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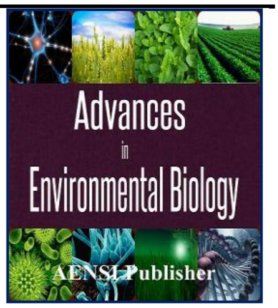

Quantification of Phenolic Compounds and Sensorial Properties of *Cosmos caudatus* Herbal Tea at Different Maturity Stages

[1]Dian-Nashiela Fatanah, [1,2]Noriham Abdullah, [1]Nooraain Hashim, [3]Azizah Abd. Hamid

[1]Department of Food Science and Technology Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[2]Malaysia Institute of Transportation (MITRANS), Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[3]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

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ABSTRACT

Background: Phenolic compounds are responsible for major sensorial characteristics of herbal tea. The consumption of *Cosmos caudatus* leaves at different maturity stages for herbal tea development constitutes an important factor in influencing phenolic compounds and sensorial properties. **Objective:** Thus, this present study was designed to identify and quantify the phenolic compounds and to evaluate sensorial properties of herbal tea prepared from *C. caudatus* leaves at different maturity stages namely young, mature and old and to compare them with *C. caudatus* herbal tea from commercial brand. **Results:** Based on the results obtained, *C. caudatus* herbal tea prepared from young leaves showed significantly higher concentration of phenolic compounds compared to other *C. caudatus* herbal tea. However, *C. caudatus* herbal tea prepared from young leaves was the least acceptable by panelists due to its strong bitter and astringent taste followed by mature leaves, old leaves and *C. caudatus* herbal tea from commercial brand. **Conclusion:** Hence, it can be concluded that, the different amount of phenolic compounds in samples contributed to the different sensorial perceptions. It is recommended to improve the acceptability of *C. caudatus* herbal tea prepared from young leaves since it provide higher antioxidant activity where antioxidant is known to possess beneficial impact to human health.

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INTRODUCTION

For a thousand years, herbal tea has been used for health care and diseases prevention in many countries [1]. Many studies reported that, herbal tea can be the major sources of phenolic compounds [2] and supplementation of diet with herbal tea offer a great amount of antioxidant that may have beneficial effects to human body [3]. Besides it also provide a specific purpose such as relaxation, rejuvenation, relief and giving negligible side effects to human body. Other important factors like convenient, easy to prepare, cheaper in price as well as available abundantly might be also the reason for the increment consumption of herbal tea worldwide [4]. Nowadays, numerous herbal tea are gaining popularity and many countries especially Asia have varieties of herbal teas, made from brewing plant leaves or other plant parts including flower [5]. In Malaysia, there are abundant of herbal teas produced by Small Medium Enterprise (SME) industry such as misai kucing *(Orthosiphon stamineus)* tea, kacip fatimah *(Labisia pumila)* tea, sabung nyawa *(Gynura procumbens)* tea, kaca beling *(Strobilanthes crispa)* tea, ginger *(Zingiber officinale)* tea and lemongrass *(Cymbopogon)* tea. Recently, *C. caudatus* or commonly known as ulam raja by the Malays is getting attention amongst Malaysian herbal industries due to its potential health-promoting properties, its unique taste and has functionality to the consumers [6]. It has been reported by several studies that, *C. caudatus* contained a number of phenolic compounds including quercetin, proanthocynidin, chlorogenic acid, catechin, epicatechin, myricetin and naringenin which have contributed to its high antioxidant activity [7]. However, the usage of different maturity stages of *C. caudatus* leaves as raw materials to produce herbal tea could affect the phenolic compounds and the

Corresponding Author: Noriham Abdullah, Department of Food Science and Technology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor Darul Ehsan.
Phone No.: +603-55438441; Email: noriham985@salam.uitm.edu.my

sensorial properties. According to [8], the phenolic compounds of herbal tea in general, are affected by several factors such as plant variety, maturity stages, processing method and brewing time, and the changes of these compounds may lead to the changes of major sensory characteristic of herbal tea, particularly colour and taste. Therefore, this study is aimed to identify and quantify the phenolic compounds and to evaluate sensorial properties of *C. caudatus* herbal tea at different maturity stages and to compare these herbal teas with *C. caudatus* herbal tea from commercial brand.

