Wahyu Widowati:

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Dear Dr. Wahyu Widowati,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**The Effect of Antioxidant and Anticancer of Cosmos** caudatus **Extract and its Compounds on Cervical Cancer**". Our decision is: **Revisions required**.
This manuscript is interesting, but it needs to be corrected before it can be published in The Indonesian Biomedical Journal. For detail corrections, you can find it in the file attached.
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Jakarta, August 10, 2018

Dear Dr. Wahyu Widowati,
Thank you for your contribution to The Indonesian Biomedical Journal. In this opportunity, we would like to inform you that your manuscript "**The Antioxidant and Cytotoxic Effects of *Cosmos caudatus* Ethanolic Extract on Cervical Cancer**" is now available online in our Article in Press section.
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I hereby certify that I am authorized to sign this document either in my own right or as a part of my team.

The ~~Effect of~~ Antioxidant and Cytotoxic Effects ~~and Anticancer~~ of *Cosmos caudatus* Extract ~~and its Compounds~~ on Cervical Cancer

Abstract

Background: Oxidative stress is closely related to all aspects of cancer from carcinogenesis to tumor-bearing state leading to chronic inflammation, which could subsequently mediate most chronic diseases including cancer. *Cosmos caudatus* extract (CEE) has been proved to have antioxidant effect that inhibited cancer cell growth due to its bioactive compounds such as catechin, quercetin, and chlorogenic acid. This study aimed to ~~observe compounds contained in CEE and also~~ evaluate antioxidant and anticancer activity of CEE and its compounds.

Methods: Total phenol was measured according to the Folin–Ciocalteu method. Catechin, quercetin, and chlorogenic acid contained in CEE were identified by High ~~Profile~~ Performance Liquid Chromatography (HPLC). Antioxidant activity was evaluated by 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)-reducing activity, 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, and Ferric Reducing Antioxidant Power (FRAP) activity test. The cytotoxic activity of CEE was determined by MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay on HeLa cells.

Results: The result showed that total phenol of CEE was 181.64 ± 0.93 µg Cathecin/mg extract. ABTS-reducing activity test showed that catechin had the highest activity (2.90 ± 0.04 µg/ml), ~~whilst~~ while CEE had moderate activity compared to other compounds. FRAP activity test demonstrated that catechin had the highest activity (315.83 µM Fe(II)/µg) compared to other compounds. DPPH scavenging activity of CEE was 22.82 ± 0.05 µg/ml. Cytotoxicity test on HeLa cell showed that CEE had lower activity (IC_{50}= 89.90 ± 1.30 µg/ml) compared to quercetin (IC_{50}= 13.30 ± 0.64 µg/ml).

Conclusion: CEE has the lowest antioxidant activity compared to quercetin, catechin, and chlorogenic acid and has the lowest anticancer activity compared to quercetin. However, CEE and its compounds has potential as antioxidant and anticancer properties.

Keywords: Antioxidant, anticancer, catechin, *Cosmos caudatus*, quercetin.

Introduction

Initiation, promotion, and progression are the three multistages of cancer [1,2]. Oxidative stress is related to the cancer initiation and progression by increasing DNA mutations or inducing DNA damage, genome instability, and cell proliferation [3]. Wide spectrum of diseases, ~~including chronic inflammation~~ such as most type of cancer are involved the role of Reactive oxygen species (ROS) [4]. Imbalance between production of free radicals and reactive metabolites called oxidants or ROS are the sign of oxidative stress, leading to damage of important biomolecules and cells which potentailly affected on the whole organism [5].

Cosmos caudatus locally known as '*Ulam raja'* and widely used as traditional medicine in Southeast Asia, is a herb of the family Compositae. Some studies reported that *C. caudatus* contains some bioactive compounds such as ascorbic acid, quercetin, chlorogenic acid, and catechin ~~reported by some studies were contained by *C. Caudatus*~~. These natural compounds have been reported to be excellent antioxidants [6-8]. *C. caudatus* is suggested to have high antioxidant capacity, antidiabetic activity, antihypertensive properties, antiinflammatory responses, bone protective effect, antimicrobial activity and anticancer properties [9,10]. This research aimed to evaluate the ~~potent~~ antioxidant potency and ~~anticancer~~ cytotoxic effect of *C. caudatus* ethanol extracts. Therefore, we also used HPLC method to observe the compounds in the *C. caudatus* extracts based on standard compound [11].

