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Nutritional Composition and Trace Elements Contents of Unfermented and Fermented Clinacanthus nutans L. Herbal Tea

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Abstract

Clinacanthus nutans L. (locally known as 'Sabah Snake Grass') has been used traditionally to treat chronic diseases. However, there is insufficient information regarding the nutritional quality of the herbal. This study was conducted to evaluate the nutritional properties and trace elements contents of unfermented and fermented herbal teas developed from C. nutans leaves using different drying techniques (microwave-oven dried and freeze dried) in different infusion time (1, 2, 5, 10, 15 and 20 min). The proximate analysis were conducted according to AOAC's standard methods, while, the colorimetric color of infusions were determined using HunterLab Color Meter. The trace elements in infusions were determined using Inductively Coupled Plasma-Optical Emission Spectrophotometric (ICP-OES) analysis. Among the C. nutans herbal teas, the freeze dried of unfermented herbal tea showed high ash (12.39 ± 0.39%) and fat (2.23 ± 0.10%) content, while, protein content was showed high in freeze dried of fermented herbal tea (23.15 ± 0.51%). Carbohydrate content was showed high in microwave-oven dried of unfermented herbal tea (63.40 ± 0.53%) with 3026.24 ± 28.23 kcal/kg of Metabolizable Energy (ME). For color infusion determination, C. nutans herbal teas displayed no significant difference ($P > 0.05$) in color darkness as compared to commercial teas (L: 3.63 to 5.77). There were no significant differences between unfermented and fermented C. nutans herbal tea for its greenish (a: -2.69 to -1.20) and yellowish (b: 3.45 to 5.59) color infusion. Sixteen elements (Al, Ba, Be, Ca, Fe, In, K, Li, Mg, Mn, Na, Zn, Se, Sr, Tl and Si) were detected in the C. nutans and commercial dried leaves. Potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) were higher in C. nutans herbal infusions as compared to commercial teas infusions. Consumption of these herbal teas as dietary intake is able to overcome nutrients and minerals deficiency.

1. Introduction

Tea developed from true tea plant, Camellia sinensis Linn. is one of the most popular beverage in the world [1].This plant is native to Southeast Asia of India and China and cultivated in at least 30 countries around the world including Indonesia, Sri Lanka, Japan and Africa [2,3]. Tea can be found in many different forms depending on the method of preparation and level of fermentation such as green (unfermented), oolong (semi-fermented) and black (fermented) tea. It is well known for its ability to promote human health that range from giving claiming effect on the mind to the extent of reducing the risk of chronic diseases such as cardiovascular disorder and cancer [4,5]. At present, other herbal infusions or herbal teas are also widely consumed for its medicinal properties. These plants are not only rich

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with phytochemicals such as phenolics, alkaloids and tannins but also proteins, enzyme, vitamins, minerals and trace elements [6].

Herbal infusions could be a good dietary source of essential trace metals for humans. However, trace element contents may have both beneficial and adverse effects on human health [7]. Mineral deficiency is a reduced level of any of the minerals essential to human health which may impair a function dependent on that mineral. Minerals are required for fluid balance, blood and bone development, maintaining a healthy nervous system, and regulating muscles, including heart muscles. However, mineral toxicity can be caused as concentration any of minerals were higher than necessary level for human body [8]. Many studies were carried out to determined level of minerals present in herbal teas [6, 9, 10]. Minerals are usually consumed in food from plants and plant-eating animals. These sources of minerals are developed by the assimilation of minerals salts into soil that nourishes edible plants. Elements such as nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, chlorine, iron, manganese, zinc, copper, molybdenum, and nickel are essential elements for plant growth. These elements are accumulated in plants depending on many factors including leaves maturity, soil, rainfall and altitude [6]. Moreover, factors affecting the minerals contents in herbs leaves may influence its concentration in the infusion. Preparation method (drying technique, time infusion and temperature) also has a great influence to maintain the mineral content in herbs leaves and their infusion [11]. Minerals such as cobalt, copper, chromium, fluorine, iron, iodine, manganese, molybdenum, selenium and zinc are considered essential elements for human body [12]. Besides, plants can be a cheaper source of energy as being the rich sources of carbohydrates, fats and proteins, which form the major portion of the human diet. The moisture, fiber and ash contents of individual plant species also have been regarded important to the human health. Energy is derived from the carbohydrate, protein, fat and alcohol found in foods and beverages [13,14,15]. Although vitamins and minerals are essential to the body and assist many human body processes, they provide no energy [16].

Clinacanthus nutans L. is locally known in Malaysia as 'Belalai gajah' or 'Sabah Snake Grass' that belongs to the plant family Acanthaceae. In Malaysia, this plant is consumed as herbal tea for its potential to cure cancer [17]. This plant is usually used in traditional herbal medicine for treating skin rashes, insects and snake bites, dysentery, lesion caused by herpes simplex virus, diabetes mellitus, fever and diuretics especially in Indonesia and Thailand [18,19]. It is claimed to consist many of essential minerals like phosphorus, calcium, magnesium, iron, zinc, copper, nickel, manganese, vitamins and 17 amino acids, however, the level of these minerals in herbal infusions were unknown. In this study, nutritional composition and infusion color of Clinacanthus nutans dried leaves and their infusion were determined depending on the level of fermentation, drying techniques and infusion time. The concentration of sixteen elements (Al, Ba, Be, Ca, Fe, In, K, Li, Mg, Mn, Na, Zn, Se, Sr, Tl and Si) were also determined. Moreover, possible impacts of these herbal teas drinking on the intake of these elements were evaluated for consumer benefits.

2. Materials and Methods

2.1. Plant materials

Clinacanthus nutans tree plants were collected from Kundasang area in Ranau which located at East of Peninsular Malaysia. Identification and authentication of *C. nutans* plant were performed by a botanist, Mr. Johnny Gisil, from Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. A voucher specimen was subjected to plant herbarium, BORNEENSIS (BorhLusia2). About 1.0 kg of fresh intermediate leaves (from 2th axis to 8th axis) of *C. nutans* was collected and rinsed with distilled water to remove soil and other foreign particles.

