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Influences of Maturity Stages and Extraction Solvents on Antioxidant Activity of *Cosmos Caudatus* Leaves

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Abstract: *Different maturity stages and extraction solvents used could affect antioxidant activity of plants. Therefore, this study investigated antioxidant activity of Cosmos caudatus leaves at three different maturity stages (young, mature and old leaves) extracted using two extraction solvents (water and 95% ethanol). The analyses of antioxidant activity conducted were total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. From the analyses, young leaves showed significantly higher antioxidant activity compared to mature and old leaves. However, C. caudatus leaves extracted using water had significantly higher in TPC, TFC and FRAP values than those of ethanolic extracts, whereas opposite trend was observed in DPPH radical scavenging assay. Based on Pearson's correlation coefficient, TPC and TFC for all extract exhibited strong correlations with antioxidant assays indicating that these leaves have potent phenolic and flavonoid compounds. In fact, most phenolic and flavonoid compounds including gallic acid, chlorogenic acid, caffeic acid, vanillic acid, ρ-coumaric acid, sinapic acid, ferullic acid, catechin, epicatechin, rutin, myricetin and quercetin, except naringenin and kaempferol were detected by High Performance Liquid Chromatography (HPLC) in all samples for both extracts. Hence, it can be concluded that C. caudatus young leaves extracted using water had the most powerful antioxidant, suggesting their potential application as health-promoting functional ingredients or natural preservatives in food and pharmaceutical products.*

Keywords: *Cosmos caudatus, maturity stages, extraction solvents, antioxidant activity, HPLC*

1. INTRODUCTION

In recent years, there has been considerable interest in findings for natural antioxidant mainly from plant sources to replace synthetic antioxidants due to their potential harmful effects on human health [1]. According to [2], plants synthesize antioxidant compounds, as secondary products which are serving in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to avoid oxidative damage. Ulam raja or scientifically named as *Cosmos caudatus* was reported to have a great potential as natural antioxidant in the previous study. [3] identified more than twenty antioxidants in *C. caudatus* including quercetin glycosides, chlorogenic acid, neo-chlorogenic acid, crypto-chlorogenic acid and catechin while [4] revealed that, at 200 ppm concentration, *C. caudatus* had the highest ability to reduce Fe(III) among herbs tested and was not significantly different with butylhyroxytoluene (BHT). Nevertheless, [5] found that, the maturity, solvent used and the interaction of maturity and extraction condition have significant impact on the phenolic compounds in the plant extracts. [6] believed that, accumulation of secondary metabolites continued from day 10 until 35 and then these compounds decreased due to the interconversion between them. In fact, the phenolic extracts are selectively soluble in the extraction solvents due to their complex mixtures [7]. Therefore, the aim of this study is to determine and to compare the effect of antioxidant activity of *C. caudatus* leaves at different maturity stages, extracted using different solvents.

2. MATERIALS AND METHODS

2.1. Chemicals

95% ethanol, acetonitrile (HPLC grade) aluminium chloride ($AlCl_3$), ethyl acetate, Folin-Ciocalteu reagent, ferric chloride ($FeCl_3$), hydrochloric acid (HCl), methanol (HPLC grade), sodium carbonate (Na_2CO_3) and sodium nitrite ($NaNO_2$) were purchased from Merck, Germany. 2,4,6-tripyridyl-s-triazine (TPTZ), ascorbic acid, DPPH, HPLC standard (gallic acid, chlorogenic acid, caffeic acid, vanillic acid, ρ-coumaric acid, sinapic acid, ferullic acid, catechin, epicatechin, rutin, myricetin, quercetin, naringenin and kaempferol), quercetin and trolox were supplied by Sigma-Aldrich Chemie, Germany. Sodium acetate and glacial acetic acid were purchased from R & M Chemicals, U.K. and Friendemann Schmidt Chemicals, U.K. respectively.

2.2. Raw materials collection and selection

The raw materials were collected and selected based on [8] method. Fresh leaves of 8-week-old *C. caudatus* plant were collected from Durian Tunggal, Malacca, Malaysia. 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 showed initial sign of senescence. Mature leaves were selected between the fifth to eighth tiers and old leaves were selected starting from ninth tiers and above.

