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Phytochemicals Composition of Medicinal Plants from Kuala Keniam National Park

Fatimah Salim[1,2*], Anis Low Muhammad Low[1,3] and Nabil Ali Al-Mekhlafi[1,4]
[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]School of Chemistry and Environment, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[3]School of Chemistry and Environment, Faculty of Applied Sciences, Universiti Teknologi MARA, Negeri Sembilan Branch, Kuala Pilah Campus, 72000 Kuala Pilah, Negeri Sembilan, Malaysia
[4]Biochemical Technology Program, Department of Chemistry, Faculty of Applied Science, Thamar University, P. O. Box 87246, Thamar, Yemen
*****Corresponding author (e-mail: fatimah2940@uitm.edu.my)

Combining ethnomedicinal knowledge with phytochemical tests is a strategically sustainable approach to selecting highly potent medicinal plants for the discovery of bioactive metabolites. This paper reports the compositions of five classes of phytochemicals (alkaloids, flavonoids, steroids, phenolics, and saponins) in 36 species of different plant parts (52 samples) collected from Kuala Keniam National Park, Malaysia. The traditional usages of each plant species were also included to rationalize the detected classes of phytochemicals. It was found that most of the collected samples were positive for at least one of the targeted classes of phytochemicals. Out of the tested 52 samples, alkaloids, flavonoids, phenolics, steroids, and saponins were found positive in 7, 11, 30, 23, and 14 total samples, respectively. Interestingly some species parts (ie. the leaves of *Uncaria* sp. and *Cratoxyallum formosus*) gave a very high '++++' composition for a particular class of phytochemicals, and interestingly their reported constituents and biological activities correlate well with the plant's traditional uses. The qualitative phytochemicals compositions and the traditional usages of the collected medicinal plant species reported in this paper provide a useful basis that could guide other researchers in selecting only high-potential plants for future in-depth chemical and pharmacological studies. These findings could also assist in managing medicinal plant resources for better sustainability.

Keywords: Medicinal plant; traditional use; phytochemical screening test; life on land; SDG

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Traditional medicines are considered promising resources for various bioactive plant chemicals or also known as phytochemicals. Plant chemical compounds are classified into 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 interaction with other species. Plant secondary metabolites have been proven as an important source of therapeutic agents [1-4]. They are categorized based on their biosynthetic origin into various classes of compounds including alkaloids, flavonoids, phenolics, glycosides, tannins, steroids, terpenes, and saponins. Thus, screening for these classes of phytochemicals is an important step that forms a logical strategy in selecting and prioritizing plants in the search for useful bioactive compounds.

A chemical reaction can be observed through several conditions which include the formation of a precipitate and/ or gas as well as changes in color, odor, temperature, and/ or taste. A similar concept is applied to the detection of plant chemicals. For instance, the alkaloids class of metabolite consists of lone pair electrons that are capable to form covalent coordinate bonding with a metal atom. In alkaloids detection, Mayer's reagent in neutral or slightly acidic conditions is used to react with the nitrogen atom giving a white precipitate of mercuric iodide complex. Whereas the chemical reaction of flavonoids with magnesium ribbon in a concentrated acidic solution will produce red coloration which is due to the formation of benzopyrylium chloride salt [5]. These observable, simple, and reliable chemical tests which can be done *in situ* were used in a qualitative phytochemical screening for the detection of alkaloids and flavonoids, respectively [6].

Kuala Keniam National Park is the largest national park in Peninsular Malaysia with a total area of 4,343 km^2 covering three states of Malaysia namely Pahang (2,477 km^2), Kelantan (1,043 km^2) and

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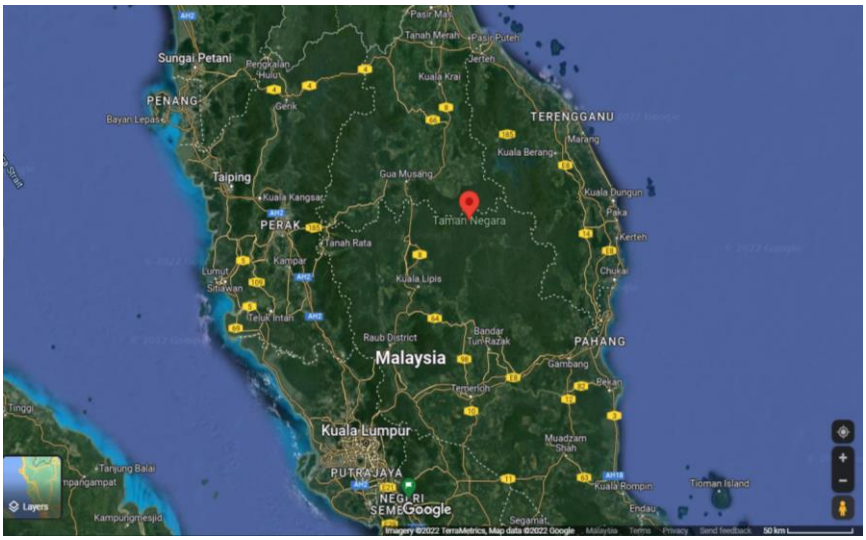