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"**The Antioxidant and Cytotoxic Effects of Cosmos caudatus Extract on Cervical Cancer**"

Because:
- This study only elucidate total phenolic content
- Since the data only cytotoxic, it is better to state that instead of "anticancer".

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Commented [User4]: Catechin did not determine the anticancer activity.

Methods

Plant Extract Preparation

Leaves of *C. caudatus* ~~plants~~ were collected from Cihideung, Lembang, West Java, Indonesia. The plants were identified by herbarium staff, Department of Biology, School of Life Science and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. The *C. caudatus* simplicia (300 g) was extracted with ethanol 70 % using maceration technique. Ethanol filtrate was filtered, and waste was re-macerated in triplicate. Using rotary evaporator [IKA RV10, 172 NW Boulevard, USA] at 50 ˚C, the filtrate was concentrated to obtain extract. The extract was stored at -20 ˚C [12-14].

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High Performance Liquid Chromatography (HPLC) Assay

CEE chemical profiling analysis was performed using HPLC. Quantification of CEE was done using the standard chlorogenic acid [Chengdu Biopurify Phytochemical 327-97-9, China], catechin [Sigma Aldrich C1251, USA], and quercetin [Sigma Aldrich Q4951, USA]. HPLC analysis used the Hitachi Pump HPLC L-6200, Hitachi L-4000 UV detector and Reverse Phase Column C-18 (Phenosphere ODS-2, Phenomenex, 4.6 mm x 250 mm). Acetonytril 70% [Merck 100030, Germany] was used as mobile phase (isocratical) with flow rate of 1.0 ml/min. The samples were then dissolved in methanol 70% (1 mg/ml), filtered through a 0.22 µm syringe, and injected (20 µl) to the column. UV absorbance was measured at 254 nm [11].

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Total Phenolic Content Assay

Total phenolic content was measured according to the Folin–Ciocalteu method. Briefly 15 µl of samples was placed into microplate then added 75 µl of Folin-Ciocalteu's reagent (2.0 M), followed by 60 µl of sodium carbonate (7.5%). The mixture was incubated at 45 ˚C for 15 min [15,16]. Subsequently, absorbance value was measured at 760 nm using microplate reader [Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Waltham, MA, USA]. Total phenolic content expressed as Eugenol equivalent was calculated by the following formula:

$$C = \frac{c * V}{m}$$

C: total content of phenolic compounds (µg/mg) of *C. caudatus* in catechin equivalent;
c: the concentration of catechin established from the calibration curve (µg/ml);
V: the volume of extract (ml);
m: the weight of extract (mg).

2,2′-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)-reducing Activity Assay

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$ABTS^+$ solution was produced by reacting 14 mM ABTS and 4.9 mM potassium persulfate [Merck EM105091, USA] (1:1 volume ratio) for 16 h in dark condition at room temperature. The mixture was then diluted with 5.5 mM PBS (pH 7.4) until the absorbance of the solution was 0.70 ± 0.02 at wavelength 745 nm. In brief, 2 µl of various concentrations of sample (0.23, 0.47, 0.94, 1.88, 3.75, 7.50, 15.00 µg/ml; µM) was added to each well at 96-well microplate, then the fresh 198 µl $ABTS^+$ solution [Sigma Aldrich A1888, USA] was added. Then, the plate was incubated for 6 min at 30˚C and calculated its absorbance at 745 nm. The ratio of reducing $ABTS^+$ absorbance in the presence of the sample relative to the absorbance in the absence of the sample (negative control) was determined as the inhibition percentage of ABTS radical (%). The calculation of the median Inhibitory Concentration (IC_{50}) was also measured [14, 16, 17].