2.3. Sample preparation

Each stage of *C. caudatus* leaves was dried at 50°C for 8 hours in cabinet dryer (Vision Scientific Enterprise, Malaysia) until constant weight in accordance to method as described by [9]. Then, the dried *C. caudatus* leaves were ground using ultra centrifugal mill (ZM 200, Retsch, Germany) at 8000 rpm and sieved through 2 mm to 1 mm mesh size prior to extraction process.

2.4. Sample extraction

According to method suggested by [10], water extracts were prepared by weighing about 10 g of ground *C. caudatus* leaves in 200 ml hot water (80°C) and stirred gently on magnetic stirrer for 10 minutes. For preparation of ethanolic extracts, 10 g of ground *C. caudatus* leaves were soaked in 200 ml 95% ethanol. The mixture was stirred gently on incubator shaker (Innova 40, New Brunswick Scientific, USA) at 120 rpm for 12 hours at room temperature (20°C). Both extracts were then filtered through a Whatman filter paper no. 41. The filtrates were evaporated using rotary evaporator (R-210, Buchi, Switzerland) and the extra water was removed by using freeze drier (Alpha 1-4 LD Plus, Martin Crist, Germany). The dried extracts were kept in air-tight container at -20°C prior to further analysis.

2.5. Total phenolic content (TPC)

The TPC of *C. caudatus* samples were determined by using the Folin-Ciocalteu assay [11]. Accurately, 0.5 ml Folin-Ciocalteu reagent, 1.5 ml 7.5% sodium carbonate and 7.9 ml distilled water were introduced in a test tube containing 0.1 ml sample/standard. The solution was mixed thoroughly and allowed to stand for 2 hours in a dark place. The absorbance at 765 nm was read by using UV-VIS Spectrophotometer (Helios Zeta, Thermo Fisher Scientific, USA). The TPC of the samples were expressed as mg of gallic acid equivalents (mg GAE)/ g of dried extract.

2.6. Total flavonoid content (TFC)

The TFC was analysed according to method as described by [12]. About 1 ml of sample/standard was diluted with 4 ml distilled water then 0.3 ml 5% sodium nitrite solution and 0.3 ml 10% aluminium chloride ($AlCl_3$) was added. The mixture was kept for 5 minutes. Then, 2 ml of 1M sodium hydroxide were added to the mixture and the mixture was vortexed thoroughly. The absorbance was measured at 510 nm using UV-VIS Spectrophotometer (Helios Zeta, Thermo Fisher Scientific, USA). This was calculated as mg quercetin equivalents (mg QE)/ g of dried extract.

2.7. Ferric reducing antioxidant power (FRAP)

The FRAP assay was carried out by the method of [13]. FRAP reagent was freshly prepared by mixing 300 mM acetate and glacial acetic acid buffer (pH 3.6), 20 mM ferric chloride and 10 mM

TPTZ was made to 40 mM HCl at a ratio of 10:1:1. Briefly, 0.1 ml sample/standard was mixed with 3 ml FRAP reagent and 3 ml distilled water. The mixture was incubated in a dark place at 37°C for 8 minutes and the absorbance at 565 nm was then read using UV-VIS Spectrophotometer (Helios Zeta, Thermo Fisher Scientific, USA). The total antioxidant activity of samples was determined against a standard of a known FRAP value and was expressed as µM trolox equivalents (µM TE)/ g of dried extract.

2.8. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH assay was performed according to the procedure as described by [14]. Accurately, 0.1 ml sample/standard was mixed with 2.9 ml of 0.05 mM DPPH in methanol and incubated in the dark at room temperature for 30 minutes. The absorbance of the sample/standard was measured using UV-VIS Spectrophotometer (Helios Zeta, Therma Fisher Scientific, USA) at 515 nm where methanol was used as blank and results were expressed as µg/g of dried extract.

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

2.9.1. 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 samples extract that were dissolved in 50 ml distilled water were passed through the SPE cartridges in order to retain phenolic and flavonoid compounds as well as to separate them from other matrix components, especially sugar 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.