2.2. Preparation of unfermented leaves

Preparation of unfermented *C. nutans* herbal tea was based on the preparation of green tea of *C. sinensis* with modification [20,21]. Half of collected leaves (0.5 kg) were used to prepare the unfermented herbal tea of *C. nutans*. The leaves were first undergoes a pretreatment to inactivate degradative enzyme by steam blanching (98 ± 2 °C) for 30 sec then immediately soaked in ice water bath for another 30 sec to prevent the leaves from overcooked. The

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leaves were ground using a blender for 5 sec to obtain relatively small particles size of leaves.

2.3. Preparation of fermented leaves

Preparation of unfermented *C. nutans* herbal tea was based on the preparation of black tea of *C. sinensis* with modification [22]. Collected *C. nutans* leaves (0.5 kg) were left to open air for 18 hours for a process known as withering to obtain 70% of leaves moisture content before grounded into small particle size for 5 sec using a blender. Blended leaves were sprayed with distilled water in 1:1 (w/v) ratio before undergone oxidation-fermentation process for 5 hours under 25 ± 1 °C.

2.4. Test 1: Effect of drying technique

Both unfermented and fermented leaves of *C. nutans* were further divided into two parts and dried using microwave oven drying and freeze drying techniques, respectively. The leaves were dried at 600 W for 5 min using a microwave-oven (Samsung Microwave Oven MW71E). Meanwhile, for the freeze drying technique, the fermented leaves were first frozen under -80 °C for 48 hours before subjected to freeze drier (Labconco Freezone 12 Liter Freeze Dry System) for 48 hours.

2.5. Test 2: Effect of infusion time

The herbal tea was prepared using hot boiling water extraction procedure in order to mimics household brewing conditions. Each 2.0 g of dried unfermented and fermented leaves of *C. nutans* were infused in 200 ml boiled distilled water (100 °C) and continuously stirred for 2 min using a magnetic stirrer under 300 rpm. The infusion left to cool to specific infusion time (1, 2, 5, 10, 15 and 20 min) before filtered using a filter paper (Wathman NO.4). Two commercial teas of *C. sinensis* ("BOH Green Tea" and "SABAH Black Tea") were used as comparison to *C. nutans* herbal teas. These teas were locally purchased and infusions were prepared with the same infusion times as *C. nutans* herbal teas.

2.6. Proximate Test

Proximate composition of unfermented and fermented *C. nutans* herbal teas were determined according to AOAC (2000) standard methods for total ash, crude fat, crude protein and crude fiber. Moisture content was determined using moisture analyzer (Mettler Toledo, HB43-s). Total carbohydrate content was calculated by differences of the sum the constituent in the dried leaves (protein, fat, moisture and fiber). Metabolizable energy was calculated after percentage of fat, protein and carbohydrate multiplied by their respective Atwater factors are summed and multiplied by 10.

Nitrogen free extract (NFE) /Carbohydrate = 100% - (% moisture + % crude protein + % crude fat + % crude fiber + % ash)

Metabolizable energy = 10[(3.5 x % crude protein) + (8.5 x % crude fat) + (3.5 x %

2.7. Infusion color range determination

Infusion colors of the unfermented and fermented *C. nutans* herbal teas were determined using a colorimeter (HunterLab Color Meter) with three-dimensional scales color (L, a, and b color space). The Hunter L, a, b color space is organized in a cube form. This scale color as follows:

L (Lightness) axis : black to white (0 to100)
a (red - green) axis : positive values are red; negative values are green; 0 is neutral
b (yellow- blue) axis : positive values are yellow; negative values are blue; 0 is neutral

2.8. Trace elements determination

The dried leaves of unfermented and fermented *C. nutans* herbal tea (1.0 g) was accurately weighed into a pre-cleaned beaker and added with 10 ml of concentrated nitric acid. The beaker covered with a watch glass and the sample boiled gently on a laboratory hot-plate until the digestion was completed. The acid digested solution was transferred to 50 ml volumetric flask and diluted to volume by addition of ultrapure water. For herbal tea infusion, each 5 ml of tea infusion were added with 0.1 ml of concentrated nitric acid before diluted to 250 ml with addition of ultrapure water. All the solutions were filtered

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through 0.45 µm nylon membrane before analyzed using Inductively Coupled Plasma-Optical Emission Spectrophotometric (ICP-OES) analyzer. The ICP-OES instrument (Optima 5300 DV ICP-OES, Perkin Elmer Instruments, USA) equipped with WinLab 32 software system was used. The operational conditions used were RF power 1300 W, plasma gas flow rate 15.00 L/min, auxiliary gas flow rate 0.20 L/min, nebulizer gas flow rate 0.8 L/min) and sample flow rate 1.50 L/min [23]. Sixteen elements were used as elements of calibration curves and analytical wavelength of each element were showed in Table 1.

Table 1 Wavelength of ICP-OES standard elements

Element	Wavelength (nm)
Al	396.153
Ba	233.527
Be	313.107
Ca	317.933
Cu	327.393
Fe	238.204
In	230.606
K	766.490
Li	670.784
Mg	285.213
Mn	257.610
Na	589.592
Ni	231.604
Se	196.026
Zn	206.200
Si	251.611

2.9. Data analysis

All data were analyzed using SPSS statistical 21 and expressed as means ± standard deviation (S.D.) of five replicate analyses in five independent experiments. One-way analysis of variance (ANOVA) followed by Tukey test was carried out to determine the significance between means. $P \leq 0.05$ level was set as statistical significant level.

