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Antimicrobial Activity against Pathogenic Bacteria, Antioxidant and Cytotoxic Activity of *Cosmos Caudatus*

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Abstract- *Cosmos caudatus* is a traditional medicinal herb commonly found in Southeast Asia. This herb has been reported as a potential antioxidant and antimicrobial agent as it contains a variety of bioactive compounds. However, its cytotoxic effects on cancer still lack empirical evidence. Thus, this study aimed to investigate antimicrobial and antioxidant potentials of *Cosmos caudatus* as well as its cytotoxic effects on cancer cell lines. Ethanol and water extracts of *Cosmos caudatus* were tested for its antimicrobial activities against pathogenic bacteria, *Staphylococcus aureus*, *Salmonella sp., Pseudomonas aeruginosa* and *Escherichia coli* using agar well diffusion method. Antioxidant properties of the extracts were evaluated by 2,2-diphenyl-1-picryl-hydrazyl radical scavenging assay while cytotoxic activity of the extracts on colorectal carcinoma cell line, HCT 116 was determined by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The findings indicated that conventional Soxhlet extraction with water as solvent extracted more efficiently than ethanolic maceration and ultrasonic assisted extraction. In antioxidants assay, both ethanolic and water extracts obtained through ultrasonic extraction exhibited the highest DPPH free radical scavenging capacity compared to maceration and Soxhlet extraction method. As for antimicrobial assay, both water and ethanol extracts showed no inhibitory action against the tested pathogenic bacteria. For anticancer activity, the F2, F7 and F8 fractions showed potent dose dependent cytotoxicity on HCT 116 cells with IC_{50} value of 15.53 ± 0.4, 32.72 ± 0.3 and 34.16 ± 1.4 µg/mL, respectively.

Indexed Terms- *Cosmos caudatus*, antimicrobial activity, antioxidant effect, cytotoxic effect

I. INTRODUCTION

Plants kingdom plays significant roles in the nutrition and sustainability of human life [1]. Based on many research projects, scientists have realised the splendid diversity of secondary metabolites found in plants as important potential biological compounds for the development of natural pharmaceutical products [2]. Malaysia is one of the richest countries in the world in terms of biodiversity. The nation has been blessed with various species of plants and in fact, is the home to one of the oldest rainforests in the world. Medicinal plants have been gaining attention because of the medicinal and nutritional benefits provided by their potential natural properties in amelioration numerous diseases. Important medicinal plants used in Malaysian traditional and modern medicine include *Zingiber officinale*, *Coleus aromaticus*, *Citrus grandis* L. Osbeck, *Amaranthus spinosus* L. and *C. caudatus* [3,4].

C. caudatus, which is known as *Ulam Raja* locally, is an annual herb of family *Asteraceae and* it can grow up to 3 meters in height and usually bears pink or purple flowers [5]. *C. Caudatus* is considered as a ubiquitous and well-known salad in Malay cuisine. It is normally served raw as a condiment due to its aroma and often being used as a food flavouring [3,6]. Through Malaysian Dietary Guidelines 2010, the Ministry of Health of Malaysia has promoted the consumption of *C. caudatus* due to its wide array of medicinal benefits [5]. Studies have demonstrated that *C. caudatus* has many pharmacological properties such as anti-diabetic, anti-inflammatory, anti-hypertensive, anti-osteoporosis as well as antibacterial activities through *in vitro* and *in vivo* studies [3,6]. Various medical advantages of this plant are generally

due to its rich sources of bioactive compounds including carbohydrate, protein, minerals, vitamins phenolic and flavonoids, quercetin, ascorbic acid and chlorogenic acids [7].

C. caudatus is also considered to have potential as a cure for various cancers. Flavonoid compounds, especially quercetin produced from endophytic bacteria can prohibit breast, colon, prostate, ovarian, endomentrial and lung cancers [8,9]. Nevertheless, there are limited investigations on *C. caudatus* as a plant which capable of treating cancer. More studies on *C. caudatus* potential in anticancer treatment should be carried out as the practice of medicine. It has the potential to be anticancer agent due to the fact the it possesses phytochemicals which act as antioxidants. There are numerous studies of *C. caudatus* concerned with its antimicrobial activity as well as its free radical scavenging activity, total phenolic content and total antioxidant status by *in vitro* techniques.