2.9.2. HPLC conditions

The identification and quantification of phenolic compounds in *C. caudatus* leaves extracts were based on the methodology described by [15] with some modifications. The HPLC apparatus was coupled with a diode array detector (DAD), a quaternary pump and an autosampler. Analyte separation was performed on a Purospher® STAR RP-C18 endcapped column with dimension of 4.6 mm x 250 mm, 5 µm. The mobile phase used was divided into phenolic acids and flavonoids. For identification of phenolic acid compounds in *C. caudatus* leaves extracts, the mobile phase used was composed of solvent A (2% glacial acetic acid, v/v) and solvent B (acetonitrile) which was run by isocratic at 12% A: 82% B. For flavonoids, the mobile phased was include solvent A (2% glacial acetic acid, v/v), solvent B (acetonitrile) and solvent C (methanol) and was run in gradient form. The gradient applied was: 10% B, 25% C (0 – 5 min), 5% B, 30%B (5 – 10 min), 10%B, 20%C (10 – 40 min) and 20%B, 20% C (40 min) followed by flushing the column. The injection volume was 20 µl with 1.0 ml/min flow rate at 60°C column temperature. The runs were monitored at 280 nm and 320 nm for phenolic acids while 280 nm and 370 nm for flavonoids. Quantification was performed using calibration curve of standards. The standard used in this study including gallic acid, chlorogenic acid, caffeic acid, vanillic acid, ρ-coumaric acid, sinapic acid and ferullic acid from phenolic acid compounds meanwhile catechin, epicatechin, rutin, myricetin, quercetin, naringenin and kaempferol from flavonoids compounds. The amount of the compound was expressed as µg/g of dried extract.

2.9.3. 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.

3. RESULTS AND DISCUSSION

3.1. Total Phenolic Content (TPC)

In this study, TPC of the sample extracts were measured by using Folin-Ciocalteu colourimeter method. This method is based on oxidation/reduction on redox properties of antioxidant compounds presence in the samples [16]. The results of TPC of water and ethanolic *C. caudatus* leaves extract at different maturity stages are shown in Table 1. *C. caudatus* young leaves for both extracts showed deeper blue colour solution after 2 hours incubation period as compared to mature and old leaves. The

addition of sodium carbonate into Folin-Ciocalteu reagent and samples containing antioxidant compounds reduced the mixture of phosphotungstic and phosphomolybdic acids in the reagent to tungsten and molybdene that produced the blue oxides via electron transfer in alkaline conditions [1]. The higher intensity of blue colour solution reflects higher phenolic compounds present in the samples [8].

From the results obtained, TPC of *C. caudatus* leaves decreased with the increment of maturity stages in which *C. caudatus* young leaves possessed significantly high antioxidant content for both water and ethanolic extracts. According to [17], the reduction of TPC with the advancing maturity is due to conversion of phenolics to other secondary metabolites by enzyme action. This is supported by [18] who reported that, *Withania somnifera* (L.) Dunal leaves extract just after flowering stage exhibited higher TPC than fully mature stage because of degradation of pigments which was then reabsorbed into the plants. [19] believed that, the aging process stimulates the formations of reactive oxygen species (ROS), which are neutralized by phenolic compounds, resulting in the lowering of their content and antioxidant capacities.

Overall, samples at all maturity stages extracted by water showed significantly higher TPC compared to ethanolic extract. [20] demonstrated that, different solvent with different polarity possess significant effect on phenolic content and antioxidant since the Folin-Ciocalteu method give different response to different phenolic compound depending on chemical structure. Commonly, TPC of plant extracts increased by increasing polarity of solvent [5]. [21] reported that, water was the solvent which generated extracts with highest TPC from brown seaweed *Stypocaulon scoparium* than ethanol extract.

3.2. Total flavonoid content (TFC)

Results of TFC showed similar trend as TPC. The reduction of TFC as the maturity stages of *C. caudatus* leaves increased were analysed through scavenging and chelating process of $AlCl_3$ colourimeter method and the results of TFC for water and ethanolic *C. caudatus* leaves extract at different maturity stages are shown in Table 1. *C. caudatus* young leaves for both extracts demonstrated higher TFC content than mature and old leaves. The values ranging from 183.69 to 438.91 mg QE/g dried extract and 83.17 to 214.00 mg QE/g of dried extract respectively.