3. Result and Discussion

3.1. Nutritional test

The nutritional value of unfermented and fermented *C. nutans* herbal tea was shown in Table 2. In unfermented *C. nutans* herbal tea, the crude protein, fat and fiber are showed higher in freeze dried leaves as compared to microwave-oven dried leaves. Same pattern also shown in fermented dried leaves. During freeze drying, freezing process at -80 °C could lead to the instant development of ice crystal within the leaves tissues matrix and resulting in better protection on protein molecules, fats and leaves fiber during drying process [24]. However, there were no significant differences ($P > 0.05$) between the unfermented and fermented of *C. nutans* dried leaves. Among the *C. nutans* herbal tea, carbohydrate content was showed higher in unfermented of microwave-oven dried with 63.40 ± 0.53 % dry weight. Both commercial teas showed a higher and significantly different ($P < 0.05$) of carbohydrate content compared to *C. nutans* herbal teas. In Fig. 1, the metabolizable energy of *C. nutans* herbal teas are lower but comparable (2988.46 to 3071.93 kcal/kg) to the commercial teas (3230.34 to 3258.71 kcal/kg). This indicated the ability of *C. nutans* herbal teas to produce similar net energy available after utilization of some energy for digestion and absorption in human body system as compared to commercial teas.

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Table 2: Nutritional value of dried leaves; (a) ash (b) crude fat (c) moisture (d) crude fiber and (e) total carbohydrate

Sample	Type	Drying Tech.	Percentage in dry weight (% DW)					
			Ash	**Crude protein**	**Crude fat**	**Moisture**	**Crude fiber**	**Total Carbo-hydrate**
C. nutans	Unfermented	MD	10.85 ± 0.51[a]	19.26 ± 0.70[a]	1.57 ± 0.23[a]	4.88 ± 0.04[ac]	17.34 ± 0.29[ad]	63.40 ± 0.53[a]
		FD	12.39 ± 0.39[bc]	22.43 ± 0.53[b]	2.23 ± 0.10[b]	3.66 ± 0.36[b]	16.40 ± 0.24[bd]	59.34 ± 0.19[b]
	Fermented	MD	11.75 ± 0.62[ac]	22.37 ± 0.13[b]	1.33 ± 0.11[a]	4.79 ± 0.13[a]	15.72 ± 0.16[b]	59.78 ± 1.00[b]
		FD	11.46 ± 0.22[a]	23.15 ± 0.51[b]	1.96 ± 0.07[c]	3.53 ± 0.04[b]	14.21 ± 0.21[c]	59.86 ± 0.68[b]
BOH Green Tea			4.85 ± 0.12[c]	20.43 ± 0.07[a]	1.47 ± 0.09[a]	4.93 ± 0.09[c]	14.46 ± 0.09[c]	68.29 ± 0.20[c]
SABAH Black Tea			5.58 ± 0.43[d]	17.31 ± 0.09[d]	1.86 ± 0.14[ac]	3.87 ± 0.06[b]	18.33 ± 0.26[a]	71.28 ± 0.62[d]

Mean ± S.D. (n = 5), Same superscripted letter within vertical columns are not significantly different (P > 0.05).

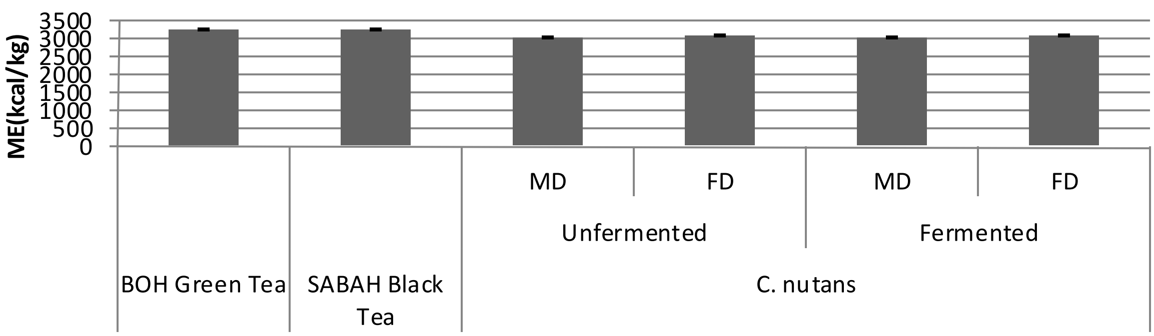

Figure 1: Metabolizable energy (ME) of unfermented and fermented *C. nutans* herbal teas

3.2. Infusion color range determination

Infusion color is one of the sensory quality parameters for herbal teas and Table 3 showed the infusion color values of *C. nutans* herbal teas and commercial teas. For color infusion determination, *C. nutans* herbal teas displayed no significant difference (P >0.05) in color darkness as compared to commercial teas (L: 3.63 to 5.77). There were no significant differences between unfermented and fermented *C. nutans* herbal tea for its greenish (a: -2.69 to -1.20) and yellowish (b: 3.45 to 5.59) color infusion. Between both drying techniques, the freeze dried *C. nutans* leaves give less darker but more green-yellowish color of infusions as compared to microwave-oven dried infusions. However, there were no significant differences (P >0.05) and clear pattern of color infusions as increasing of time infusion in different drying techniques of unfermented and fermented *C. nutans* herbal infusions. Previous study by Vanderhaegen *et. al*. [25] stated the formation of color and flavor compounds during fermentation of *C. nutans* leaves also might reduce the polyphenol concentration. Polyphenols are proved to contribute for antioxidant action and anticancer mechanism. Some metals, especially copper, play an important role as transitions metal for these processes [26].