Ferric Reducing Antioxidant Power (FRAP) Assay

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The FRAP reagent was prepared by mixing acetate buffer (10 ml) 300 mM, ferric chloride hexahydrate [Merck 1.03943.0250, USA] (1 ml) 20 mM dissolved in distilled water, and 1 ml of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) [Sigma Aldrich 3682- 35-7, USA] 10 mM dissolved in HCl 40 mM. In 96-well microplate, 7.5 µl of various concentrations of sample (1.17, 2.34, 4.69, 9.38, 18.75, 37.50, 75.00 µg/mL; µM) was mixed with 142.5 µl FRAP reagent, and incubated at 37 ˚C for 30 min. The absorbance value was measured at 593 nm with a microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Waltham, MA, USA). The standard curve was created using $FeSO_4$, between 0.019 and 95 µg/ml $FeSO_4$. The measurement results were expressed in µM Fe(II)/µg extract [14,16,18].

2,2-Diphenyl-1-picrylhydrazil (DPPH) Scavenging Assay

The DPPH scavenging assay was used to measure the radical scavenging activity of the samples [14]. Samples (50 µl) with various concentrations were added to each well in a 96-well microplate. It was followed by addition of 200 µl of 2,2-Diphenyl-1-picrylhydrazil (DPPH) [Sigma D9132, USA] solution (0.077 mmol/L in methanol) into the well. The mixture was then incubated in the dark for 30 min at room temperature. The absorbance was read using a microplate reader [Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Waltham, MA, USA] at 517 nm wavelength. The radical scavenging activity was measured using the following formula:

$$\% \text{ Scavenging} = (Ac - As) / Ac \times 100$$

Ac = negative control absorbance (without sample).
As = sample absorbance.

~~Anticancer~~ Cytotoxicity Assay

The cervical cancer cells (HeLa- ATCC CCL-2) were obtained from Stem Cell and Cancer Institute, Jakarta, Indonesia. The cells were maintained in Dulbecco modified Eagle's medium containing 10% FBS [Invitrogen, California, USA], 100 U/ml penicillin [Invitrogen, California, USA], and 100 mg/mL streptomycin [Invitrogen, California, USA]. Then, the cells were incubated at 37 ˚C, 5% CO_2 [12]. Briefly, 5 x 10^3 of cells were seeded in 96 well-plates for 24 h [12, 13]. The medium was discarded, then 180 µl of fresh medium was added into each well. The cells was treated with 20 µl of *C. caudatus* ethanol extract in various concentrations (1000, 500, 250, 125, 62.5, 31.25, 16.125 µg/ml) and quercetin in various concentrations (200, 100, 50, 25, 12.5, 6.25, 3.125 µM). DMSO 10% was added in different well as blank. All samples and blank were set in triplicate and incubated for 24 h. Untreated cells were employed as control. MTS assay [Promega, Madison,WI, USA] was used to determine cell viability [12]. 20 µL MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] was added to each well. The plate was incubated in 5% CO_2at 37 ˚C for 4 h. The absorbance was measured at 490 nm with a microplate reader [Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Waltham, MA, USA]. The data was then presented as percentage of viable cells (%) [12, 13].

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Results

Total Phenolic Content

Total phenolic content of the sample was measured, this study show that CEE has total phenolic content is 181.64 ± 0.93 µg Cathecin/mg extract.

HPLC Assay

The compounds content of *C. caudatus* extract was evaluated using HPLC with quercetin, catechin, and chlorogenic acid as standard. Figure 1 shows that quercertin, catechin, and chlorogenic acid had retention time at 1.64 min, 1.40 min, and 1.30 min, respectively. CEE has

peak at 1.403 min, it is close with catechin peak (1.40 min) which was assumed as catechin. This HPLC assay indicated that CEE contained catechin compound.

ABTS-reducing Activity

ABTS-reducing activity of CEE, catechin, quercetin, and chlorogenic acid can be seen in Figure 2A and Table 1. Figure 2A shows ABTS-reducing activity in concentration-dependent manner, where higher concentration of sample increased ABTS-reducing activity. At the highest concentration of sample (15 µg/ml), catechin has the highest percentage of ABTS-reducing activity (73.53%) compared to quercetin (62.10%), CEE (24.94%) and chlorogenic acid (22.46%). This indicated that CEE had low ABTS-reducing activity among other compounds except chlorogenic acid.

