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Alkaloids and Flavonoids Compositions of Traditional Medicinal Value Plants from Imbak Canyon Conservation Area (ICCA)

Fatimah Salim[1,2]*, Nor Hadiani Ismail[1,2], Nurunajah Ab. Ghani[1,2], Norrizah Jaafar Sidik[2], Amalina Mohd Tajuddin[1,2] and Ahmad Kambali B. Khalil[2]
[1]Atta-ur-Rahman Institute for Natural Product Discovery (AuRIns), Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia
[2]Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, 40450, Selangor, Malaysia
*Corresponding author (e-mail: fatimah2940@uitm.edu.my)

Imbak Canyon Conservation Area (ICCA) represents a land rich in plants that are used traditionally by the locals as remedies for various ailments, however yet to be reported. The ethnomedicinal knowledge inspired researchers to search for novel compounds which might be developed into therapeutic agents and this could start with simple *in situ* phytochemical tests on the identified plant sample. The present paper reports on the alkaloids and flavonoids compositions in different plant parts of 46 species (57 samples) collected from five trails of Imbak Canyon Study Centre (ICSC) and three trails of Gunung Kuli Study Centre (GKSC) of ICCA. The collection was based on the traditional medicinal usage described by the ICCA rangers and some plants of particular interest to the group. The phytochemical detection for alkaloids and flavonoids was conducted based on Mayer's reagent and Shinoda test, respectively. Of the 57 samples, 11 samples were found to be positive for both alkaloids and flavonoids. While 12, and 13 samples tested were positive for alkaloids, and flavonoids only, respectively. Of the positive samples, the leaves of *Eusideroxylon zwageri* (belian), *Synsepalum dulcificum* (buah ajaib), *Pinanga jambusana* (pinang bumburing), *Pycnarrhena cauliflora* (pokok ajinamoto), *Cinnamomum* sp. (madang sarsi), *Ixora capillaris* (jenjarum), *Mallotus mollissimus* (bayor) and the flowers of *Curculigo latifolia* (lamba) showed high content of alkaloids, whereas *Phaleria macrocarpa* (mahkota dewa) fruits, *Aeschynanthus* sp. (hoya) flowers, and the stems of *Uncaria calophylla* (kalait or talait), *Orophea hexandra* (karai) as well as *Bauhinia diftera* (bunga api) were rich in flavonoids. It was found that the detected classes of phytochemicals in most of the plant species are consistent with their previously reported constituent and subsequently could correlate to the plant's traditional uses. The findings could provide a basis for the selection of high-potential plants from ICCA for future in-depth chemical and pharmacological studies.

Key words: Phytochemical test; plant traditional use; medicinal plant; alkaloid; flavonoid

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Sabah, "The Land below the Wind" is the second largest state in Malaysia with a land area comprising 73,631 square kilometers, accounting for 22% of the total land area in Malaysia. Of this area, 51% is forested with rich tropical flora, touted to be among the oldest in the world. It is estimated that Sabah has 8000 species of higher plants [1]. In ensuring 30% of Sabah's land area is protected by 2025, several pristine areas have been conserved by the State of Sabah and the last was Imbak Canyon Conservation Area (ICCA) in 2009. ICCA is located in the heart of Sabah (Figure 1), and represents 30,000 hectares of land rich in biodiversity and unique in terms of geomorphologic attributes [2]. Within these rich bio-resources, there are many plants of medicinal value which are used traditionally by the locals as remedies for various ailments [3]. This indigenous knowledge inspired researchers to search for novel phytochemicals that could be developed into therapeutic agents. In addition, there could also be some plants which the potential has not been explored.

Plants contain chemical compounds in the form of primary and secondary metabolites. While the primary metabolites are necessary for plant growth, the secondary metabolites may play much more diverse functions such as in defense mechanisms or interactions with other species [4]. Plant secondary metabolites have been proven as an important source of therapeutic agents. They are categorized based on their biosynthetic origin into various classes of compounds including alkaloids, flavonoids, phenolic, glycoside, terpenes, and saponins. Among these classes of plant secondary metabolites, alkaloids and flavonoids are known to exert various biological properties [5]. Thus, screening for alkaloids and flavonoids forms a logical strategy for selecting and prioritizing plants in the search for useful bioactive compounds.