Figure 1. Kuala Keniam National Park, Pahang, Malaysia (Source: *Google Map, 2022*)

Terengganu (853 km^2). The park is located at the mouth of and along the Keniam River of Pahang (Figure 1), and lies between 80 to 2,187 m above sea level where Mount Tahan, the highest mountain in Peninsular Malaysia is the highest peak [7]. Interestingly, although the park is managed by the Department of Wildlife and National Parks, Peninsular Malaysia (PERHILITAN), its facilities and services are managed by local communities as well as local and private sectors including Universiti Teknologi MARA (UiTM) who has established a field research station in 2007 focusing on scientific research, conservation, and management of the biological resources [8]. This park is considered a primary forest and it is estimated that at least 280 tree species are available per hectare by extrapolation [8].

Within Kuala Keniam's unexploited bio-resource, there could be many plants of medicinal value used traditionally by the locals and ethnic communities as remedies for various ailments. This paper reports the qualitative phytochemical components of 36 medicinal plant species collected from Kuala Keniam National Park. The medicinal plants' traditional uses were also included to correlate with their previously reported phytochemical constituents and biological activities subsequently rationalizing their detected classes of phytochemicals.

EXPERIMENTAL

Chemicals and Materials

General

Solvents and reagents used for the phytochemical tests were of analytical grade. The data was presented based on the visual observation made including changes in appearance, and color intensity of the solution. These observations were relatively scaled as '+' for trace, '++' for moderate, '+++' for high, and '++++' for very high amounts, accordingly.

Sample Collection and Identification

The plant samples were collected randomly from Kuala Keniam National Park during the three days expedition (5-7 September 2020). Some plants of particular interest to the group were also collected. Each species was tagged and numbered accordingly. A total of 36 species, most with different parts (52 samples) were collected. The plant species were identified by botanists from the Forest Research Institute of Malaysia (FRIM), Siti Munirah Mat Yunoh and Imin Kamin. The information on the traditional uses of the species was obtained from scientific journals, books, and reports searched through available platforms and databases such as GLOBinMED, Tropical Plants, PROSEA, MyBIS, Google Scholar, PubMed, Directory of Open Access Journals, and Bioline International.

Sample Processing

The different fresh plant parts (leaves, stems, and flowers) were cleaned, chopped, crushed, and ground using a mortar and pestle. Approximately 500 mg of each sample was transferred into different test tubes for alkaloids, flavonoids, steroids, phenolic, and saponins tests.

Qualitative Phytochemical Tests

Test for Alkaloids (Mayer's Reagent): The ground sample was extracted for 2 minutes with 3 mL of ammoniacal chloroform solution (1:9) in a test tube. Then, the extract was transferred into another test tube where 2 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 in a test tube. 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.

Test for Steroids (Salkowski Test): The ground sample was extracted for about 2 minutes with 3 mL of chloroform in a test tube. The extract was then transferred into another test tube. Two mL of concentrated H_2SO_4 was carefully added from the side of the test tube and the formation of a red-brown color ring at the junction of two phases that turns shortly into red indicates the presence of steroids.

Test for Phenolics (Ferric Chloride Test): The ground sample was extracted with 3 mL of an equal mixture of water and methanol (5:5) solution and shaken in a test tube. The extract was transferred into another test tube. Then, five drops of 2 % ferric chloride aqueous solution were added and the formation of an intense greenish-black color indicates the presence of phenolic compounds.

Test for Saponins (Froth Test): The ground sample was extracted with 3 mL of water. The extract was transferred into another test tube. Then, 10 drops of olive oil were added and the mixtures were vigorously shaken. The formation of persistent foam indicates the presence of saponins.

RESULTS AND DISCUSSION

Plant chemicals exhibit diverse array of structures and characteristics that are classified into different skeletal types or classes of compounds including alkaloids, flavonoids, phenolics, steroids, saponins, tannins, lignin and others. This classification allowing the natural plant-derived chemicals to be extracted, concentrated and consequently detected from the plant sample. The confinement to detect important classes of plant's chemicals is through a specific phytochemical test. In this study, five classes of plant chemicals which are alkaloids, flavonoids, phenolics, steroids, and saponins were tested in a total of 52 samples of different plant parts from 36 species collected in the two-day expedition. These classes of plant chemicals were chosen to be tested in the collected samples due to their potential to exert various biological activities. The collected species were taxonomically diverse belonging to 33 genera and 21 families (Table 2). Some of the collected samples are shown in Figure 2.