Extraction is a significant step for separating active parts from the inert components by treating with a specific solvent [10]. It aims to provide the maximum yield of extracts and produce the high concentration of target compounds with the highest quality [11]. The obtained extract may be further processed and fractionated in order to isolate particular chemical entities for characterization. The extraction of plants results in obtaining compounds with biological properties [12]. The commonly employed methods for extraction of phytochemicals from plants include maceration and Soxhlet extraction. These are the conventional extraction which initially involve diffusion of solvent into the plant cells, followed by the solubilization of bioactive compounds within the plant matrix and eventually diffusion of phytochemical-rich solvent out of the plant cells [13]. However, great improvements can be achieved with the use of advanced extraction method such as ultrasound-assisted extraction. The improved features of this 'green' extraction method often offer low consumption of solvent, short extraction time and high extraction yield [14].

Malaysia is rich in flora and fauna resources while major of them are underexplored and the findings on potential of medicinal products from these resources are limited. This is one of the reasons to carry out this study. There are plenty of herbs and plants that pose various medicinal properties which can be processed into medicinal products for curing different diseases. For instance, many studies are now actively committed to search the best remedy to treat diseases especially cancer. One alternative method for curing cancer is by using herbal medicine and there is an increasing trend of using medicinal products that derived from plants and herbs. However, various plant-based products in Malaysia which purchased by hospital and pharmacies are imported from other countries. This issue has to be addressed as it can influence the country's economy.

One of the aim of this study was to extract the leaves of *C. caudatus* by three different extraction methods, namely maceration, Soxhlet and ultrasonic. Furthermore, it also aims to investigate the antimicrobial activity of water, ethanolic and methanolic extracts of *C. caudatus* against *Staphylococcus aureus (S. aureus), Salmonella, Pseudomonas aeruginosa (P. aeruginosa) and Escherichia coli (E .coli)* as bacteria strains and evaluate the antioxidant potential and cytotoxic effects against human colorectal carcinoma cells (HCT 116).

II. METHODOLOGY

2.1 Chemicals

Ethanol, methanol, dimethyl sulfoxide (DMSO), phosphate buffered saline (PBS), hydrochloric acid (HCl), sodium hydroxide pellets, Dulbecco's modification of Eagle medium (DMEM), sodium bicarbonate, Penstrep, fetal bovine serum (FBS), trypsin-EDTA, trypan blue solution, nutrient broth, nutrient agar, 2,2-diphenyl-1-pocrylhydrazyl (DPPH), ascorbic acid. All the chemicals were obtained from the Chemistry Laboratory in Universiti Malaysia Pahang, Faculty of Chemical and Process Engineering Technology.

2.2 Plant material

C. caudatus was purchased from a local market in Kuantan, Pahang. The digestible part of fresh plants was cleaned and washed under running tap water.

2.3 Preparation of C. caudatus extracts

The collected leaves were dried at 50 ℃ for three days until complete removal of moisture [7]. The dried leaves (152 g) were ground into powder and extracted three times using different extraction methods (maceration, Soxhlet and ultrasonic) with water and ethanol as solvents. The extracts were filtered and concentrated by distillation and freeze-drying processes. The dried extracts were stored in the dark at 4 ℃ until further utilization. The extraction yield was calculated using the following Equation (1).

$$Y_T\ (\%) = \frac{Total\ mass\ of\ extract}{Total\ mass\ of\ sample} \times 100 \quad (1)$$

2.3.1 Maceration extraction

The technique used by Cheng et al. [5] was adopted with slight modification. A 10 g of plant sample was weighed and immersed in a 200 ml of solvent. The plant materials were extracted with organic solvents in an incubator shaker at constant stirring of 120 rpm for 2 h. Then, it was filtered using Whatman No. 1 filter paper.

2.3.2 Soxhlet extraction

The samples of dried leaves, roots and stems were removed from the refrigerator and kept at room temperature (28°C) for 30 minutes to gently release the water vapour from the samples. This was done to retain the quality and amount of oil content in its original condition. A total of 8 grams of dried samples were placed into thimble. The cotton was placed into the thimble to ensure the presence of samples inside the thimble during the experiment while 240 ml water was placed in the flask of Soxhlet apparatus as extraction solvents. After this, the thimble containing the sample was placed into the extraction chamber. Lastly, the condenser was placed on top of the extraction flask and all these parts were fixed vertically. The temperature of the process that corresponded to boiling point of solvent was used and the extraction time was set for 24 hours. The process was repeated until the colour of chamber become colourless. Upon completion of the extraction process, the volume of the mixture in boiling flask was measured.