MATERIALS AND METHOD

Chemicals:

HPLC standards (gallic acid, trans sinapic acid, chlorogenic acid, caffeic acid, ferullic acid, sinapic acid, vanillic acid, ρ-coumaric acid, catechin, epicatechin, rutin, myricetin, quercetin, naringenin, kaempferol) were supplied by Sigma-Aldrich Chemie, Germany.

Raw materials collection and selection:

The fresh leaves of 8-week-old *C. caudatus* plant were collected from Durian Tunggal, Malacca, Malaysia. The 8-week-old *C. caudatus* plant was selected based on the previous findings from [9] where 8-week-old plant were reported to contain higher antioxidant activity compared to 10 and 12-week-old *C. caudatus* plant. The leaves were divided into 3 groups, classified as young, mature and old leaves. Young leaves were selected from the first four tiers where the leaves are still tender, newly emerged and not attaining full expansion. Mature leaves are located at the middle part of *C. caudatus* plant where the leaves are fully developed while old leaves are located at the lower part of the plant and the leaves had showed initial sign of senescence. Mature leaves were selected between the fifth to eighth tiers and old leaves were selected starting from ninth tier and above.

Fig. 1: Selection of 8-week-old C. caudatus leaves based on maturity. (A) Young leaves (B) Mature leaves (C) Old leaves

Sample preparation:

Each group of *C. caudatus* leaves were prepared according to the normal procedure as being conducted for herbal tea preparation by Small Medium Enterprise (SME) industry in Malaysia. The leaves at different maturity stages were dried at 50°C for 8 hours in a cabinet dryer until constant weight. *C. caudatus* herbal tea powder was prepared according to method described by [10] with some modifications. The dried leaves were milled using centrifugal mill, which were then screened through different sized sieves ranged from 2 mm to 1 mm to separate the milled leaves. Then, 2 g of milled leaves were collected and packed in a tea bag. The *C. caudatus* herbal teas were infused in 200 ml boiling distilled water for 3 minutes according to method suggested by [11]. The infused teas were filtered through a Whatman filter paper No. 41 prior for further analyses.

Identification and quantification of phenolic and flavonoid compounds using High Performance Liquid Chromatography (HPLC)

Solid Phase Extraction (SPE):

Sep-Pax® cartridges devoted for SPE were conditioned with 12 ml methanol and washed with 18 ml distilled water. Then, about 5 g *C. caudatus* herbal tea samples extract that dissolved in 50 ml distilled water were passes through the SPE cartridges in order to retain phenolic and flavonoid compounds as well as to separate them from other matrix components, especially sugars and peptides. The SPE cartridges were washed again with 10 ml of distilled water to remove remnants of samples matrices and finally, 30 ml of ethyl acetate were passed through the cartridges to elute compounds retained. Resulting extracts containing phenolic and flavonoid compounds isolated from the samples were analysed by HPLC.

HPLC conditions:

High performance liquid chromatography (HPLC) was used to identify phenolic and flavonoid compounds in *C. caudatus* herbal tea samples. The method used was as described by [12] with some modification. This method was performed using a reverse phase HPLC with a diode array detector (DAD) by Agilent Technologies 1200 Series model. Analyte separation was carried out on a RP-C18 column (5μm × 250mm). The mobile phase used for the identification and quantification of phenolic compounds were 18% acetonitrile (solvent A) and 82% acidified water (2%, solvent B). Meanwhile, the mobile phase used for the flavonoid compounds were 10% acetonitrile (solvent A), 65% acidified water (2%, solvent B) and 25% methanol (solvent C). The injection volume was 20 μl with 1.000 ml/min flow rate at 60^{0}C column temperature. The standards used in this study were gallic acid, trans sinapic acid, chlorogenic acid, caffeic acid, ferulic acid, vanillic acid, ρ-coumaric acid, catechin, epicatechin, rutin, myricetin, quercetin, naringenin and kaempferol. The amount of the compound was expressed as μg/ml of herbal tea.