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Table 3 Herbal infusion color of three-dimensional scales

Sample	Type	Drying tech.	Infusion time	L	a	b
C. nutans	Unfermented	MD	1	4.91 ± 0.05[ac]	-0.91 ± 0.04[a]	4.08 ± 0.07[a]
			2	4.24 ± 0.07[b]	-2.18 ± 0.03[b]	4.15 ± 0.05[a]
			5	4.28 ± 0.03[b]	-2.11 ± 0.10[b]	4.16 ± 0.05[a]
			10	4.29 ± 0.02[b]	-2.13 ± 0.05[b]	4.61 ± 0.02[ab]
			15	4.49 ± 0.02[bc]	-2.18 ± 0.04[b]	4.30 ± 0.02[a]
			20	4.57 ± 0.04[bc]	-2.29 ± 0.03[b]	4.79 ± 0.03[b]
		FD	1	5.08 ± 0.11[ac]	-2.42 ± 0.04[a]	5.43 ± 0.05[a]
			2	4.72 ± 0.06[a]	-2.17 ± 0.04[a]	4.71 ± 0.08[b]
			5	5.03 ± 0.09[ac]	-2.69 ± 0.06[a]	5.34 ± 0.32[a]
			10	5.77 ± 0.29[bc]	-2.56 ± 0.08[a]	6.23 ± 0.47[c]
			15	4.85 ± 0.10[a]	-2.27 ± 0.04[a]	5.45 ± 0.09[a]
			20	4.91 ± 0.02[a]	-2.61 ± 0.03[a]	5.59 ± 0.02[a]
	Fermented	MD	1	4.16 ± 0.06[a]	-1.20 ± 0.05[a]	3.45 ± 0.03[a]
			2	4.73 ± 0.05[ac]	-2.15 ± 0.23[b]	4.78 ± 0.09[b]
			5	4.54 ± 0.07[a]	-1.43 ± 0.04[a]	4.29 ± 0.02[b]
			10	4.31 ± 0.04[a]	-1.34 ± 0.03[a]	4.43 ± 0.06[b]
			15	5.09 ± 0.10[bc]	-1.38 ± 0.10[a]	4.97 ± 0.11[b]
			20	4.69 ± 0.07[a]	-1.52 ± 0.03[a]	4.76 ± 0.11[b]
		FD	1	4.60 ± 0.03[a]	-1.90 ± 0.06[a]	4.82 ± 0.03[a]
			2	4.37 ± 0.05[a]	-2.34 ± 0.07[b]	4.19 ± 0.02[a]
			5	5.53 ± 0.04[b]	-2.39 ± 0.02[b]	5.51 ± 0.03[c]
			10	5.21 ± 0.03[b]	-2.12 ± 0.06[b]	5.45 ± 0.07[bc]
			15	5.10 ± 0.04[bc]	-2.27 ± 0.05[b]	5.07 ± 0.04[ab]
			20	4.73 ± 0.05[ac]	-2.07 ± 0.07[b]	4.90 ± 0.03[a]
BOH Green Tea			1	4.13 ± 0.09[a]	-0.34 ± 0.02[a]	0.45 ± 0.08[a]
			2	4.44 ± 0.08[a]	-1.12 ± 0.15[b]	0.89 ± 0.06[a]
			5	4.73 ± 0.10[ac]	-1.45 ± 0.13[b]	1.39 ± 0.18[b]
			10	4.36 ± 0.08[a]	-1.25 ± 0.10[b]	1.46 ± 0.07[b]
			15	4.30 ± 0.06[a]	-1.10 ± 0.14[b]	0.58 ± 0.06[a]
			20	5.02 ± 0.20[bc]	-1.32 ± 0.22[b]	1.58 ± 0.22[b]
SABAH Black Tea			1	3.63 ± 0.07[a]	-0.18 ± 0.06[a]	3.55 ± 0.08[a]
			2	4.16 ± 0.09[b]	-0.38 ± 0.04[b]	3.93 ± 0.07[b]
			5	3.89 ± 0.06[bd]	-0.15 ± 0.10[a]	3.26 ± 0.18[a]
			10	3.93 ± 0.04[bd]	-0.48 ± 0.04[c]	4.15 ± 0.09[b]
			15	3.83 ± 0.09[bd]	-0.34 ± 0.02[b]	3.83 ± 0.04[b]
			20	3.57 ± 0.04[ad]	-0.25 ± 0.03[a]	3.83 ± 0.08[b]

Mean ± S.D. (n = 5), Same superscripted letter within vertical columns of same sample and color scale are not significantly different ($P > 0.05$).

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3.3. Trace elements determination

The essential elements in *C. nutans* dried leaves were shown in Table 4. Elements such as K, Ca, Mg and Zn were higher in *C. nutans* dried leaves as compared to both commercial teas regardless of types and drying techniques used. However, K, Ca, Fe, Mg, Zn, Cu and Ni showed no significant differences ($P > 0.05$) between the unfermented and fermented *C. nutans* dried leaves. In unfermented dried leaves, K, Mg, Zn, Cu and Ni were found higher for microwave-oven dried leaves compared to freeze dried leaves. Meanwhile, in fermented dried leaves, all these essential elements were higher for freeze dried leaves as compared to microwave-oven dried leaves.

Beneficial elements are the elements which promote plant growth in many plant species but are not absolutely necessary for completion of the plant life cycle. The beneficial elements in *C. nutans* dried leaves were shown in Table 5. Na and Se were relatively higher than both commercial teas. However, only Na was showed significantly different ($P < 0.05$) to commercial teas. Se and Si were showed significant differences ($P < 0.05$) between the unfermented and fermented type. In unfermented dried leaves, freeze dried leaves showed higher Na, Se and Si compared to microwave-oven dried leaves. Contrarily, these elements were higher in microwave-oven dried leaves for fermented type. Other elements especially Al was found higher compared to Ba, Be, In and Li (Table 6). Elements of Al, Ba and In were found lower but significantly different ($P < 0.05$) than commercial teas. These elements might be contributed during preparation and drying process of leaves.

