-	0.29	11.644	0.28	11.639
Caryophyllene	Terpenoid	1.79	12.081	-	-	-	-
D-Fruktosa	Lipopolisacharide	12.54	14.641	-	-	-	-
Ethyl oleate	Fatty acid	2.17	19.956	7.59	10.775	3.83	10.772
Ethyle stearate	Fatty acid	-	-	1.9	10.939	-	-
Heptadecane	Hydrocarbone	-	-	0.9	6.8178	-	-
Kopsinin	Alkaloid	-	-	2.87	13.92	5.3	13.915
Matrine	Alkaloid	2.35	21.611	-	-	-	-
17-Metiloctadecanoic acid	Fatty acid	-	-	-	-	0.3	10.937
Octadecane	Hydrocarbone	-	-	1.64	9.8.723	-	-
Pyrazino[2,3-c]pyrimidino	Nitrogen-containing compound	29.67	21.74	-	-	-	-
Quebrachamin	Alkaloid	-	-	0.75	13.339	-	-
Tabersonin	Alkaloid	-	-	2.67	13. 835	-	-
Thiophene	Sulfur containing aromatic compound	12,39	15,486	-	-	-	-

Note : RT (Retention Time, minute) ; - (not detected)

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Table 2: Chemical composition of *K. arbore* leaves collected from Jimbaran, Mambal, and Bedugul (Bali)

Compositions	Type of Compound	Bedugul		Mambal		Jimbaran	
		Peak area	RT	Peak area	RT	Peak area	RT
(Z)-3-Phenylacrylaldehide	Aldehyde	1.05	9.93	-	-	-	-
12-Olean-3-ethyl acetate	Terpenoid	-	-	-	-	13.98	15.338
1-Nonadecene	Hydrocarbone	-	-	-	-	1.22	16.118
2-Furancarboxylaldehid	Aldehyde	-	-	0.47	5,157	-	-
3-Dibenzofuranamine	Benzofurane	-	-	24.24	15.166	-	-
4-O-Methylmannose	Lipopolisacharide	10.57	15.721	-	-	-	-
5-Nitrouracil	Pyrimidinones	-	-	0.21	9,964	-	-
Eicosatetraenoic acid	Fatty acid	-	-	-	-	1.35	16.516
Linoleic acid	Fatty acid	-	-	2.54	10,574	2.08	10.786
Palmitic acid	Fatty acid	-	-	2.78	9,544	1.71	9.724
Pentadecanoic acid	Fatty acid	-	-	-	-	0.55	9.22
Stearic acid	Fatty acid	-	-	0.46	10.695	0.41	10.938
Trans-13- octadecanoic acid	Fatty acid	-	-	-	-	0.64	10.568
Caulophylline	Terpenoid	13.64	18.91	-	-	-	-
D-Fruktosa	Lipopolisacharide	33.49	15.685	-	-	-	-
Dibutyl Phthalate	Phthalic acids	-	-	0.68	9,625	0.31	9.625
Ethyl heptadecanoic	Fatty acid	-	-	-	-	0.13	10.281
Ethyl linoleic	Fatty acid	-	-	0.82	10,787	-	-
Phytol	Terpenoid	-	-	-	-	1.49	10.429
Caryophyllene oxide	Terpenoid	-	-	-	-	0.43	7.683
Matrine	Alkaloid	23.48	21.705	-	-	-	-
Propyl-1-d1 dodecyl ether	Dialkyl ether	-	-	2.3	8,477	-	-
Cychlopentana	Hydrocarbone	-	-	-	-	1.11	14.934
Squalene	Terpenoid	-	-	5.33	14,627	-	-
Trans-caryophyllene	Terpenoid	-	-	-	-	0.1	6.639

Note : RT (Retention Time, minute) ; - (not detected)

Aspidospermidin, quabrachamin, and tabersonin were the three alkaloid compounds identified in the chromatogram of the Mambal sample, with a peak of 0.29, 0.75, and 2.67%, respectively. The dominant compounds appearing in Mambal purnajiwa were oleic acid (9.38%), ethyl oleate (7.59%), and palmitic acid (5.19). The only alkaloid identified in purnajiwa leaves from Bedugul was matrine. Meanwhile, the Jimbaran and Mambal purnajiwa leaves did not show any alkaloidal compound.