Table 1 shows that catechin had the lowest IC_{50} value (2.90 ± 0.04 µg/ml) compared to quercetin (3.64 ± 0.05 µg/ml), chlorogenic acid (12.70 ± 0.76 µg/ml), and CEE (31.97 ± 1.42 µg/ml). This finding supported the result of ABTS-reducing activity that demonstrated the low activity of CEE.

FRAP Activity

FRAP activity of CEE, catechin, quercetin, and chlorogenic acid can be seen in Figure 2B. The antioxidant activity of CEE, catechin, quercetin, and chlorogenic acid were evaluated using FRAP activity assay. Catechin had the highest activity (315.83 µM Fe(II)/µg) compared to quercetin (306.00 µM Fe(II)/µg), chlorogenic acid (241.67 µM Fe(II)/µg), and CEE (18.33 µM Fe(II)/µg). This indicated that CEE had the lowest antioxidant activity compared to other compounds (Figure 2B).

DPPH Scavenging Activity

The median inhibitory concentration (IC_{50}) of DPPH scavenging activity of CEE, catechin, quercetin, and chlorogenic acid can be seen in Table 1. Table 1 shows that the IC_{50} value of DPPH scavenging activity of CEE (22.82 ± 0.05 µg/ml) indicated antioxidant activity through scavenging DPPH free radical.

Cytotoxic Activity

~~The relationship between CEE anticancer activity and HeLa cell viability can be seen in Figure 3~~. Figure 3 shows the correlation between CEE and quercetin concentration and its cytotoxicity on HeLa cell. Viability of cells decreased in concentration-dependent manner. The increased concentration was correlated with increased toxicity (<90% viable cells). The highest extract concentration (1000.00 µg/ml and 200.00 µM) demonstrated the lowest of viability of cells by CEE was 19.23% and quercetin 34.07%, respectively. CEE and quercetin can inhibited the growth of HeLa cancer cell line with minimum inhibitory concentration (IC_{50}) values 89.90 ± 1.30 µg/ml and 43.99 ± 2.15 µM (13.30 ± 0.64 µg/ml), respectively. This indicated that CEE had lower cytotoxicity compared to quercetin.

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Answer: Yes its correct, beacuse it has been conversion from µM to µg/ml

Discussion

C. caudatus has been known as a potential herb that has antioxidant and anticancer activity [19]. *C. caudatus* has been reported to have high antioxidant capacity, mainly due to its polyphenol content [7]. The rich-phenolic foods are the sources of natural antioxidants [19, 20]. The total phenolic content of CEE in this study was 181.64 µg Cathecin/mg extract. The result of other study showed that *C. caudatus* has high total phenolic content (1274 ± 98 GAE mg/100 g fresh weight) in the acetone/water system [7]. The aqueous extract of *C. caudatus* has also been known to have the highest phenolic content [21]. Other study showed that the total phenolic content of *C. caudatus*

ethanol extract (1144.6 mg/100g) was higher than *C. caudatus* water solvent (844.8 mg/100g) [22]. High phytochemical contents, antioxidants, proteins, amino acids, vitamins, and minerals are associated with risk reduction of free radical-related degenerative diseases [23].

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In this study HPLC analysis was evaluated to determine compounds content of *C. caudatus*. Quercetin, catechin, and chlorogenic acid were used as standards. Figure 1 shows that quercertin, catechin, and chlorogenic acid had retention time at 1.64 min, 1.40 min, and 1.30 min, respectively. CEE peaked at 1.403 min which was assumed as catechin. This indicated CEE contain catechin compound. Based on Noriham et al. (2015) study, *C. caudatus* ethanol extract measured by HPLC show presence of catechin [24].