Table 4: Essential elements in herbal tea dried leaves

Sample	Type	Drying tech.	Element concentration (mg/kg dry weight)			
			K	Ca	Fe	Mg
C. nutans	Unfermented	MD	35807.93 ± 25^{a}	18326.63 ± 5^{a}	101.31 ± 0.54^{a}	9036.95 ± 4^{a}
		FD	29500.90 ± 92^{bc}	20074.97 ± 28^{b}	94.41 ± 1.04^{b}	8072.95 ± 5^{b}
	Fermented	MD	30116.81 ± 94^{b}	23729.59 ± 99^{c}	94.77 ± 0.69^{b}	7968.51 ± 13^{b}
		FD	31963.56 ± 81^{c}	18664.02 ± 94^{a}	103.46 ± 0.01^{a}	8323.53 ± 15^{c}
BOH Green Tea			18346.95 ± 145^{d}	4282.44 ± 43^{d}	152.03 ± 0.05^{c}	2046.08± 15^{d}
SABAH Black Tea			19536.38 ± 22^{d}	5381.57 ± 28^{e}	73.75 ± 0.81^{d}	2016.36 ± 13^{d}
			Mn	Zn	Cu	Ni
C. nutans	Unfermented	MD	51.57 ± 0.2^{a}	85.61 ± 0.13^{a}	8.84 ± 0.05^{a}	1.08 ± 0.01^{a}
		FD	75.93 ± 0.7^{b}	81.79 ± 1.01^{b}	6.28 ± 0.11^{b}	0.82 ± 0.08^{a}
	Fermented	MD	97.21 ± 0.8^{c}	86.87 ± 0.78^{a}	5.61 ± 0.06^{b}	1.06 ± 0.05^{a}
		FD	99.45 ± 0.1^{c}	99.42 ± 0.03^{c}	8.62 ± 0.02^{a}	1.98 ± 0.11^{b}
BOH Green Tea			764.12 ± 7^{d}	27.69 ± 0.02^{d}	15.73 ± 0.01^{c}	3.13 ± 0.07^{c}
SABAH Black Tea			860.98 ± 3^{e}	16.24 ± 0.03^{e}	14.44 ± 0.13^{c}	3.93 ± 0.01^{c}

Mean ± S.D. (n = 5), Same superscripted letter within vertical columns are not significantly different ($P > 0.05$).

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Table 5: Beneficial elements in herbal tea dried leaves

Sample	Type	Drying tech.	Element concentration (mg/kg dry weight)		
			Na	Se	Si
C. nutans	Unfermented	MD	338.07 ± 1.18^{a}	4.78 ± 0.29^{a}	86.34 ± 0.04^{a}
		FD	353.28 ± 7.10^{a}	4.86 ± 0.62^{a}	88.56 ± 0.86^{a}
	Fermented	MD	336.87 ± 3.69^{a}	4.17 ± 1.59^{b}	95.21 ± 0.72^{b}
		FD	275.50 ± 4.14^{b}	3.78 ± 0.17^{bc}	78.39 ± 0.52^{c}
BOH Green Tea			201.32 ± 0.75^{c}	3.58 ± 0.58^{c}	82.80 ± 0.31^{a}
SABAH Black Tea			195.30 ± 5.27^{c}	3.29 ± 0.06^{c}	88.26 ± 0.10^{a}

Mean ± S.D. (n = 5), Same superscripted letter within vertical columns are not significantly different (P > 0.05).

Table 6: Other elements in herbal tea dried leaves

Sample	Type	Drying tech	Element concentration (mg/kg dry weight)		
			Al	Ba	Be
C. nutans	Unfermented	MD	64.90 ± 0.43^{a}	4.89 ± 0.01^{a}	1.46 ± 0.01^{a}
		FD	65.26 ± 0.80^{a}	10.12 ± 0.01^{b}	1.71 ± 0.01^{a}
	Fermented	MD	53.07 ± 0.63^{b}	12.11 ± 0.01^{b}	1.97 ± 0.06^{ac}
		FD	77.16 ± 0.52^{c}	7.57 ± 0.04^{c}	2.07 ± 0.04^{bc}
BOH Green Tea			1120.12 ± 8.49^{d}	23.12 ± 0.05^{d}	2.91 ± 0.02^{d}
SABAH Black Tea			1498.49 ± 11.7^{e}	50.37 ± 0.51^{e}	2.41 ± 0.09^{b}
			In		Li
C. nutans	Unfermented	MD	1.08 ± 0.03^{a}		0.195 ± 0.003^{a}
		FD	0.82 ± 0.07^{a}		0.262 ± 0.003^{b}
	Fermented	MD	1.06 ± 0.03^{a}		0.249 ± 0.003^{b}
		FD	1.98 ± 0.03^{b}		0.176 ± 0.002^{a}
BOH Green Tea			3.13 ± 0.06^{c}		0.169 ± 0.001^{a}
SABAH Black Tea			3.93 ± 0.05^{d}		0.243 ± 0.001^{b}

Mean ± S.D. (n = 5), Same superscripted letter within vertical columns are not significantly different (P > 0.05).

Elements concentration in herbal infusion plays a significant measurement on the level of these elements intake for consumers. The minerals contents in dried leaves may influence its concentration in the infusion depending on the preparation method (drying technique, time infusion and temperature) [27]. There was a reduction and significant difference (P < 0.05) of the essential elements concentration from the *C. nutans* dried leaves to their infusions. Reduction of these elements mainly depends on the solubility of these elements in water. Each element was initially in the plant cellular and those water-soluble elements would infuse into water. Besides, solubility of these elements in infusion also dependent on the accessibility of hot water to leaves cellular. The concentration of elements in infusions were high, however, differently from the concentration in dried leaves which fully digested by acid causing much higher concentration of elements.