Quantitative Descriptive Analysis (QDA):

The QDA was carried out in accordance to [13] procedure. A sensory panel composed of 10 Food Science & Technology postgraduate's students were trained for 2 weeks. Training session was divided into two sessions where during the first session, panelists were trained using only reference sample (commercial sample) while for second session, panelists were given reference and *C. caudatus* herbal tea at different maturity samples. Seven attributes had been evaluated including colour, herbal aroma, herbal taste, bitterness, astringency, after taste and overall acceptability. The panelists were given water and lemon juice in between the sample tasting in order to eliminate bitter, astringent and after taste. Reference sample was coded as 'R' while the *C. caudatus* herbal tea samples were coded with three digital codes in random. Each panelist was served with 20 ml samples at about 60^{0}C. Then, panelists recorded their evaluation by making a vertical line on 15 cm line scale with 1.5 cm anchor at each end. The line scale was drawn from left to the right with the increasing intensity. The average scores given by panelists were provided as evaluation results.

Statistical analysis:

All experiments were run in triplicates. Statistical analyses were conducted with the Statistical Analysis System (SAS) 9.1.3 software package. Analyses of variance were performed by ANOVA procedures. Significant differences ($p<0.05$) were determined by least square means comparison.

RESULTS AND DISCUSSION

Identification and quantification of phenolic acid and flavonoid compounds

The quantification of phenolic compounds of *C. caudatus* herbal tea at different maturity stages is presented in Table 1. Fourteen selected standards of phenolic compounds which are from phenolic acid and flavonoid compounds were prepared for HPLC analysis. The identification results showed that, these compounds eluted at different retention time based on polarity. In total, twelve from fourteen compounds were identified with remarkable difference among *C. caudatus* herbal tea samples by comparing retention time and UV spectra with authentic standards. The detected compounds are gallic acid, chlorogenic acid, caffeic acid, vanillic acid, ρ-coumaric acid, sinapic acid, ferullic acid, catechin, epicatechin, rutin, myricetin and quercetin. In agreement with study by [14] study, they reported that *C. caudatus* are rich in variety of phenolic compounds such as chlorogenic acid, catechin, quercetin and its derivatives. However, two compounds not detected in all *C. caudatus* herbal tea samples were naringenin and kaempferol. This is in contrast with previous study, where the presence of naringenin and kaempferol in *C. caudatus* plant were detected. According to [15], sample preparation and extraction, climate differences, soil conditions and sample geographical area might effect the phenolic compounds in the plants. Amongst the detected compounds, chlorogenic acid, ρ-coumaric acid and sinapic acid were found to be the major contributors of phenolic acid compounds while catechin, rutin and quercetin were quantified as the main representatives of flavonoid compounds in all *C. caudatus* herbal tea samples. [16] found that, chlorogenic acid was the most enriched phenolic acid in *C. caudatus* leaves while [17] stated that, the major flavonoid compounds of *C. caudatus* leaves are quercetin and rutin.

When comparing among the *C. caudatus* herbal tea at different maturity stages, it clearly shown that, the concentration of each phenolic compounds decreased as the maturity stages increased in which the highest amount was in *C. caudatus* herbal tea prepared from young leaves. According to [9], the reduction of phenolic compounds with the increasing maturity might due to the conversion of these compounds to other secondary metabolites such as sugar by enzymes action. The reduction of these phenolic compounds might cause the mature and old leaves possess inadequate antioxidant to scavenge the overproduction of reactive oxygen species (ROS) produced during aging process, eventually causing senescence of plant tissues [6]. In addition, [18] believed that, the stoppage of new biosynthesis of phenolic compounds with the advancing maturity might be another possible explanation to the reduction of phenolic compounds. Generally, most of this new biosynthesis took place at the early stages of plant growth which provide highest antioxidant activity to react with the ROS produced [19]. With the highest level of antioxidant activity in young leaves, phenolic compounds could acts as reductone to react with free radicals by converting them to more stable products and terminating radical chain reaction [20]. In comparison between the *C. caudatus* herbal tea at different maturity stages and *C. caudatus* herbal tea from commercial brand, it showed that all *C. caudatus* herbal tea at different maturity stages had significantly higher phenolic compounds compared to the commercial brand. [21] demonstrated that, the growing environment largely influences the phenolic compounds distribution in tea while [22] stated that the decrement of phenolic compounds and antioxidant activity in teas is due to the processing technique and aging process.