In this study, the alkaloid identified in the highest concentration is kopsinine. Kopsinine was first isolated from *Kopsia longiflora* Merr.[31] Kopsinine has been identified in several species of the genus Kopsia, from the twig and stem bark of *K. arborea*[32], fruit and stem bark of *K. arborea* [27]; leaf, stem bark, and twig of *K. hainanensis*[23], root, stem, twig, leaf, and fruit of *K. officinalis*[33–36], leaf and stem bark of *K.griffithi*[37], stem bark of *K. dasyrachis*[38], stem bark, and leaf of *K. pauciflora.*[39] Kopsinine has also been identified in *K. fructicosa, K. jasminiflora, K. grandifolia, K. singapurensis*, and *K. teoi*[2]. Research shows that kopsinine has hepatoprotective effects.[40,41]

Kopsinine, aspidospermidine, matrine, quebrachamin, and tabersonin were found in the purnajiwa samples. Aspidospermidine is an indole alkaloid identified in *Aspidosperma pyrifolium* and *Vinca minor.*[42,43] It is usually found in the genus Aspidoseperma. Aspidosperma species are traditionally used to treat malaria, dysentery, appendicitis, wound healing, fever, dyspnea, asthma, urinary tract inflammation, and other health conditions.[44] Matrine is a quinolizidine alkaloid compound to be distributed in various plant genera. *Sophora flavescens* is considered a matrine source.[45] Matrine shows activity related to pathways signalling of various biomarkers such as PI3K/AKT/mTOR, TGF-β/Smad, NF-κB, Wnt/β-catenin, MAPKs, JAK/STAT, which indicates its activity on various biological processes such as cell proliferation, differentiation, apoptosis, and regulation of immunity. Matrine also showed biological activity on cancer cells and inflammatory conditions through several mechanisms.[46] Numerous studies have also reported the therapeutic effects of matrine on Alzheimer's syndrome, encephalomyelitis, asthma, myocardial ischemia, rheumatoid arthritis, osteoporosis, and various other conditions related to inflammatory responses. Matrine activity is associated with inhibition of inflammatory response and apoptosis.[47,48]

Quebrachamine has been identified in *K. arborea, K. hainanensis, K. pauciflora*, and *K. officinalis.*[30,49–51] Meanwhile, quebrachamine compounds have only been reported to be identified in the three species of Kopsia. The study showed the cytotoxic effect of quebrachamine.[49]

Tabersonin is an indole alkaloid that has been detected in several plants, including *Alstonia yunnanensis, Catharanthus trichophyllus, Catharanthus roseus, Melodinus hemsleyanus*, and *Amsonia brevifolia.*[52–55] These compounds are also distributed in many species of the genus Tabernaemontana, such as *Tabernae citrifolia, Tabernae catharinensis, Tabernae alternifolia, Tabernae cymose, T. grandiflora*, and *T. divaricate.* [56–60] Research shows the cytotoxic effect of tabersonin against various cancer cell cultures.[61–63]

Terpenoids are other secondary metabolites identified in the purnajiwa sample. The terpenoids identified in the Jimbaran fruits sample is aristolin, while 12-Olean-3-ethyl acetate, Caryophyllene oxide, and Trans-caryophyllene were identified in the Jimbaran leaves sample. Two types of terpenoids are identified in Bedugul samples: alpha

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cubebene and caryophyllene in fruits and caulophylline in leaves. No terpenoidal compounds were identified in the fruits of Mambal samples, while squalene compounds were identified in the Mambal leaves sample. Terpenoidal compounds were in fairly high concentrations in the *K. arborea* and *E.horsfieldii*. Triterpenoids and sterols are compounds that are primarily identified in Kopsia. β-amyrin, β-amyrin acetate, β-amyrone, lupeol, lupeol acetate, and stigmasterol are terpenoids and sterols that have been identified in the leaf and bark of *K. singapurensis.*[64]

Fatty acids are another class of compounds identified in purnajiwa samples. Fatty acids play an important role in biological systems for various biological functions. Plants synthesize various fatty acid compounds, even though only a few fatty acids are present as the main constituents. They are commonly distributed in plant species, such as palmitic acid, oleic acid, linoleic acid, and linolenic acid.[65] Different amounts and variations of both saturated and unsaturated fatty acid compounds were identified in the *K. arborea* and *E.horsfieldii* samples. Environmental factors may influence these differences. In general, an increase in unsaturated fatty acids is associated with colder climates. Cold climates increase antioxidant production in a plant.

Conclusion

This study identified the phytochemical profile of purnajiwa collected in different locations in Bali. It showed variations in the phytochemical distribution in fruit and leaves of purnajiwa samples, which was predicted to be influenced by geographical and climate conditions, affecting metabolites' biosynthesis. Purnajiwa samples showed several types of alkaloids, along with other secondary metabolites, especially terpenoids. Alkaloids showed a wide range of possible pharmacological effects, as numerous studies prove. This study demonstrated purnajiwa's potential for development into various pharmaceutical products with various biological activities, including antiproliferative agents. Further research is needed to extract or isolate alkaloids from purnajiwa and investigate their pharmacological activities.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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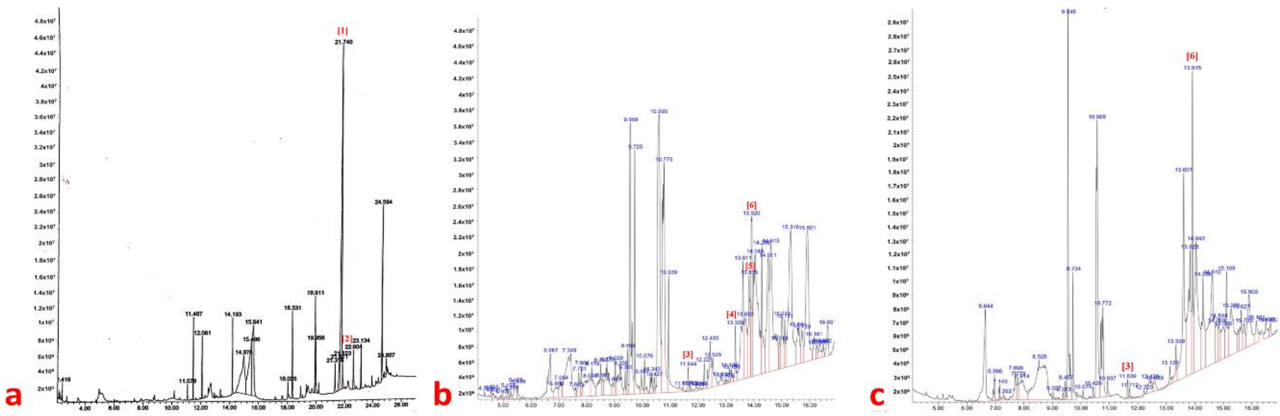