In the herbal infusion, only six essential elements (K, Ca, Fe, Mg, Mn and Zn) were detected

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compared to eight elements in dried leaves. Based on Table 7, only K, Ca and Fe in *C. nutans* herbal infusions were higher as compared to both commercial teas. Whereas, only Mn concentration was showed lower and significantly different ($P < 0.05$) than the commercial teas. K function in human body mainly associated with Na. K is the principal positively charged ion (cation) in the fluid inside cells, while Na is the principle cation in the fluid outside cells which basically to maintain the body's internal balance of fluids and chemicals [28]. The adequate intake (AI) of K is 4700 mg per day for adults. Ca mostly is important for nerve transmission, muscle contraction, glandular secretion and the contraction and dilation of the blood vessel. Up to 99 % of body calcium is in the bones and teeth and its deficiency can caused the osteoporosis development. The Adequate Intake (AI) of Ca is 1000 mg per day for adults. Fe as a part of hemoglobin plays an important role in transporting oxygen to body tissues and its deficiency can cause anemia. The Recommended Dietary Allowanced (RDA) of Fe is 18 mg per day for women of childbearing age and 8 mg per day for men and postmenopausal women, while 27 mg per day are suggested for pregnant women. Fe concentrations in *C. nutans* herbal infusions were range from 12.23 - 35.67 mg/L, which must be reduced for daily intake especially for men, postmenopausal women and pregnant women. Mg acts as important cofactor for more than 300 enzymes for energy production in human body. The RDA of Mg is set at 320 mg per day for adult women and 420 mg per day for adult men. Chronic Mg deficiency may cause in hyperexcitability of nerves and muscles. Mn is essential component for bone formation and involve in metabolism of amino acids, lipids and carbohydrate. The AI of Mn is 2.3 mg per day for men and only 1.8 mg per day for women. Zn is an essential element for enzymes to prevent impaired growth. The AI for Zn is 8 mg per day for women and 11 mg per day for men. The concentration on which Zn effect human health are ranges from 100 to 500 mg/L [31]. Among these essential elements, the concentrations of K, Ca, Mg, Mn and Zn in *C. nutans* herbal infusions were not ranged above the level suggested for continuing daily intake [28,29]. In unfermented herbal infusions, there were no significant differences ($P > 0.05$) of all essential elements between both drying techniques, meanwhile in fermented herbal infusions, the K and Mn in microwave oven dried infusions were higher and significantly different ($P < 0.05$) compared to freeze dried infusions. Regardless of drying techniques and infusion times, the Zn showed no significant differences between the unfermented and fermented herbal infusions. Ca and Mg were higher in unfermented as compared to fermented, while Mn was lower in unfermented herbal infusions. However, there were no significant differences ($P > 0.05$) and clear pattern of any element concentration as increasing of time infusion in different drying techniques of unfermented and fermented *C. nutans* herbal infusions.

The concentration of beneficial elements (Na, Se and Si) in herbal infusions were also lower and significantly different ($P < 0.05$) from their concentration in dried leaves. Se is a component of enzyme gluthione peroxidase which protect against oxidative stress. The RDA for Se is 55 µg per day for both men and women [29]. In *C. nutans* herbal infusions, Se were ranged from 0.015 - 1.039 mg/L, which some of the infusions contain higher concentration than recommended level. Si is a trace element that is critical for healthy bone, however, the exact biological role of Si in human bone health is still not clear, although a number of possible mechanisms have been suggested, including the synthesis of collagen and/or its stabilization, and matrix mineralization [32]. There was no information on the RDA or AI of Si for human intake. However, there is no evidence that silicon that occurs naturally in food and water produces adverse health effects.

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Table 7: Essential elements in herbal tea infusion

Sample	Type	Drying tech	Infusion time	Element concentration (mg/L)		
				K	Ca	Fe
C. nutans	Unfermented	MD	1	382.67 ± 2.00[a]	947.54 ± 2.65[a]	35.67 ± 0.17[a]
			2	339.62 ± 5.45[b]	935.42 ± 8.43[a]	31.62 ± 0.01[b]
			5	353.56 ± 2.77[c]	943.33 ± 4.90[a]	36.26 ± 0.01[a]
			10	364.50 ± 4.94[a]	946.09 ± 1.75[a]	31.71 ± 0.10[b]
			15	350.22 ± 1.66[c]	941.46 ± 3.10[a]	25.87 ± 0.06[c]
			20	383.32 ± 5.96[a]	965.65 ± 5.79[b]	33.97 ± 0.17[ab]
		FD	1	355.18 ± 3.32[ad]	928.68 ± 2.90[a]	31.28 ± 0.01[a]
			2	331.18 ± 30.3[b]	947.92 ± 6.56[b]	23.36 ± 0.02[b]
			5	319.89 ± 3.27[c]	944.10 ± 4.92[b]	32.17 ± 0.19[a]
			10	363.27 ± 1.08[a]	953.15 ± 6.04[c]	21.52 ± 0.03[b]
			15	349.90 ± 3.58[d]	943.45 ± 2.49[b]	29.01 ± 0.01[ac]
			20	363.54 ± 3.27[a]	947.03 ± 6.01[b]	26.30 ± 0.04[bc]
	Fermented	MD	1	438.83 ± 4.60[a]	862.88 ± 1.76[a]	24.40 ± 0.13[a]
			2	401.81 ±1.27[b]	841.60 ± 6.58[b]	22.90 ± 0.07[b]
			5	460.97 ± 4.21[c]	863.54 ± 1.81[a]	24.42 ± 0.05[a]
			10	461.91 ± 2.78[c]	861.29 ± 4.32[a]	26.14 ± 0.01[c]
			15	416.71 ± 2.56[b]	855.48 ± 3.94[a]	28.34 ± 0.01[d]
			20	505.89 ± 0.94[d]	865.72 ± 1.18[a]	22.54 ± 0.02[b]
		FD	1	361.72 ± 0.59[a]	839.12 ± 1.06[a]	20.26 ± 0.13[a]
			2	387.61 ±1.54[b]	849.28 ± 7.98[b]	12.23 ± 0.06[b]
			5	348.15 ± 1.56[c]	852.10 ± 7.71[b]	22.27 ± 0.17[a]
			10	361.05 ± 1.27[a]	839.92 ± 7.70[a]	20.37 ± 0.01[a]
			15	346.36 ±1.57[c]	835.41 ± 4.52[a]	23.51 ± 0.04[a]
			20	385.73 ± 1.56[b]	846.28 ± 1.02[b]	14.29 ± 0.13[b]
BOH Green Tea			1	206.58 ± 2.76[a]	825.96 ± 5.52[a]	18.76 ± 0.05[a]
			2	218.82 ± 1.66[a]	816.76 ± 2.76[b]	23.52 ± 0.15[b]
			5	206.83 ± 0.26[a]	815.31 ± 1.95[b]	12.32 ± 0.07[cd]
			10	211.15 ± 1.32[a]	814.02 ± 1.01[b]	9.24 ± 0.02[c]
			15	249.42 ± 2.24[b]	816.84 ± 7.33[b]	14.83 ± 0.22[d]
			20	219.28 ± 0.50[a]	819.13 ± 1.06[b]	14.94 ± 0.13[d]
SABAH Black Tea			1	207.41 ± 0.30[a]	829.29 ± 4.92[a]	7.35 ± 0.06[a]
			2	240.40 ± 1.77[bd]	816.22 ± 3.23[b]	8.31 ± 0.14[a]
			5	259.70 ± 4.58[b]	825.91 ± 1.10[a]	11.94 ± 0.19[b]