Table1. *Antioxidant content and antioxidant activity of C. caudatus leaves at different maturity stages using different extraction solvent.*

Sample		Assay			
		TPC (mg GAE/g of dried extract)	**TFC (mg QE/g of dried extract)**	**FRAP (µM TE/g of dried extract)**	**DPPH (µg/g of dried extract)**
Water extract	**Young leaves**	325.83 ± 7.22^{a}	438.91 ± 57.02^{a}	429.58 ± 34.86^{a}	326.17 ± 46.00^{c}
	Mature leaves	260.00 ± 2.50^{b}	221.07 ± 16.53^{b}	337.44 ± 36.56^{b}	470.89 ± 1.69^{b}
	Old leaves	205.83 ± 1.44^{c}	183.69 ± 14.17^{c}	241.74 ± 4.87^{c}	535.51 ± 40.24^{a}
Ethanol extract	**Young leaves**	149.33 ± 4.21^{d}	214.00 ± 6.61^{b}	313.77 ± 22.00^{b}	166.25 ± 11.87^{f}
	Mature leaves	99.00 ± 4.70^{e}	116.50 ± 2.50^{d}	240.00 ± 14.08^{c}	204.30 ± 5.42^{e}
	Old leaves	79.67 ± 5.33^{f}	83.17 ± 14.43^{e}	175.00 ± 33.37^{d}	242.21 ± 2.74^{d}

Values are expressed as mean ± standard deviation. Means with different letters are significantly different ($p<0.05$)

In agreement with [22], the flavonoid content followed the same trend as for the phenolic content, increasing maturity stages seems to decrease the total flavonoid content in the samples. [23] found that, the immature part of *Graptopetalum paraguayense* (E.) Walther exhibited highest flavonoid content while the mature part had the lowest flavonoid content. The lower level of flavonoids in mature and old plant might be due to overproduction of reactive oxygen species (ROS) and the declining of secondary metabolites in plants which leads to senescence of plant tissues [8]. [24] strongly believed that, the components of both the enzymatic and non-enzymatic antioxidant correlate well with oxidative stress during senescence and plant development.

The comparison between the extracts showed that water extract had significantly higher TFC compared to ethanolic extract for all samples tested. Table 1 clearly shows that the solvents used influence the extractability of the TFC in samples, which are selectively soluble in the solvents [25]. The higher TFC in *C. caudatus* leaves extracted using water could possibly due to the presence of

more water-soluble bioactive compounds in the samples. [26] emphasised that water will extract only water-soluble compounds. [20] revealed that *B. refusa* water extract has higher antioxidant activity than ethanolic extract.

3.3. Ferric reducing antioxidant power (FRAP)

In line with the declining in TPC and TFC, FRAP also showed significant decreased in values as the maturity progressed. Table 1 presented results for water and ethanolic *C. caudatus* leaves extract at different maturity stages. The FRAP assay is actually a method that directly measured the reductive ability of antioxidant by reacting ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to produce a coloured ferrous tripyridyltriazine (Fe^{2+}-TPTZ) where, the reducing properties are associated with the presence of antioxidant compounds in the extracts by breaking the free radical chain through donating of hydrogen atom [16]. The highest antioxidant activity is owned by *C. caudatus* young leaves followed by mature leaves and the lowest possessed by old leaves.

In *C. caudatus* young leaves, most of new biosynthesis took place at the early stages of plant growth which provide highest phenolic and flavonoid compounds [27]. Then, these compounds could act as reductone and react with ROS produced in the plants by converting them to a more stable products and terminating the radical chain reaction [10]. The ROS will be chelated and disengaged by the antioxidant in young leaves from participating in the initiation of oxidative stress [28]. [29] stated that, the part of flowering stage of *Opuntia microdasys* (Lehm) Pteiff give most powerful antioxidant activity compared to intermediate and fully mature stages.

The variation in the FRAP value between the extracts was notable to have significant difference to each other. The FRAP value ranged from 241.74 to 429.58 µM TE/g of dried extract and 175.00 to 313.77 µM TE/g of dried extract for water and ethanolic extract respectively. When compared with previous work by other researchers, water extract were recorded to have low content of phenolics and flavonoids than ethanolic extract [30]. However, in this study *C. caudatus* leaves extracted with water had significantly higher antioxidant activity compared to ethanol. This might due to the different extraction methods and chemical structures of plant compounds since phenolic compounds respond differently depending on the number of phenolic groups they have [25]. This study was confirmed by [5], where they stated that the phenolic contents in plants depend on plant species, ripeness, environmental conditions, post-harvest treatments and extraction method.