Figure 2: Chromatogram of Bedugul (a), Mambal (b), and Jimbaran (c) fruit sample**.** The alkaloid and nitrogen-containing substances represented by numbers 1 through 6 on the chromatogram are [1] Pyrazino[2,3-c]pyrimidino, [2] Matrine, [3] Aspidospermidin, [4] Quebrachamin, [5] Tabersonin, and [6] Kopsinin.

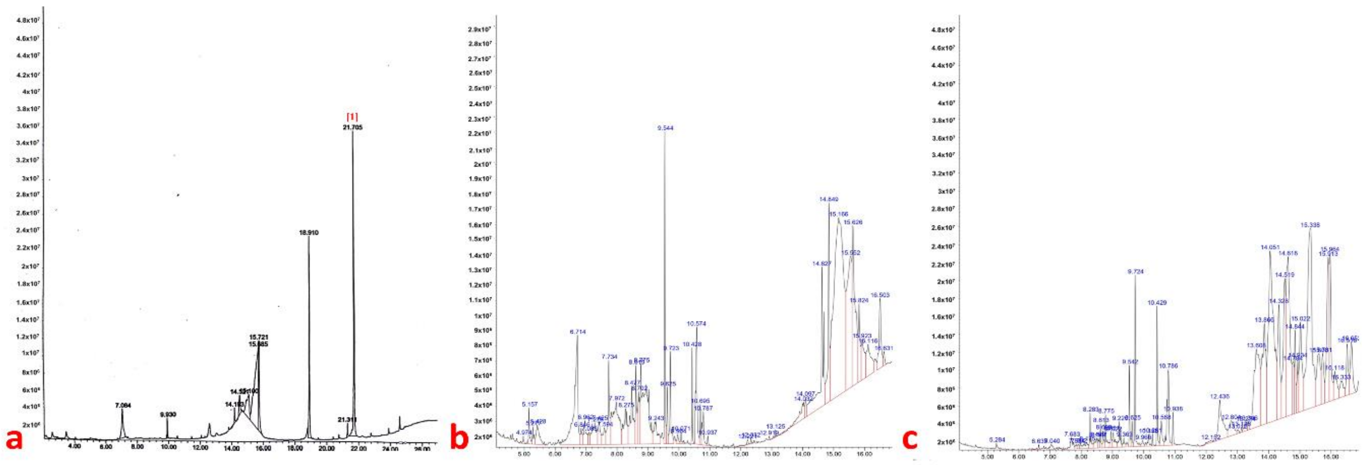

Figure 3: Chromatogram of Bedugul (a), Mambal (b), and Jimbaran (c) leaves sample. Number [1] in the chromatogram showed the peak for the matrine alkaloid.

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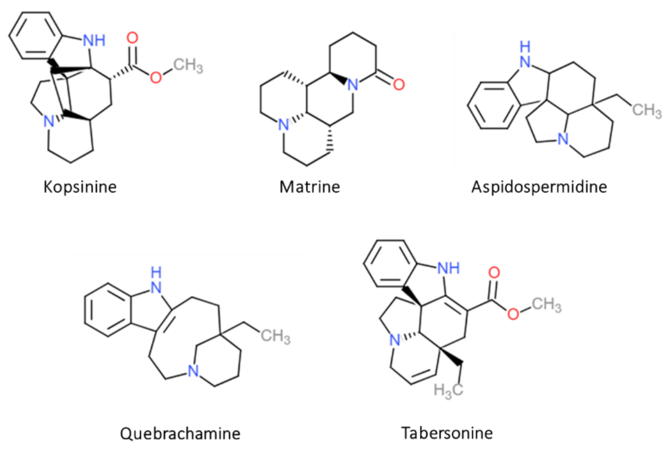

Figure 4: Molecular structure of five alkaloids identified in purnajiwa samples

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