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			10	223.82 ± 0.71^{c}	817.51 ± 6.37^{b}	10.14 ± 0.18^{b}
			15	238.51 ± 3.72^{cd}	827.09 ± 3.78^{a}	11.34 ± 0.03^{b}
			20	232.56 ± 1.91^{cd}	821.89 ± 1.31^{a}	15.62 ± 0.01^{c}
				Mg	Mn	Zn
C. nutans	Unfermented	MD	1	281.38 ± 0.12^{ad}	0.097 ± 0.006^{ad}	5.79 ± 0.05^{a}
			2	271.11 ± 2.42^{b}	0.102 ± 0.003^{a}	6.23 ± 0.01^{b}
			5	264.60 ± 0.96^{c}	0.090 ± 0.001^{ad}	5.95 ± 0.01^{cd}
			10	268.50 ± 3.23^{c}	0.088 ± 0.001^{bd}	5.87 ± 0.01^{ac}
			15	271.66 ± 0.55^{b}	0.108 ± 0.002^{a}	6.07 ± 0.01^{d}
			20	306.18 ± 3.58^{d}	0.140 ± 0.007^{c}	5.75 ± 0.04^{a}
		FD	1	262.45 ± 1.29^{a}	0.145 ± 0.003^{a}	6.33 ± 0.01^{a}
			2	273.06 ± 0.97^{a}	0.146 ± 0.003^{a}	6.17 ± 0.03^{bd}
			5	270.30 ± 1.54^{a}	0.130 ± 0.003^{b}	5.96 ± 0.01^{c}
			10	288.30 ± 0.58^{b}	0.135 ± 0.004^{b}	6.38 ± 0.01^{a}
			15	271.98 ± 0.29^{a}	0.139 ± 0.001^{c}	5.99 ± 0.01^{c}
			20	283.51 ± 0.90^{b}	0.144 ± 0.001^{ac}	6.24 ± 0.01^{d}
	Fermented	MD	1	244.64 ± 0.92^{a}	0.186 ± 0.005^{a}	6.20 ± 0.01^{a}
			2	241.26 ± 1.37^{a}	0.236 ± 0.009^{b}	6.04 ± 0.04^{b}
			5	247.19 ± 1.09^{a}	0.207 ± 0.001^{a}	6.42 ± 0.02^{c}
			10	244.28 ± 1.79^{a}	0.231 ± 0.005^{b}	6.01 ± 0.01^{b}
			15	248.23 ± 1.83^{a}	0.248 ± 0.005^{c}	5.79 ± 0.06^{d}
			20	253.29 ± 0.57^{b}	0.234 ± 0.001^{b}	6.11 ± 0.01^{a}
		FD	1	247.68 ± 1.78^{a}	0.296 ± 0.008^{a}	5.78 ± 0.01^{a}
			2	251.21 ± 1.54^{b}	0.315 ± 0.005^{bc}	6.25 ± 0.02^{b}
			5	252.43 ± 1.04^{b}	0.289 ± 0.006^{a}	5.92 ± 0.02^{ad}
			10	253.89 ± 1.40^{b}	0.292 ± 0.003^{a}	6.07 ± 0.01^{c}
			15	244.97 ± 1.35^{a}	0.297 ± 0.002^{a}	5.85 ± 0.01^{a}
			20	245.34 ± 1.30^{a}	0.307 ± 0.001^{ac}	6.00 ± 0.02^{cd}
BOH Green Tea			1	239.00 ± 2.11^{a}	2.75 ± 0.04^{ac}	5.78 ± 0.02^{a}
			2	234.27 ±2.43^{a}	2.98 ± 0.03^{b}	5.77 ± 0.01^{a}
			5	228.93 ± 0.78^{b}	2.88 ± 0.15^{bc}	6.30 ± 0.03^{b}
			10	234.35 ± 2.04^{a}	3.05 ± 0.02^{a}	6.04 ± 0.03^{c}
			15	235.28 ± 0.05^{a}	3.68 ± 0.09^{d}	6.32 ± 0.01^{b}
			20	231.08 ± 0.41^{a}	3.21 ± 0.22^{e}	5.78 ± 0.01^{a}
SABAH Black Tea			1	240.67 ± 2.62^{a}	1.81 ± 0.004^{ae}	6.11 ± 0.04^{a}
			2	229.24 ± 0.37^{b}	2.33 ± 0.10^{b}	5.94 ± 0.04^{b}

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			5	233.58 ± 1.71[c]	2.48 ± 0.07[b]	6.38 ± 0.03[c]
			10	233.10 ± 1.31[c]	2.06 ± 0.14[ce]	6.15 ± 0.01[ab]
			15	236.05 ± 0.19[a]	2.27 ± 0.03[d]	6.55 ± 0.01[d]
			20	237.93 ± 2.57[a]	2.26 ± 0.05[d]	6.23 ± 0.02[c]

Mean ± S.D. (n = 5), Same superscripted letter within vertical columns of same sample and element are not significantly different (P > 0.05).