The phytochemicals composition data shown in Table 1 revealed that most of the collected samples gave positive results for the presence of at least one of the tested class of phytochemicals. In fact, alkaloids, flavonoids, phenolics, steroids, and saponins were found to be positive in 7, 11, 30, 23, and 14 total tested samples, respectively. Interestingly, some of the plant parts gave high '+++', and very high '++++' positive results for a particular class of phytochemicals. For instance, a very high content of flavonoids, and phenolics were detected in the leaves of *Uncaria* sp, (**1**) and *Cratoxyallum formosus* (**14**), respectively.

Uncaria species are known for their mono-terpenoid indole alkaloids, and triterpenes constituents [9]. Although chemotaxonomically these classes of phytochemicals are prevalent in *Uncaria* species, a number of flavonoids have also been isolated. They include uncariechin, epiafzelechin, epicatechin, rutin, catechin, quercetin, kaempferol, and taxifolin [10-12]. These flavonoids were reported likewise exhibit important biological activities comparable to those of the alkaloids such as antioxidant, neuroprotective, and antidiabetic effects [9,10,12]. The phytochemicals in

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

Uncaria species are also known to vary based on geographical and seasonal collections due to the existence of ecotypes and chemotypes thus further justifying the present findings [13-14]. The species *Cratoxyallum formosus* has also been reported to be rich in phenolic compounds such as flavonoids, xanthone, and anthranoids [15-16]. These phenolic compounds showed promising antimicrobial and strong anti-neuroinflammatory effects [15-16]. These show that phytochemical composition and the reported biological activities of these species could provide evidence to support their traditional uses. To verify this hypothesis, the information on the traditional uses of the tested plants' species was searched through trusted available online platforms and databases such as GLOBinMED, Tropical Plants, PROSEA, MyBIS, Google Scholar, PubMed, Directory of Open Access Journals, and Bioline International. As shown in Table 2, almost all of the collected species have been utilized traditionally to treat various ailments. It was found that the detected classes of phytochemicals in most of the plant species correlate well with their previously reported constituent and biological activities subsequently rationalizing the plant's traditional uses.

For instance, the *Uncaria* species (**1**) that gave a very high amount of flavonoid composition is used traditionally to treat rheumatism, a disease affecting the joints, ligaments, bones, tendons, and muscles. Dietary flavonoids have been reported to control joint inflammation and alleviate arthritis symptoms in both human and animal models [17]. Similarly, *Cratoxyallum formosus* (**14**) which contains a very high amount of phenolic is used traditionally to treat skin problems and colic. Phenolic compounds have been found to exert multifunctional roles in human health particularly to exert an anti-inflammatory effect on metabolic disorders such as eczema [18]. However, further chemical and biological exploration studies need to be carried out on the species to confirm this correlation.

There are also several other species that gave high '+++' positive results for the therapeutic class of phytochemicals tested. These include the leaves of *Bauhinea tomentosa*, *Xanthophyllum flavescens*, *Staurogyne merguensis*, *Codonoboea malayana*, *Smythea lanceata*, *Caesalpinia crista*, *Cocculus petiolaris*, *Bouea macrophylla*, *Schefflera oxyphylla*, *Zanthoxylum alatum* and *Sonneratia griffithii*, the stems of *Cratoxyallum formosus*, *Bouea macrophylla*, *Mollotus dispar* and *Chroesthes longifolia*, the flowers of *Schefflera oxyphylla*, the leaves and stems of *Ampolecissus cinnamomea*. The qualitative phytochemicals composition data here would provide useful information and could guide other researchers in the selection of potential medicinal plants worth for further investigation. Indeed, the rich biological resources of Kuala Keniam primary forests certainly provides a tremendous opportunity for the discovery of bioactive compounds.