2.3.3 Ultrasound-assisted extraction

Ultrasound-assisted extraction was carried out as the procedure developed by Yang & Zhang [15] with slightly modification. In this study, BANDELIN Sonorex Digitec ultrasonic bath was used as an ultrasound source. The bath is a rectangular container with 35 kHz transducers which are annealed at the bottom. The bath power rating was set at 100W on the scale of 1-9. The extraction of *C. caudatus* desirable compounds was performed by adding 4 g of ground dried leaves into 100 mL of solvent on a 250 mL conical flask. The flask was partially immersed into the ultrasonic bath with 2 L of water. Water in the ultrasonic bath was circulated and regulated at constant desired temperature to prevent the rising of water temperature caused by ultrasonic exposure.

2.4 Antimicrobial activity

2.4.1 Bacterial strains

A total of four microbial strains were used in the study. *Staphylococcus Aureus*, *Salmonella*, *Pseudomonas aeruginosa*, and *Escherichia coli* which were taken from Microbiology laboratory of Universiti Malaysia Pahang, Faculty of Chemical and Process Engineering Technology (Pharmaceutical) were used in this study. The strains were kept in Microbiology laboratory and cryopreserved at -80 ℃.

For experiments, bacteria were inoculated onto Mueller Hinton agar plates and incubation was performed at 37 ℃, under anaerobic conditions for a day in incubator (Ecotron, Swirtzerland). After harvesting the bacteria, the bacterial suspensions were prepared in the sterilized centrifuge tubes containing Mueller Hinton broth and the inoculum, which contained a loopful of a single colony for each tested strain. The concentration of bacterial suspensions was adjusted according to turbidity of 0.5 McFarland standard (0.06-0.10). The density of bacterial suspensions was determined by an UV-Vis spectrophotometer.

2.4.2 Agar well diffusion method

The agar well diffusion method was employed for this assay. The antimicrobial assay used by Balouiri et al. [16] was adopted with slight modification. The Mueller Hinton agar plate surface was inoculated by spreading 100 µl of microbial inoculum over entire agar surface. After that, a hole with a diameter of 4mm was punched aseptically with a sterile dropper and a volume (80-100 µl) of the plant extracts at desired concentration (75, 100, 125 and 150 mg/ml) was added into these holes separately. Then, the agar plates were incubated at 37 ℃ for a day. After incubation, the inhibition zones were measured and the total mean numbers were calculated for the extracts.

2.5 Antioxidant activity

2.5.1 DPPH radical scavenging activity

The DPPH radical scavenging activity assay used by Chan et al. [17] was adopted with slight modification. A 0.004% (w/v) of DPPH solution was prepared by dissolving 4 mg of DPPH in 100 ml of 95 % methanol in a dark room. Extract (2 mg) was dissolved in 2.5 methanol in order to prepare 800 µg/ml solution. After that, serial dilution of extract was performed by mixing 100 µl of methanol and 100 µl of extract into 96-well plate. Then, 150 µl of DPPH solution was added into each well. A 100 µl of methanol was added into 150 µl of DPPH solution to be prepared as a control. Ascorbic acid was used as standard in this assay. The mixture was then left to stand in the dark for 30 min. The absorbance of the resulting solution was measured at 517 nm by microplate reader system. The scavenging activity of each extract on DPPH radical was calculated as a percentage using Equation (2).

$$Scavenging\ activity = (1 - \frac{Absorbance\ of\ sample\ at\ 517nm}{Absorbance\ of\ control\ at\ 517nm}) \times 100 \quad (2)$$

2.6 Cytotoxicity analysis

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was performed in 96-well plate to determine cell viability based on previous study [18]. The $1x10^4$ cells were seeded in each well in 100 µl culture medium. After overnight incubation, 100 µl of fresh medium containing different concentrations of fractions or the positive control (F1 - F10 and betulinic acid) were added and further incubated for 48 h. Next, MTT with the concentration of 0.5 mg/mL was prepared in RPMI medium and the old medium was aspirated before 100 µL of MTT was added to each well. After 5 h incubation at 37˚C, supernatant was aspirated and 200 µL dimethyl sulfoxide (DMSO) added into each well. The absorbance (OD) was measured at 570 nm by microplate reader. The cell viability was calculated using Equation (3). IC50 values were determined from the respective dose response curves ± SD (n=3).

$$Cell\ viability\ (\%) = \frac{OD570\ samples\text{-}blank}{OD570\ negative\ contol\text{-}blank} \times 100 \quad (3)$$

III. RESULTS AND DISCUSSION

3.1 Extraction yield of Cosmos caudatus extracts

In order to investigate the optimum conditions for extraction, the effect of three variables (extraction method, solvent to solid ratio and extraction time) on yield were analysed as shown in Table 1.