Table 8. Beneficial elements in herbal tea infusion

Sample	**Type**	**Drying tech**	**Infusion time**	**Element concentration (mg/L)**		
				Na	Se	Si
C. nutans	Unfermented	MD	1	224.34 ± 1.54[a]	0.063 ± 0.006[a]	20.16 ± 0.20[a]
			2	215.14 ± 2.57[b]	0.063 ± 0.002[a]	26.28 ± 0.12[b]
			5	219.71 ± 1.42[b]	0.080 ± 0.001[b]	20.14 ± 0.16[a]
			10	223.32 ± 9.83[ac]	0.068 ± 0.002[a]	19.69 ± 0.30[a]
			15	218.21 ± 2.54[bc]	0.080 ± 0.001[b]	26.08 ± 0.07[b]
			20	224.81 ± 1.12[a]	0.082 ± 0.003[b]	10.76 ± 0.17[c]
		FD	1	212.35 ± 5.20[ac]	0.067 ± 0.004[a]	16.92 ± 0.03[a]
			2	212.95 ± 4.41[ac]	0.087 ± 0.013[b]	29.62 ± 0.15[b]
			5	211.22 ± 2.53[a]	0.033 ± 0.003[c]	17.07 ± 0.20[a]
			10	221.54 ± 1.52[b]	1.000 ± 0.005[d]	27.12 ± 0.27[c]
			15	216.81 ± 1.18[c]	0.017 ± 0.009[e]	12.36 ± 0.45[d]
			20	218.55 ± 1.96[c]	0.056 ± 0.003[a]	16.35 ± 0.07[a]
	Fermented	MD	1	225.39 ± 3.32[a]	0.086 ± 0.001[a]	20.54 ± 0.43[a]
			2	202.61 ± 0.29[a]	0.065 ± 0.001[b]	16.53 ± 0.19[b]
			5	222.11 ± 3.46[a]	0.036 ± 0.002[c]	12.36 ± 0.36[c]
			10	221.16 ± 3.76[a]	0.072 ± 0.003[d]	8.51 ± 0.17[d]
			15	210.27 ± 6.55[b]	1.025 ± 0.011[e]	13.14 ± 0.19[c]
			20	232.55 ± 1.71[c]	1.039 ± 0.003[f]	5.24 ± 0.08[e]
		FD	1	197.06 ± 0.74[a]	0.067 ± 0.011[a]	15.20 ± 0.28[a]
			2	203.87 ± 1.10[b]	1.039 ± 0.009[b]	12.96 ± 0.27[b]
			5	196.69 ± 2.74[a]	0.015 ± 0.002[c]	12.18 ± 0.02[b]
			10	196.42 ± 0.43[a]	0.087 ± 0.006[d]	8.36 ± 0.08[c]
			15	195.48 ± 1.37[a]	0.052 ± 0.005[e]	8.75 ± 0.02[c]
			20	202.16 ± 0.81[b]	1.003 ± 0.006[f]	12.21 ± 0.43[b]
BOH Green Tea			1	148.80 ± 4.44[ac]	1.076 ± 0.001[a]	7.23 ± 0.41[a]
			2	152.01 ± 0.63[b]	0.086 ± 0.002[b]	14.25 ± 0.47[b]
			5	152.44 ± 1.23[b]	0.035 ± 0.004[c]	16.75 ± 0.13[c]
			10	153.20 ± 6.76[bc]	1.016 ± 0.009[d]	18.07 ± 0.40[d]
			15	154.62 ± 2.45[bc]	1.014 ± 0.005[d]	8.94 ± 0.11[e]

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	20	152.32 ± 2.38^{bc}	0.064 ± 0.006^{e}	12.79 ± 0.06^{f}
SABAH Black Tea	1	149.11 ± 1.17^{a}	0.088 ± 0.013^{a}	23.18 ± 0.44^{a}
	2	149.49 ± 0.16^{a}	1.039 ± 0.001^{b}	14.01 ± 0.10^{b}
	5	151.80 ± 7.44^{a}	1.056 ± 0.001^{c}	18.50 ± 0.28^{c}
	10	149.80 ± 0.51^{a}	0.080 ± 0.001^{a}	19.840 ± 0.31^{c}
	15	149.92 ± 0.58^{a}	0.016 ± 0.002^{d}	16.16 ± 0.09^{d}
	20	151.50 ± 4.52^{a}	1.005 ± 0.001^{e}	22.83 ± 0.50^{a}

Mean ± S.D. (n = 5), Same superscripted letter within vertical columns of same sample and element are not significantly different (P > 0.05).

Conclusion

The unfermented and fermented *C. nutans* dried leaves gave similar net energy as commercial teas of *C. sinensis*. Freeze dried technique contribute higher crude protein, fat and fiber as compared to microwave-oven dried leaves in both unfermented and fermented *C. nutans* dried leaves. However, fermentation process gave less effect in energy production based on the proximate composition of dried leaves. The essential and beneficial elements in *C. nutans* dried leaves were reduced in their infusions which might be affected from preparation, element solubility in water and water accessibility to plant cellular. The concentrations of K, Ca, Mg, Mn and Zn in *C. nutans* herbal infusions were not ranged above the level suggested for continuing daily intake. Future study will be carried out to determine bioactive compounds such as phenolics, flavonoids and catechins and its biological activities for human health benefits. Since this plant is orally consumed, these findings showed the ability of *C. nutans* herbal tea as a potential nutrient source especially for energy, minerals and trace elements. It is also important as scientific information on levels of minerals and trace elements intake in order to overcome nutrients deficiency and to prevent minerals toxicity.

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