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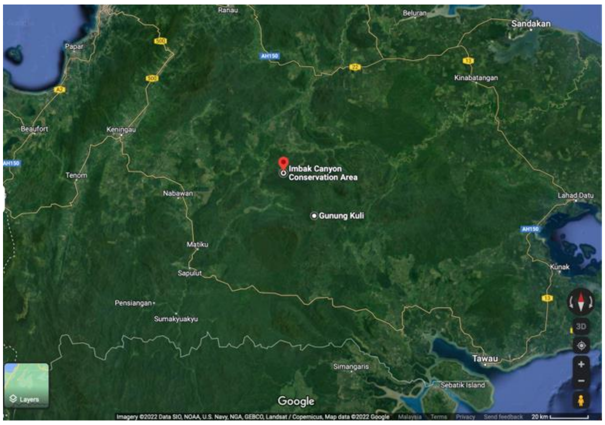


Figure 1. ICCA and Gunung Kuli, Sabah (*Source: Google Map, 2022*)

Alkaloids are nitrogen-containing secondary metabolites. This class of phytochemicals is well-known for their biological potential and these include some available alkaloidal drugs such as morphine, strychnine, quinine, ephedrine, and nicotine. Flavonoids, on the other hand, are important plant metabolites that are known for antioxidant properties. The presence of alkaloids and flavonoids in plants can be detected using simple and reliable chemical tests which can be done *in situ* [6]. For instance, alkaloids will react with Mayer's reagent in neutral or slightly acidic conditions to give a white precipitate of mercuric iodide complex. Whereas the chemical reaction of flavonoids with magnesium ribbon in a concentrated acidic solution will produce red colouration which is due to the formation of benzopyrylium chloride salt.

Many parts of ICCA are largely unexplored, thus offer as a new frontier for exciting nature discoveries. The present paper reports on the outcome of phytochemical screening of alkaloids and flavonoids for 46 plant species collected from five trails of Imbak Canyon Study Centre (ICSC) and three trails of Gunung Kuli Study Centre (GKSC). To the best of our knowledge, this is the first report for the phytochemical tests on ICCA plants of traditional medicinal value.

EXPERIMENTAL

General

All the solvents and reagents used for the phytochemical screening were of analytical grade. The data was presented based on a visual observation made on the thickness of the white precipitate formed for alkaloids and on the intensity of the reddish solution formed for flavonoids. These observations were relatively scaled as '+' for trace, '++' for moderate, and '+++' for high amounts, accordingly.

Sample Collection and Identification

The plant samples were collected between 10 – 17 December 2019 from five trails of ICSC (Herbs Garden, Nepenthes, Belian, Phenology, and Ara) and three trails of GKSC (Kawang, Maya Waterfall, and Kuli Waterfall). The collection was based on the traditional medicinal usage described by the ICCA rangers (Mr. Idris Arsan, Mr. Roziman Mi, Mr. Alasri Asni, and Mr. M. Shahril). Some plants of particular interest to the group were also collected. Each species was tagged and numbered according to its location of the collection. A total of 46 species, some with different parts (57 samples) were collected and deposited at the specimen laboratory of ICCA. The plant species were identified by botanist, Prof Madya Dr Norrizah Jaafar Sidik, assisted by officers of the Sabah Forestry Department (SFD).

Sample Processing

The plant samples were cleaned, cut into small pieces, and crushed using mortar and pestle. Approximately 500 mg of each sample was transferred into test tubes for alkaloids and flavonoids tests.

Phytochemical Qualitative Screening

Test for Alkaloids (Mayer's Reagent):

The ground sample was extracted for 2 minutes with 3 mL of ammoniacal chloroform solution (1:9). Then, the extract was transferred into another test tube where 3 mL of 5% HCl was then added, and shaken. Next, six drops of Mayer's reagent were added and the formation of a white precipitate indicating the presence of alkaloids was recorded.

Test for Flavonoids (Shinoda Test):

The ground sample was extracted for 2 minutes with 3 mL of methanol. The extract was transferred into another test tube. Then, a piece of magnesium ribbon was added, followed by three drops of concentrated HCl. The presence of flavonoids was indicated by the formation of a reddish solution.

RESULTS AND DISCUSSION

During the expedition, 46 plant species were collected and identified from eight trails surrounding the area of ICCA. Figure 2 shows some of the collected samples prior to processing. The information on the species, family, local name, traditional uses, and parts collected are summarized in Table 1, whereby species numbered 1-30 and 31-46 were collected from ICSC and GKSC trails of ICCA, respectively.