Table 1. Phytochemical composition of different plant parts species collected from Kuala Keniam National Park

No.	Plant species	Plant Parts	Phytochemicals				
			Alkaloids	Flavonoids	Phenolics	Steroids	Saponins
1.	Uncaria sp.	Leaves	-	++++	-	-	-
		Stems	-	+++	-	-	-
2.	*Bauhinea*	Leaves	-	-	+++	-	-
	tomentosa	Stems	-	-	-	-	-
3.	*Ardisia elliptica*	Leaves	-	-	++	-	-
4.	*Xanthophyllum*	Leaves	-	-	+	-	+++
	flavescens	Stems	-	-	-	-	++
5.	*Gnetum*	Flower	+	-	+	+	+
	latifolium	Stems	-	-	-	-	-
6.	Uncaria sp.	Leaves	++	+	-	-	-
		Stems	++	-	++	-	-
7.	*Cissus hastata*	Leaves	-	-	++	+	-
8.	*Ampolecissus*	Leaves	-	++	+++	++	-
	cinnamomea	Stems	-	+	+++	+	-
9.	*Staurogyne*	Leaves	-	+	+++	+	+
	merguensis	Flower	-	++	-	-	-
10.	Uncaria sp.	Leaves	-	-	+++	+	+++
		Stems	+	-	+++	-	-
11.	*Trevesia burckii*	Leaves	-	-	+	++	++
		Stems	-	-	-	-	-
12.	*Codonoboea*	Leaves	-	-	-	+	+++
	malayana						

No.	Plant species	Plant Parts	Phytochemicals				
			Alkaloids	Flavonoids	Phenolics	Steroids	Saponins
13.	*Carallia suffruticosa*	Leaves	-	-	++	-	-
14.	*Cratoxyallum formosus*	Leaves	-	-	++++	-	-
		Stems	-	+++	-	-	-
15.	*Cytandra pilosa*	Leaves	-	-	++	+	-
		Stems	-	-	++	+	-
16.	*Smythea lanceata*	Leaves	-	-	+++	-	-
17.	*Xylopia fusca*	Leaves	-	-	++	+	-
18.	*Caesalpinia crista*	Leaves	-	-	+++	-	-
19.	*Cocculus petiolaris*	Leaves	-	-	+++	+	-
		Stems	-	-	++	-	-
20.	*Stemona curtisii*	Leaves	-	-	-	-	-
21.	*Bouea macrophylla*	Leaves	-	-	+++	-	+++
		Stems	-	+	+++	-	+++
22.	*Pavetta napieri*	Leaves	-	-	+	+	-
23.	Uncaria sp.	Leaves	-	-	+	-	-
24.	*Chassalia chartacea*	Leaves	-	-	+	+	-
25.	*Aglaonema simplex*	Leaves	-	-			
26.	*Clerodendrum deflexum*	Leaves	-	-	-	+	++
27.	*Schefflera oxyphylla*	Leaves	-	-	-	+++	+++
		Flower	-	-	-	-	+++
28.	*Spelaeanthus chinii*	Leaves	+	-	-	+	-
29.	*Morinda citrifolia*	Leaves	-	-	-	+	-
30.	*Diospyros diepenhorstii*	Stems	-	-	+	-	-
31.	*Mollotus dispar*	Leaves	-	-	-	-	-
		Stems	+	+++			
32.	*Chroesthes longifolia*	Leaves	-	-	-	+	-
		Stems	-	+	-	-	+++
33.	*Andrographis paniculata*	Leaves	-	-	-	+	-
34.	*Zanthoxylum alatum*	Leaves	+++	-	+	+	-
35.	*Sonneratia griffithii*	Leaves	-	-	+++	+	-
36.	*Peliosanthes teta*	Leaves	-	-	++	++	+

Note: Qualitative approximation scale: '+' trace, '++' moderate, '+++' high, '++++' very high, and '-' negative

Table 2. Traditional uses, family and local names of plant species collected from Kuala Keniam National Park

No.	Plant species	Family	Local Name	Traditional Uses	Ref.
1.	*Uncaria sp.*	Rubiaceae	Kekait	Rheumatism and framboesia	[19]
2.	*Bauhinea tomentosa*	Fabaceae	Tapak kuda	General pain, inflammation and infections	[20]
3.	*Ardisia elliptica*	Myrsinaceae	Mata pelanduk	To assuage retrosternal pains	[21]
4.	*Xanthophyllum flavescens*	Polygalaceae	Gading jantan	Treat colic	[22]
5.	*Gnetum latifolium*	Gnetaceae	Akar melinjau	Seeds are eaten roasted or cooked, and young leaves are cooked as vegetable	[23]
6.	*Uncaria sp.*	Rubiaceae	Kekait	Rheumatism and framboesia	[19]