3.4. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The stable free radical DPPH has been used widely as the magnitude of the antioxidant capacity for determination of primary antioxidant activity in food materials particularly plant and fruit samples [31]. The DPPH scavenging activity is a kinetic antioxidant method which based on the reduction of DPPH• free radical into $DPPH_2$ by the action of antioxidant [32]. The EC_{50} value was determined from the plotted graph of scavenging activity against the concentration of extracts which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% [16]. The lowest EC_{50} value reflects strongest antioxidant power.

Based on Table 1, the highest EC_{50} value is possessed by *C. caudatus* young leaves with the value of 326.17 µg/g of dried extract for water and 166.15 µg/g of dried extract for ethanol. According to [33], total phenolics and flavonoids prevailed during the early maturity but their contents and antioxidant activity decreased with the advancing maturation. [22] revealed that, the radical scavenging effects in sample of *Capsicum chinese* Habanero on DPPH radical showed the highest activity at the younger stage while at the older stage the activity was lower.

In comparison between the extraction solvents, contradicted to TPC, TFC and FRAP results, *C. caudatus* leaves extracted using ethanol had higher antioxidant power compared to those extracted with water. Study by [26] also found similar trends, in which ethanolic *Tamarix aphylla* (L.) Karst extract possessed good DPPH scavenging capacity against free radicals than water extract. [10] reported that, DPPH can only solubilised in organic media, especially alcohol, but not in water, which is significant limitation when interpreting the role of hydrophilic antioxidant. [30] demonstrated that, solvents used for polyphenols extraction had significant effects on DPPH scavenging capacity.

3.5. Pearson's correlation coefficient

The correlation analyses by using Pearson's correlation coefficient were conducted to determine the interrelationship between antioxidant content (TPC and TFC) with antioxidant activity (FRAP and

DPPH) of *C. caudatus* leaves extract. The correlations are shown in Table 2. Regardless of extraction solvents, TPC and TFC showed strong correlation with antioxidant activity indicating that these phenolic and flavonoid compounds are major contributor in the antioxidant activity. [24] believed that these strong correlations are mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers.

Table2. *Pearson's correlation coefficient (R) between antioxidant activity and antioxidant content of C. caudatus leaves at different maturity stages using different extraction solvent*

Extraction solvent	FRAP vs TPC	DPPH vs TPC	FRAP vs TFC	DPPH vs TFC
Water extract	0.998	-0.987	0.922	-0.986
Ethanol extract	0.999	-0.998	0.971	-0.962

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

By comparing retention time and UV spectra with fourteen standards, twelve compounds were identified with remarkable difference in the amount between maturity stages and extraction solvents. Table 3 shows the amount of phenolic and flavonoid compounds presence in *C. caudatus* leaves extracts. Chlorogenic acid, ρ-coumaric acid and sinapic acid were detected as major compounds of phenolic acid while catechin, rutin, myricetin and quercetin were found to be the major flavonoid compounds in all samples. In agreement with previous studies, chlorogenic acid and quercetin were identified as the most enriched compounds that contributed to antioxidant activity in *C. caudatus* plant [34]; [35]. However, in another study, naringenin and kaempferol were not detected in *C. caudatus* leaves extracted by water and ethanol. Different sample preparation and extraction, climate differences, soil conditions and sample geographical areas used might be the possible explanation [36].

Table3. *Amount of phenolic and flavonoid compounds in C. caudatus leaves at different maturity stages using different extraction solvent.*