Table 1: Percentage yields of three different extraction methods on *C. caudatus*

Extract (g/ml)	Percentage yields of three different extraction methods (%)		
	Maceration	Soxhlet	Ultrasonic
Sample-to-solvent ratio	1:50	1:30	1:25
Time (h)	2	24	1
Water	16.78 ± 1.57	20.62 ± 0.77	14.11 ± 1.21
Ethanol	5.81 ± 0.59	9.36 ± 1.21	8.98 ± 1.29

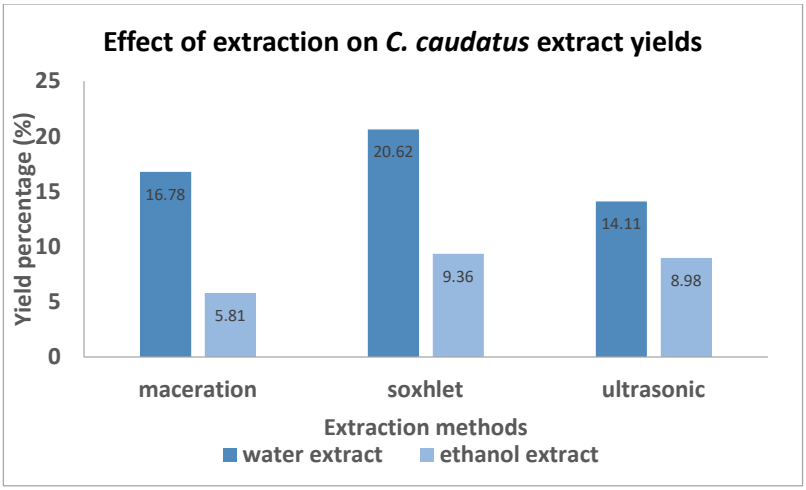

Figure 1: Effect of extraction methods of *C. caudatus* extract yields

The result of percentage yield of three different extraction methods is presented in Table 1 and Figure 1. It revealed that the yields of *C. caudatus* using Soxhlet extraction method were 20.62 % and 9.36 % for water and ethanol extracts respectively. While ultrasonic assisted extraction showed lowest value (14.11%) for water extracts among three extraction methods and yield a total of 8.98% for its ethanol extract. Percentage yields of maceration for water extract and ethanol extract were 16.78 % and 5.81 % respectively. The percentage yield differs from three of the methods may be due to some parameters such as solvent polarity, extraction time and temperature which can affect the extraction efficiency of a compound. The highest percentage yields of extraction method of *C. caudatus* was 20.62% from Soxhlet extraction with water.

3.2 Antimicrobial activity of C. caudatus extracts

In the present study, antimicrobial activity of the extracts and DMSO (control) were carried out by agar well diffusion method. The antimicrobial activity of the extracts was found at 75-150 mg/ml concentration range. The inhibition zones of bacteria were in the range of 6 to 12.5 mm. The results revealed that ethanol extract was potentially effective in suppressing the microbial growth of bacteria with variable potency. However, it was found that aqueous extract did not show any inhibitory effect on the tested microorganisms and thus no antimicrobial effect. Ethanol extract was found as the most effective extract in 75-100 mg/ml against *P. aeruginosa* by comparing with three different extraction

methods. There was only ultrasonic extraction using ethanol had antimicrobial effect against *S. aureus* at extract concentration of 100 mg/ml and no inhibition zone being observed for ethanol maceration and Soxhlet extraction technique. This indicated that ultrasonic extraction was more efficient in extracting the desired bioactive compounds for antimicrobial effect against *S. aureus*. Figure 2 showed that ethanolic extraction of *C. caudatus* using maceration at 100 and 125 mg/ml extract concentration and UAE at extract concentration of 75, 125 and 150 mg/ml had antimicrobial activity against *S. sp.* However, ethanolic extraction of *C. caudatus* by Soxhlet had no antimicrobial activity against *S. sp.* Soxhlet extraction was more effective in extracting bioactive compounds that exhibited antimicrobial activity against *E. coli* as this method showed inhibitory action at 75 and 100 mg/ml concentration.