No.	Plant species	Family	Local Name	Traditional Uses	Ref.
7.	*Cissus hastata*	Vitaceae	Akar kerayong, akar asam riang, akar iang-iang	Leaves are used to poultice boils, and put on the abdomen for ague	[24]
8.	*Ampolecissus cinnamomea*	Vitaceae	Akar lipang	Treat cholera, wound healing, and dye cotton for brownish colour	[25]
9.	*Staurogyne merguensis*	Acanthaceae	Lemba hutan	Entire plant is used treating post-partum depression	[26]
10.	*Uncaria sp.*	Rubiaceae	Kekait	Rheumatism and framboesia	[19]
11.	*Trevesia burckii*	Araliaceae	Tapak hantu	Relief small sores, skin complaints, fractured bones, and, rub to treat rheumatism, ague and fever	[27]
12.	*Codonoboea malayana*	Gesneriaceae	Malayan Cowslip	-	[28]
13.	*Carallia suffruticosa*	Rhizophoraceae	Sisek puyu, meransi, redip pepuyoh	Used against worms and cough, after childbirth, and a bath to treat fever	[29]
14.	*Cratoxyallum formosus*	Hypericaceae	Gerunggung, pink mempat, kemutul, mempitis	Skin complaints, and colic	[30]
15.	*Cytandra pilosa*	Gesneriaceae	-	-	[28]
16.	*Smythea lanceata*	Rhamnaceae	Bulang akar	Branches used for tying fish traps and in boat-building	[31]
17.	*Xylopia fusca*	Annonaceae	Mempisang, jangkang paya	Wood is used in making pineapple box and bark is used for walls of huts	[32]
18.	*Caesalpinia crista*	Fabaceae	Kuku tupai	For colic and to treat sharp gouty pains in hands and feet	[31]
19.	*Cocculus petiolaris*	Menispermaceae	-	-	-
20.	*Stemona curtisii*	Stemonaceae	Ubi kemili utan, galak tua	Aphrodisiac, treatment for skin diseases	[28]
21.	*Bouea macrophylla*		Kundang, gandaria	Young fruits and leaves are eaten raw as ulam. Ripe fruits consumed fresh	[30]
22.	*Pavetta napieri*	Rubiaceae	-	-	[28]
23.	*Uncaria sp.*	Rubiaceae	Kekait	Rheumatism and framboesia	[19]
24.	*Chassalia chartacea*	Rubiaceae	Pengugur, mata ketam, beras-beras, kembang bulan	Roots for menstrual cramps	[33]
25.	*Aglaonema simplex*	Araceae	Sumpuh bulan, sumpuh kering, penggeheh	Speed up labour delivery and pain relief.	[30]
26.	*Clerodendrum deflexum*	Verbenaceae	Setawor bukit	Roots use for fever and stomach complaints	[34]
27.	*Schefflera oxyphylla*	Araliaceae	Kukau	Roots decoction as a sedative to calm frightened children and used externally against fever	[23]
28.	*Spelaeanthus chinii*	Gesneriaceae	-	-	[28]
29.	*Morinda citrifolia*	Rubiaceae	Mengkudu	To treat cough, enlarged spleen, in nausea, colic and fever	[22]
30.	*Diospyros diepenhorstii*	Ebenaceae	Kayu arang	Ripe and unripe fruits are eaten	[30]

No.	Plant species	Family	Local Name	Traditional Uses	Ref.
31.	*Mollotus dispar*	Euphorbiaceae	Balik angin	-	[28]
32.	*Chroesthes longifolia*	Acanthaceae	Peluruh darah/ gugur anak	The roots decoction as an abortifacient	[26]
33.	*Andrographis paniculata*	Acanthaceae	Hempedu bumi, bidara, akar cerita	To treat snake-bites and stings of insects	[35]
34.	*Zanthoxylum alatum*	Rutaceae	Minyak beruk	Tooth complaints and relief fever	[36]
35.	*Sonneratia griffithii*	Lythraceae	Perapat bumi, pedada	Fruits are eaten	[30]
36.	*Peliosanthes teta*	Asparagaceae	Derhaka mertua, serenget, tadah embun, akar suntong	-	[28]

CONCLUSION

The phytochemicals composition and the traditional uses reported for 36 species of medicinal plants from Kuala Keniam National Park in this paper would provide guidance for better prospects of searching for novel therapeutic agents as well as managing medicinal plant resources for better sustainability. The present work also revealed several interesting medicinal plant species including *Uncaria* sp., *C. formosus*, *C. malayana*, *C. crista*, *C. petiolaris*, *C. longifolia*, *B. tomentosa*, *B. macrophylla*, *X. flavescens*, *S. merguensis*, *S. lanceata*, *S. oxyphylla*, *S. griffithii*, *Z. alatum*, *M. dispar*, and *A. cinnamomea* are worthy of further chemical and biological investigations. To the best of our knowledge, this is the first *in-situ* report on the five classes of phytochemical composition of Kuala Keniam National Park plants with their traditional medicinal values.

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