The samples which include different plant parts were subjected to qualitative phytochemical screening to detect the presence of alkaloids and flavonoids. As shown in Table 1, the results indicate that out of 57 samples screened, 12 samples were positive for alkaloids only, 13 samples were positive for flavonoids only, and 11 samples were positive for both alkaloids and flavonoids. The remaining 21 samples were negative for both phytochemical classes.

Figure 2. Some of the collected samples prior to processing.

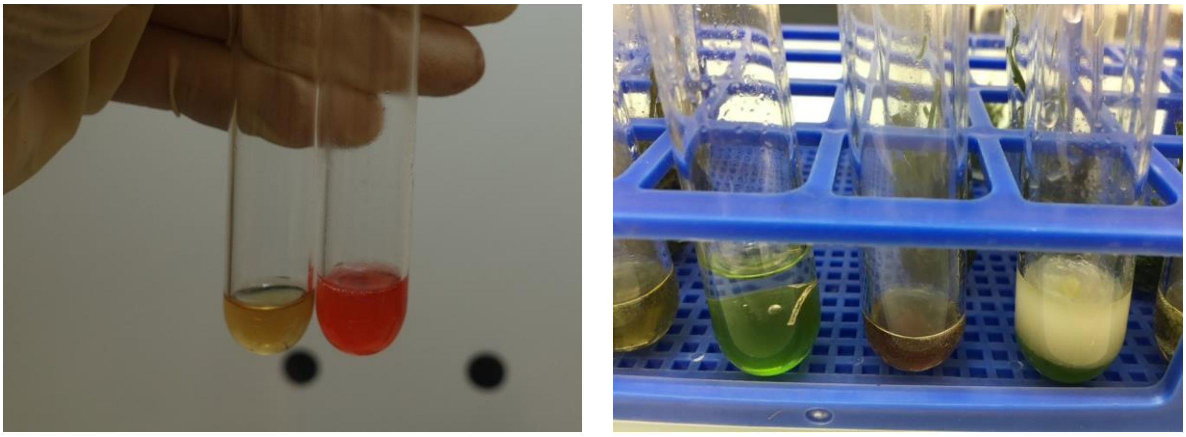
Table 1. Alkaloids and flavonoids phytochemical screening on different plant species parts collected from ICCA.

No.	Plant species	Family	Local Name	Traditional Uses	Plant Parts	Phytochemical Screening	
						Alkaloids	Flavonoids
1.	*Rennellia borneensis*	Rubiaceae	Ginseng Sabah	Backache, energizer	Leaves	-	-
2.	*Dryobalanops keithii*	Dipterocarpaceae	Kapur gumpait	-	Leaves	-	-
3.	*Dryobalanops beccarii*	Dipterocarpaceae	Kapur merah	Wounds, cuts	Leaves	-	-
4.	*Ficus septica*	Moraceae	Lintotobau	-	Leaves	-	-
5.	*Lycopodium cernum*	Lycopodiaceaeceae		Haircare	Leaves	-	+
6.	*Orchid sp.*	Orchidaceae		-	Leaves	-	-
7.	*Asplenium nidus*	Aspleniaceae	Tapako kolindid Langsuir	Blood circulation, get rid toxic from the body	Leaves	-	-
8.	*Eusideroxylon zwageri*	Lauraceae	Belian	-	Leaves	+++	+
9.	*Tetracera scandens*	Dilleniaceae	Mempelas kasar	-	Leaves	-	-
10.	*Dryobalanops lanceolata*	Dipterocarpaceae	Kapur paji	Stimulates heart, spleen and lung. Promote	Leaves	-	-