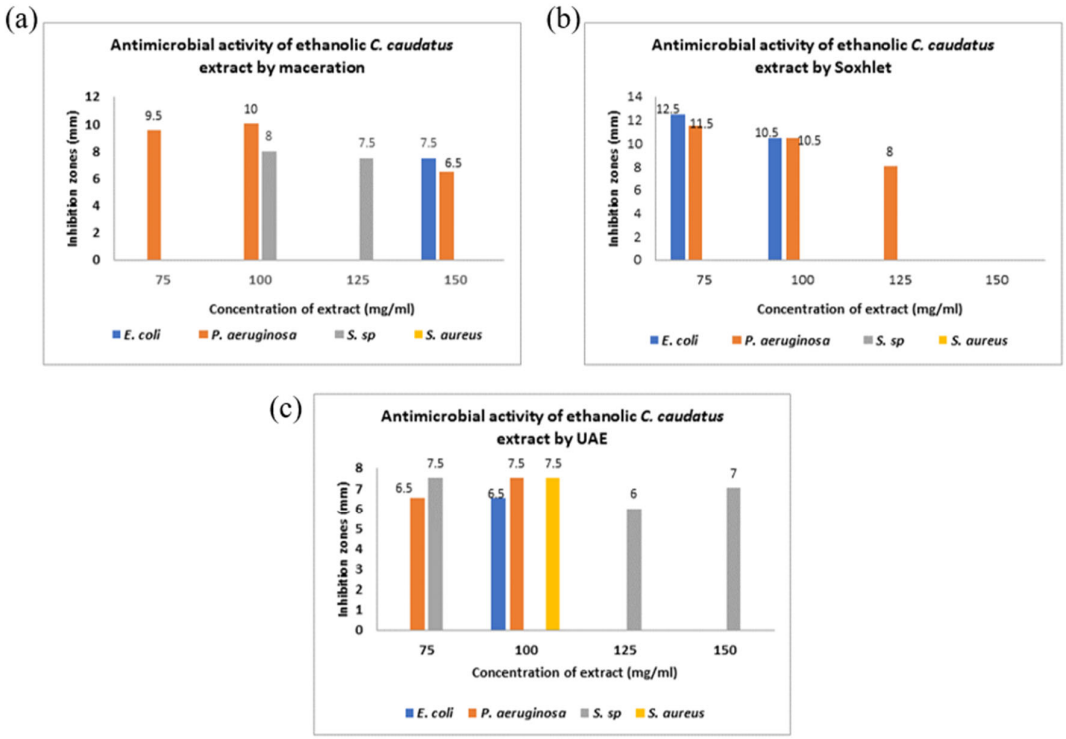

Figure 2: Antimicrobial activity of ethanolic *C. caudatus* by different extraction techniques: (a) maceration; (b) Soxhlet; (c) UAE

DMSO (solvent used for dissolving ethanolic extract of *C. caudatus*) was used as a negative control by agar well diffusion method. The inhibition zones exhibited by DMSO was tabulated in Table 2.

Table 2: Diameter of inhibition zones by DMSO as control

Pathogenic bacteria	Diameter of inhibition (mm)
E. coli	9.5 ± 0.71
P. aeruginosa	13 ± 1.41
S. sp.	12.5 ± 0.71
S. aureus	12 ± 1.41

Values represent as mean ± standard deviation for duplicate experiments.

Generally, antimicrobial effect of the extracts increases according to concentration [19]. However, the results from this research did not aligned with this statement. The inhibition zones being observed in this study for ethanol extract were mostly due to the solvent (DMSO) itself and not the ethanol extract of *C. caudatus*. This was because inhibition zones were present for the controls of this test, indicating that DMSO produced the inhibitory action on the tested bacteria strains. This might be caused by the synergistic effect between the solvent used and ethanol extract samples which resulted in the formation of inhibition zones. Lower concentration of ethanol extract sample (75 mg/ml) used lesser amount of solvent as compared to that of higher concentration of extract (150 mg/ml). The extract at 75 mg/ml diluted with higher amount of solvent and thus produced bigger diameter of inhibition zones as compared to the higher concentration of extract which diluted with low amount of solvent. When there was low amount of solvent used in the extract sample, the solvent produced little inhibitory effect towards the susceptible pathogenic bacteria and hence resulted in small diameter of inhibition zones.