ABTS-reducing activity of CEE had moderate activity compared to catechin, quercetin and chlorogenic acid. Based on the result above, CEE had moderate activity compared to catechin, quercetin and chlorogenic acid, meanwhile in previous study, CEE had the highest ABTS-reducing activity compared to other plants (4.71 µmol TE/g fw) [25]. High antioxidants activity of *C. caudatus* was associated with the ability to reduce oxidative stress [7]. Another study also proved that *C. caudatus* had extremely high antioxidant compared to other plants through total antioxidant capacity (AEAC value) [10].

In this research, DPPH scavenging activity of CEE (IC_{50}= 22.82 µg/ml), indicated that antioxidant activity through scavenging DPPH free radical. In our previous studies, DPPH values of catechin were 7.02 µg/ml [26] and 8.11 µM [27], while DPPH values of quercetin were 4.279 µg/ml [28], 3.244 µg/ml [29], and 19.200 µg/ml [21]. The FRAP activity value of CEE was the lowest among other compounds. Some studies reported that *C. caudatus* had greater antioxidant activity than *S. androgynus* (L) Merr and *C. asiatica* in DPPH and FRAP assays [30]. Other study showed that *C. caudatus* had the greatest FRAP activity among other plants [25], also CEE had the highest DPPH scavenging activity as supported by Andarwulan (2010), which was correlated with flavonoid content in the plants. *C. caudatus* had been reported to possess the highest flavonoid and phenolic content [25]. *C. caudatus* aqueous extract is a good source of antioxidant because it has the highest DPPH and FRAP values [10]. In other study, *C. caudatus* had the highest free radical scavenging potential extract (86.85%) [31]. *C. caudatus* extract also showed beneficial activities in reducing number of parameters such as peroxyl value as an antioxidant. Phenolic content in CEE plays a key role in scavenging free radicals which cause oxidative stress [19]. In addition, phenolic compounds have been shown to possess antioxidant ability which facilitates scavenging electrophiles and active oxygen species, slows down nitrosation and chelates metal ions to limit auto-oxidation, and increases the ability to adjust some enzyme actions [32,33].

Tumorigenesis occurs due to the increasing free radicals that lead to DNA damage and mutation, apoptosis inhibition, cell cycle/proliferation stimulation, and DNA repair inhibition [34]. The role of ROS in cancer development can be determined in three different stages. Firstly, generating DNA damage including mutations and structural alterations is the ROS first role, followed by the second stage which is the promotion stage where ROS blocks cell-cell comunication leading to abnormal gene expression and modification of second messanger, resulting in increased cell proliferation or decreased cell apoptosis. Last stage, furthermore, is the progression of cancer caused by oxidative stress affecting further DNA alterations [1]. Free radicals can react with membrane fatty acids and form lipid peroxides, accumulation of which leads to production of carcinogenic agents [35]. In this study, CEE had lower cytotoxicity on HeLa cell compared to quercetin. These results were confirmed by Lee and Vairappan (2011) that found a weak cytotoxic activity of the ethanolic extract of *C. caudatus* against P388 murine leukemia cells [9]. However, in other study, *C. caudatus* exhibited the highest DPPH free radical scavenging, ABTS-reducing activity, FRAP, and inhibition of linoleic acid [24].

Conclusion

C. caudatus and its compounds showed antioxidant activities as measured through ABTS-reducing activity, DPPH scavenging activity, and FRAP activity. CEE has the lowest antioxidant activity compared to quercetin, catechin, and chlorogenic acid. CEE also has the lowest cytotoxic activity compared to quercetin. However, CEE and its compounds has potential as antioxidant and anticancer properties.

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Acknowledgements

This study was supported by the Grants-in-Aid from Penelitian Unggulan, Riset Pembinaan Tenaga Kesehatan (2017), Ministry of Health, Republic of Indonesia. The author also thankful to Annisa Amalia, Yukko Arinta, Fajar Sukma Perdana, Ni Luh Wisma Ekayanti, Annisa Arlisyah, and Rismawati Laila Q from Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, Indonesia for their valuable assistance.

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Figure/Tables

Table 1.