Table 1: Quantification of Phenolic Acid and Flavonoid Compounds of herbal tea prepared from *C. caudatus* leaves at different maturity stages

Compounds (µg/ml of herbal tea)		Retention time (min)	*C. caudatus* herbal tea			
			Young leaves	Mature leaves	Old leaves	Commercial brand
Phenolic acid	Gallic acid	3.77	8.08 ± 0.32^{a}	6.20 ± 0.72^{b}	4.12 ± 0.28^{c}	2.78 ± 0.04^{d}
	Chlorogenic acid	4.62	18.88 ± 0.93^{a}	16.21 ± 0.58^{b}	13.12 ± 0.65^{c}	9.01 ± 0.78^{d}
	Caffeic acid	7.09	2.86 ± 0.25^{a}	1.70 ± 0.13^{b}	1.39 ± 0.06^{b,c}	1.31 ± 0.06^{c}
	Vanillic acid	7.52	7.72 ± 0.42^{a}	5.85 ± 0.70^{b}	4.14 ± 0.07^{c}	4.17 ± 0.37^{c}
	ρ-coumaric acid	12.23	31.35 ± 6.18^{a}	26.09 ± 1.81^{a,b}	23.96 ± 2.87^{b}	13.21 ± 1.43^{c}
	Sinapic acid	13.34	23.21 ± 1.76^{a}	16.67± 0.78^{b}	13.07 ± 0.65^{c}	8.73 ± 0.89^{d}
	Ferullic acid	14.29	8.07 ± 0.41^{a}	7.83 ± 0.38^{a}	7.74 ± 0.86^{a}	6.76 ± 0.50^{b}
Flavonoid	Catechin	3.83	220.16 ± 5.53^{a}	179.94 ± 14.00^{b}	146.92 ± 5.23^{c}	53.44 ± 7.96^{d}
	Epicatechin	4.65	27.31 ± 2.28^{a}	21.37± 1.14^{b}	14.60 ± 1.24^{c}	8.90 ± 1.21^{d}
	Rutin	11.66	280.92 ± 10.79^{a}	194.43 ± 17.65^{b}	10.5.16 ± 3.46^{c}	101.13 ± 8.62^{d}
	Myricetin	15.05	64.94 ± 4.73^{a}	54.50 ± 3.81^{b}	41.88 ± 2.70^{c}	32.26 ± 3.03^{d}
	Quercetin	21.64	369.22 ± 17.19^{a}	268.76 ± 25.23^{b}	255.86 ± 25.14^{c}	216.77 ± 32.25^{d}
	Naringenin	25.84	ND	ND	ND	ND
	Kaempferol	34.10	ND	ND	ND	ND

Quantitative Descriptive Analysis (QDA):

In terms of sensory evaluation, most of the panelists most preferred *C. caudatus* herbal tea from commercial brand, which has been used as reference sample compared to all *C. caudatus* herbal tea at different maturity stages. The results of sensory evaluation in the form of spider web diagram for 8 different attributes of *C. caudatus* herbal tea samples are shown in Figure 2. It should be noted that the panelists' acceptability towards *C. caudatus* herbal tea from commercial brand than other *C. caudatus* herbal tea is due to the weak herbal aroma, weak herbal taste, mild bitter taste and mild astringent taste. According to [23], the existence of attributes like bitterness and astringency in herbal tea could be the reasons to the unacceptable taste to consumers' preference. [24] believed that, bitterness and astringency are highly positively correlated with the phenolic compounds, suggesting that these compounds in samples contribute to strongly sensory perceptions. [22] suggested that bitter taste of tea is more related to catechin while [25] found that quercetin contributed to bitter taste and rutin give the astringent taste to the herbal tea samples. However, [24] summarised that, the bitterness and astringency in samples is due to phenolic compounds such as gallic acid, catechin, epicatechin, kaempferol, naringenin and quercetin. Based on the colour, as expected, *C. caudatus* herbal tea prepared from young leaves has darker colour compared to other *C. caudatus* herbal tea samples due to higher antioxidant activity. According to [26], the colour intensity of sample is related to pigments such as flavonoids, in fact, the increase of the colour intensity seems to be related to an increase in the concentration of these compounds. In agreement with the previous studies, therefore the results of sensory seems to relates with the quantification of phenolic compounds in which different concentration of phenolic compounds detected in *C. caudatus* herbal tea contributed to the different sensory perceptions.