Extraction solvents		Amount of compound (µg/g of dried extract)					
		Water extract			Ethanolic extract		
	Sample / Compound	Young leaves	Mature leaves	Old leaves	Young leaves	Mature leaves	Old leaves
Phenolics	Gallic acid	38.76 ± 0.59^{a}	25.60 ± 1.12^{b}	15.33 ± 0.06^{d}	23.98 ± 2.18^{b}	17.97 ± 0.94^{c}	12.73 ± 0.40^{e}
	Chlorogenic acid	95.71 ± 3.02^{a}	64.92 ± 4.62^{b}	34.09 ± 1.16^{d}	54.29 ± 3.32^{c}	39.25 ± 2.60^{d}	21.40 ± 0.77^{e}
	Caffeic acid	6.50 ± 0.09^{a}	5.00 ± 0.20^{b}	3.32 ± 0.19^{d}	3.74 ± 0.04^{c}	1.95 ± 0.03^{e}	1.58 ± 0.04^{f}
	Vanillic acid	11.27 ± 0.91^{a}	11.17 ± 0.94^{a}	11.23 ± 0.60^{a}	6.80 ± 0.21^{b}	5.23 ± 0.13^{c}	5.15 ± 0.02^{c}
	ρ-coumaric acid	113.43 ± 1.33^{a}	45.38 ± 1.42^{b}	29.97 ± 0.06^{c}	13.15 ± 1.43^{d}	5.94 ± 0.29^{e}	3.07 ± 0.23^{f}
	Sinapic acid	86.72 ± 1.04^{a}	54.99 ± 1.17^{b}	23.12 ± 0.08^{c}	10.22 ± 0.93^{d}	6.03 ± 0.64^{e}	3.29 ± 0.27^{f}
	Ferullic acid	25.95 ± 0.29^{a}	17.84 ± 2.25^{b}	9.60 ± 0.01^{c}	5.59 ± 0.36^{d}	5.27 ± 0.27^{d}	5.95 ± 0.53^{d}
Flavonoids	Catechin	735.97 ± 2.76^{a}	356.31 ± 8.82^{b}	150.93 ± 2.05^{c}	49.27 ± 0.92^{d}	37.33 ± 0.18^{e}	22.66 ± 1.17^{f}
	Epicatechin	13.54 ± 0.51^{a}	13.05 ± 0.33^{a}	13.03 ± 0.29^{a}	10.51 ± 0.54^{b}	7.28 ± 0.43^{c}	6.75 ± 0.14^{c}
	Rutin	1318.09 ± 112.06^{a}	1024.64 ± 20.58^{b}	87.64 ± 3.10^{c}	140.88 ± 8.44^{d}	105.73 ± 13.61^{e}	76.73 ± 9.36^{f}
	Myricetin	814.84 ± 5.12^{a}	552.05 ± 2.38^{b}	404.29 ± 11.81^{c}	64.59 ± 0.63^{d}	39.84 ± 1.01^{e}	20.20 ± 0.96^{f}
	Quercetin	360.11 ± 33.31^{a}	279.23 ± 1.67^{b}	162.09 ± 15.15^{c}	137.78 ± 10.61^{c}	122.09 ± 3.38^{d}	102.11 ± 9.86^{e}
	Naringenin	nd	nd	nd	nd	nd	nd
	Kaempferol	nd	nd	nd	nd	nd	nd

Values are expressed as mean ± standard deviation. Means with different letters are significantly different ($p<0.05$)

As shown in Table 3, the concentration of each compound decreased as the maturity stages increased. According to [37], the higher levels of the compounds in the young leaves are in accordance with the role of secondary metabolites in a plant's defence mechanisms over the extensive production of ROS. For mature and old leaves, they possessed inadequate antioxidant to react with the ROS produced [24]. However, between the extracts, *C. caudatus* leaves at different maturity stages extracted using water showed significantly higher phenolic and flavonoid compounds than the ethanolic extract. The differences in concentration of these compounds were influenced by the polarity of extracting solvents and the solubility of these compounds in the solvent used for the extraction process [38]. [5] stated that, the characteristics of the extraction solvents noticeably affected TPC and antioxidant activity of the plants.

4. CONCLUSION

As a conclusion, maturity stages and extraction solvents significantly influenced antioxidant activity in *C. caudatus* leaves. From the study, *C. caudatus* young leaves exhibited highest antioxidant activity compared to mature and old leaves. However, all samples extracted using water possessed significantly higher antioxidant activity than ethanolic extract. Nevertheless, samples from both extraction solvents showed strong correlations between TPC and TFC and antioxidant activity representing that, major compounds that contributed to antioxidant activity in *C. caudatus* leaves are phenolics and flavonoids. It is proven through the identification and quantification of these compounds using HPLC where twelve from fourteen compounds were detected in these samples.

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