Sample	ABTS-reducing Activity		DPPH Scavenging Activity	
	IC_{50} (µM)	IC_{50} (µg/ml)	IC_{50} (µM)	IC_{50} (µg/ml)
CEE	-	31.97 ± 1.42	-	22.82 ± 0.05
Catechin	10.00 ± 0.15	2.90 ± 0.04	-	-
Quercetin	12.04 ± 0.16	3.64 ± 0.05	-	-
Chlorogenic Acid	35.94 ± 2.14	12.70 ± 0.76	-	-

Table 2.

Sample	Cytotoxic Activity	
	IC_{50} (µM)	IC_{50} (µg/ml)
CEE	-	89.90 ± 1.30
Quercetin	43.99 ± 2.15	13.30 ± 0.64

Commented [User19]: It is better if the author can provide IC50 data for the compounds based on DPPH assay (if data available).
Answer :
Really we have measured DPPH scavenging activity of Catechin, Quercetin and Chlorogenic Acid and have been published in previous study. (I have mentioned this result discussion section)

Commented [User20]: Please added the standard deviation value.

Commented [User21]: It is better if the author can provide IC50 data for catechin and chologenic acid as well.

Commented [User22]: Please added the standard deviation value

Commented [User23]: Please added the standard deviation value

Figure 1.

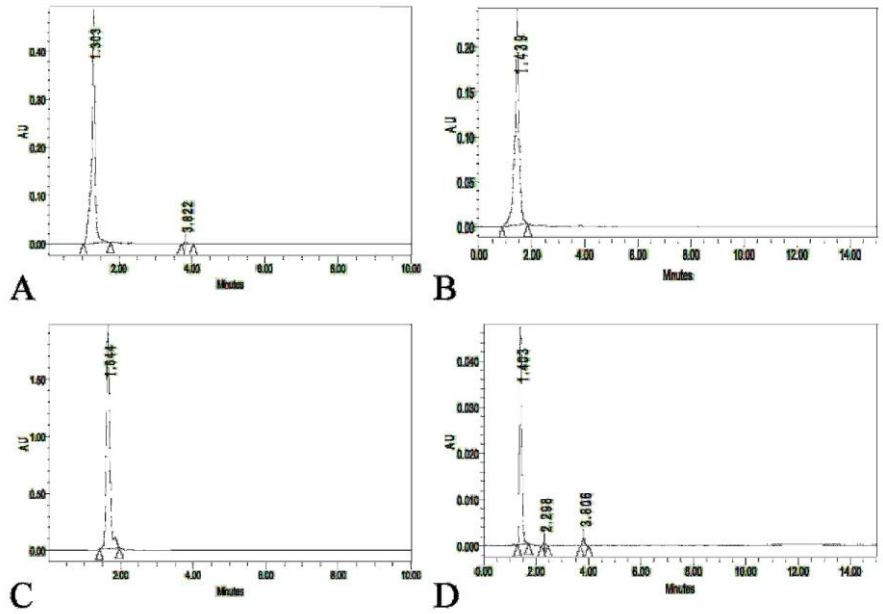
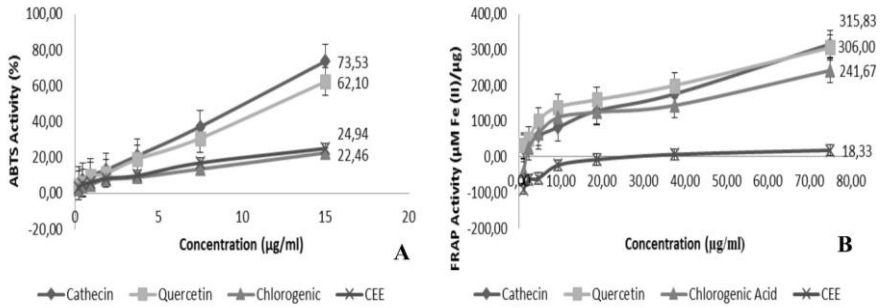
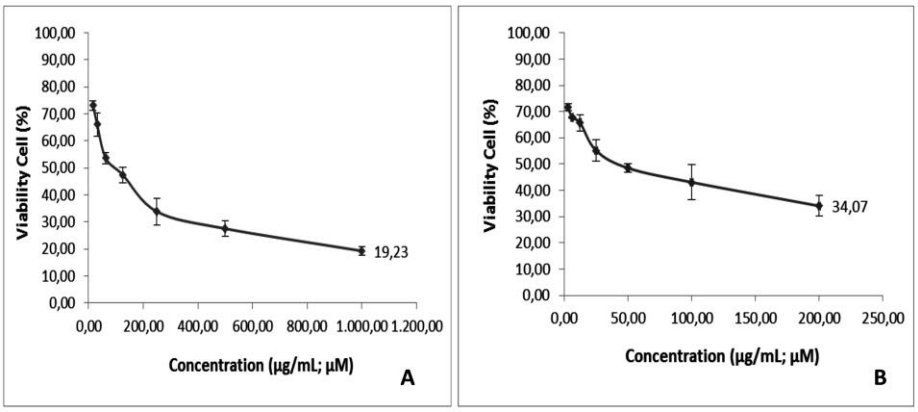