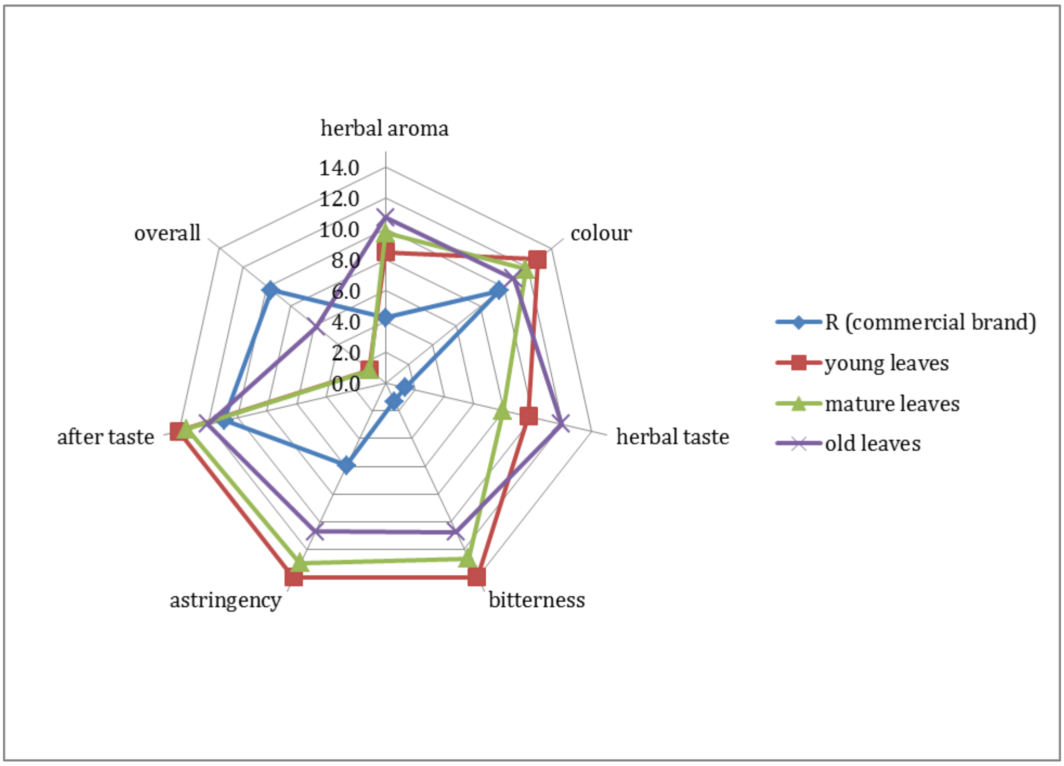

Fig. 2: Spider web diagram for the different attributes of herbal tea prepared from *C. caudatus* leaves at different maturity stages

Conclusion:

Based on the results obtained, it can be concluded that the amount of phenolic compounds were correlated well with the sensorial properties of *C. caudatus* herbal tea samples. The panelists most preferred *C. caudatus* herbal tea from commercial brand instead of all *C. caudatus* herbal tea at different maturity stages due to its mild bitter and astringent taste which contributed by lower concentration of phenolic compounds. Therefore, it is recommended to enhance the acceptability of *C. caudatus* herbal tea prepared from young leaves for example, by adding natural sweetener to mask the bitter and astringent taste since this herbal tea possessed highest phenolic compounds which is associated with beneficial health effects to consumers.

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