				wounds and cuts healing.			
11.	*Koompassia excels*	Leguminosae	Mengaris, Tualang	-	Leaves Stems	++ +	- -
12.	*Selaginella ingens* sp	Selaginellaceae	Cakar ayam	Promote wound healing	Leaves	+	-
13.	*Scorodocarpus borneensis*	Olacaceae	Bawang hutan	-	Leaves	+	-
14.	*Synsepalum dulcificum*	Sapotaceae	Buah ajaib	-	Leaves	+++	+
15.	*Phaleria macrocarpa*	Thymelaceae	Mahkota dewa	Lowers blood pressure, and glucose level	Leaves	++	-
16.	*Gmelina elliptica*	Lamiaceae	Buak-buak	-	Leaves Stems	+ +	- +
17.	*Amorphophallus* sp.	Araceae	Keladi ular, Todopon lapong	Reduce pain on bitten parts	Leaves	-	++
18.	*Pycnarrhena cauliflora*	Menispermaceae	Pokok ajinomoto	Flavour and to treat snake bite	Leaves	+++	-
19.	*Curculigo latifolia*	Hypoxidaceae	Lamba	-	Flowers	+++	-
20.	*Pinanga jambusana*	Arecaceae	Pinang bumburing	-	Leaves	+++	+
21.	*Begonia* sp.	Begoniaceae		-	Leaves	+	++
22.	*Cinnamomum* sp.	Lauraceae	Pengolaban madang, Madang sarsi	Headache	Leaves	+++	-
23.	*Ixora capillaris*	Rubiaceae	Jarum jarum, Jenjarum, Ixora hutan	-	Leaves Flowers	+++ -	- +
24.	*Mallotus mollissimus*	Euphorbiaceae	Dahu, Bayor	-	Leaves	+++	-
25.	*Stachytarpheta jamaicensis*	Verbenaceae	Tali-tali	Blood circulation for high blood pressure and gastro-intestinal tract health	Leaves	+	-
26.	*Diplazium esculentum*	Athyriaceae	Paku pakis	-	Leaves	-	-
27.	*Hedychium* sp.	Zingiberaceae	Halia	-	Leaves	-	-
28.	*Oldenlandia corymbosa*	Rubiaceae	Rumput Fatimah	-	Leaves	-	-
29.	*Polygala* sp.	Polygalaceae	Rumput akar minyak pati	-	Leaves	++	+
30.	*Orchid sp.*	Orchidaceae	-	-	Leaves	++	+
31.	*Aeschynanthus* sp.	Gesneriaceae	Hoya, gincu monyet	Malaria	Flowers Leaves	- -	+++ +
32.	*Etlingera* sp.	Zingiberaceae	Tolidus	Haemorrhoid	Stems Leaves Roots	- - -	- - -
33.	*Uncaria calophylla*	Rubiaceae	Talait	Gastric, general body health Rheumatism and the water inside the stem use to reduce body heat.	Leaves Stems	- +	- +++
34.	*Selaginella* sp.	Selaginellaceae	Sali Ganila	Stop bleeding, wound healing	Leaves	-	-

35.	*Orophea hexandra*	Annonaceae	Karai	-	Leaves Stems	- -	+ +++
36.	*Akar Aristolochia*	Aristolochlaceae	Akar rundap, Kagos posod	Navel pain, hypertension, reduce hangover of alcohol effect	Stems Bark Leaves	- -	- +
37.	*Bauhinia diftera*	Fabaceae	Bunga-api	-	Stems Bark	-	+++
38.	*Bauhinia* sp	Fabaceae	-	-	Leaves	+	++
39.	*Uncaria* sp. 4	Rubiaceae	Talait	-	Stems	-	++
40.	*Uncaria sp. 5*	Rubiaceae	Talait	-	Stems	-	-
41.	*Scindapsus* sp.	Araceae	Keladi pemanjat, pagarong	Paste for joint pain	Stems	-	+
42.	*Blechnum orientalis*	Blechnaceae	Pakis gajah	To treat boils	Stems	+	++
43.	*Lasianthus conocarpus*	Rubiaceae	Big bim	-	Stems Leaves	- -	- -
44.	*Uncaria longiflora* var. *longiflora*	Rubiaceae	Talait	-	Stems Leaves	+ -	++ -
45.	*Uncaria longiflora* var. *pteropoda*	Rubiaceae	Talait	-	Leaves	-	+
46.	*Uncaria barbata*	Rubiaceae	Talait	-	Leaves Stems	- -	+ ++

Note (a): Collection location along ICSC trails: Hanging bridge and Nepenthes (1-13), Herbs Garden (14-18), Belian (19-25), Phenology (26-29) and Ara (30), and GKRS trails: Kawang (31-40), Kuli Waterfall (41-45) and Maya Waterfall (46).
Note (b): Qualitative approximation scale: '+' trace, '++' moderate, '+++' high, and '-'negative.



(a) (b)

Figure 3. Phytochemical screening for detection of (a) flavonoids, and (b) alkaloids.

The presence of alkaloids is indicated by the formation of a white precipitate, while the red colouration shows the presence of flavonoids in the plant samples (Figure 3). Qualitative approximation was made based on the thickness of the white precipitate or the intensity of the reddish solution obtained. Scale of '+++' represents high content, '++' for moderate content, and '+' for trace amount. Of the positive samples, the leaves of *Eusideroxylon zwageri*, *Synsepalum dulcificum*, *Pinanga jambusana*, *Pycnarrhena cauliflora*, *Cinnamomum* sp., *Ixora capillaris*, *Mallotus mollissimus* and the flowers of *Curculigo latifolia* showed high content of alkaloids.