1.303
3.622
AU
Minutes
A
1.439
AU
Minutes
B
1.044
AU
Minutes
C
1.493
2.298
3.606
AU
Minutes
D

Figure 2.



Commented [User24]: Both of these assays aimed to investigate the antioxidant effects. However, the results for cholorgenic acid were contradicitive in those two assays. How can the author explain this?
Answer :
These methods are different mechanism
ABTS reducing activity : Chlorogenic Acid reduce $ABTS^{*+}$ to ABTS is lowest than all samples
FRAP assay : Chlorogenic Acid reduce of Fe^{3+} to Fe^{2+} is higher than CEE

Figure 3.



Commented [User25]: It will be better if the control cell (0 concentration of CEE/catechin) is set as 100% cell viability.

Table and Figure Legends

Table 1. IC_{50} Value of ABTS-reducing Activity of CEE, Catechin, Quercetin, and Chlorogenic Acid

*CEE= *C. caudatus* Ethanolic Extract, ABTS= 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid). The data was presented as mean ± standard deviation. The ABTS-reducing activity assay were measured in triplicate for each sample.

Table 2. IC_{50} value Cytotoxicity HeLa Cells of CEE and Quercetin

* CEE= *C. caudatus* Ethanolic Extract. IC50 of CEE and quercetin was presented as µg/ml and µM, respectively. The data was presented as mean ± standard deviation. This research was conducted in triplicate for each treatment.

Figure 1. Chromatogram of Extract and Compounds with HPLC. A) Chlorogenic acid, B) Catechin, C) Quercetin, D) CEE

*CEE= *C. caudatus* Ethanolic Extract, HPLC= High Performance Liquid Chromatography. This research was conducted in triplicate for each treatment.

Figure 2. ABTS and FRAP Activity of CEE, Catechin, Quercetin, and Chlorogenic Acid

*CEE= *C. caudatus* Ethanolic Extract, ABTS= 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid), FRAP= Ferric Reducing Antioxidant Power. This research was conducted in triplicate for each treatment. CEE, catechin, quercetin, and chlorogenic acid in ABTS assay were diluted in DMSO to reach the final concentration of 0.23; 0.47; 0.94; 1.88; 3.75; 7.50; 15.00 (µg/ml for CEE and µM for compounds), while in FRAP assay were diluted in DMSO to reach the final concentration of 1.17; 2.34; 4.69; 9.38; 18.75; 37.50; 75.00 (µg/ml for CEE and µM for compounds).

Figure 3. Viability of HeLa Cell of CEE and Quercetin. A) CEE, B) Quercetin

*CEE= *C. caudatus* Ethanolic Extract. This research was conducted in triplicate for each treatment. CEE was diluted in DMSO to reach the final concentration of 16.125; 31.25; 62.50; 125.00; 250.00; 500.00; 1000.00 (µg/ml); Quercetin was diluted in DMSO to reach the final concentration of 3.125; 6.25; 62.50; 12.50; 25.00; 50.00; 100.00 200.00 (µM).

Commented [User26]: Figure legend should contain more information, such as the methods, at least generally. i.e. incubation time for the treatment, assay that had been used, etc…

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