E. zwageri (Lauraceae), a timber species locally known as *pokok belian*, is a dominant species in the surrounding area of ICCA justifying the name of the Belian trail. The timber is utilised for making bridges, power line poles, masts, piles, and house posts. No known medicinal usage was however described by the rangers. Interestingly, the leaves of *E. zwageri* (No.8) in the collection were found to be rich in alkaloids (+++) and a trace amount of flavonoids (+). Previously, the stem bark of this species collected from East Kalimantan, Indonesia was reported to contain flavonoids [7].

Another species with high content of alkaloids is the leaves of *P. cauliflora* or locally known as pokok Ajinamoto. The leaves of this plant are used traditionally to treat snake bite, and as flavour enhancer [3]. A literature search on the species showed that besides taxonomic description, there was no report on plant chemistry. It must be noted that *P. cauliflora* is synonym to *Antitaxis cauliflora* Miers., *Pycnarrhena longifolia*, (Decne. ex Miq.), *Antitaxis longifolius* (Decne. ex Miq.) Mier., and *Gabila longifolia* (Decne. ex Miq, B) [8]. Among these synonyms, only *P. longifolia* was reported to contain isoquinoline alkaloids [9, 10]. Other species that are worth highlighting are the leaves of *P. jambusana* (No.20) and *M. mollissimus* (No.24) which also show high content of alkaloids.

Six *Uncaria* species (No.33, 39, 40, 44, 45, and 46) were collected from GKRS trails. The *Uncaria*s are easily recognized from their hooks on the side of the shoots, explaining the local names of *talait* or *kalait*, which mean hooks. The liquid in the bark of *talait* plants is traditionally drunk to reduce body heat by the locals. Interestingly, only stems of *U. calophylla* contain traces of alkaloids (+). On the other hand, flavonoids were detected in five out of the six collected species including the stems of *U. calophylla* (+++) (No.33). Chemotaxonomically, *Uncaria* species are known to contain indole alkaloids [11, 12]. Alkaloid content, even though prevalent in *Uncaria* species, is also known to vary based on geographical and seasonal [13] due to the existence of ecotypes and chemotypes [14]. Several flavonoids have been isolated from the species including rutin, catechin, epicatechin, quercetin, epiafzelechin, kaempferol, taxifolin, and uncariechin [15-17].

A high content of flavonoids was also observed in the flowers of *Aeschynanthus* sp. (No.17), the stems of *O. hexandra* (No.35), and *B. diftera* (No.37). Previously, *O. hexandra* was reported to contain alkaloids but no flavonoids in both stems and leaves of the plant [18]. Whereas there were no previous phytochemical data found on *B. diftera* and the *Aeschynanthus* sp.

Generally, the detected alkaloids and flavonoids in most of the plant species correlate well with their previously reported phytochemical constituent subsequently rationalizing the plant's traditional uses. For instance, *Uncaria* species that are used traditionally to treat rheumatism, a disease related to joints ligaments, bones, tendons, and muscles, are rich in flavonoids constituent. Dietary flavonoids have been reported to control joint inflammation and alleviate arthritis symptoms in both human and animal models [19]. Similarly, the species *Pycnarrhena cauliflora* was found to contain a high amount of alkaloids and was used traditionally to treat snake bites. Alkaloids have been reported to neutralize enzymes from snake venom [20].

CONCLUSION

This work has revealed that combining ethno-medicinal knowledge and phytochemical tests provided useful lead information on several interesting plant species, particularly *E. zwageri* (belian), *S. dulcificum* (buah ajaib), *P. jambusana* (pinang bumburing), *P. cauliflora* (pokok ajinamoto), *Cinnamomum* sp. (madang sarsi), *I. capillaris* (jenjarum), *M. mollissimus* (bayor), *C. latifolia* (lamba), *P. macrocarpa* (mahkota dewa), *Aeschynanthus* sp. (hoya), *U. calophylla* (kalait or talait), *O. hexandra* (karai), and *B. diftera* (bunga api) are worthy of further investigation. This regards that rich biological resources of Sabah virgin forests certainly provide ample opportunity for the discovery of bioactive compounds. This is the first report on the alkaloids and flavonoids compositions of traditional medicinal value plants of ICCA, thus would be handy for researchers to select only highly potential medicinal plants for in-depth chemical and pharmacological studies.

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