3.3 Antioxidant activity of C. caudatus extracts (DPPH assay)

In this study, DPPH is used to assess antioxidant activity of water and ethanol extract extracts *C. caudatus in vitro*. Reduction of this radical by hydrogen-donating antioxidant is monitored as a decrease in optical density [20]. DPPH assay showed an increase in concentration increased the free radical scavenging activity for reference standard, ascorbic acid and crude aqueous and ethanolic extracts of *C. caudatus* as illustrated in Figure 3 and Figure 4. The DPPH assay of present study showed a dose dependent manner for standard and both the extracts. From the results illustrated in Figure 3 and Figure 4, the highest DPPH scavenging activity was shown in extracts obtained from the highest concentrations (400 µg/ml).

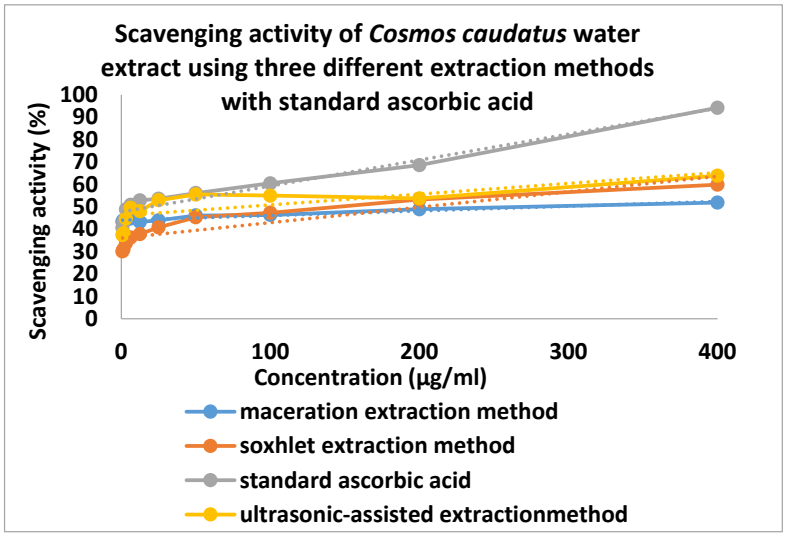

Figure 3: DPPH scavenging activity shown by standard and crude water extracts of *C. caudatus*

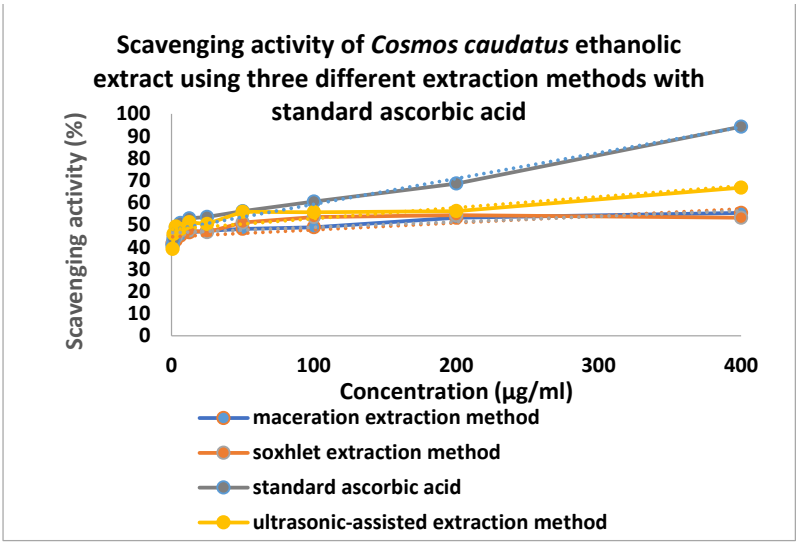

Figure 4: DPPH scavenging activity shown by standard and ethanolic extracts of *C. caudatus*

The results of the DPPH free radical scavenging assay suggest that plant of *C. caudatus* have potent antioxidant property which may be due to presence of various phytochemicals in the extracts mainly phenols and flavonoids. Percentage of DPPH scavenging activity of aqueous and ethanolic extracts using three different methods were comparable with standard ascorbic acid respectively and ultrasonic technique was reported to have highest scavenging activity among three extraction methods for both water extract and ethanol extract. For its aqueous extract 83.85 µg/ml was required to scavenge 50% of DPPH radical while it required less than 50 µg/ml ethanolic extract to scavenge 50% of DPPH radical. This may be due to the ultrasonic method involved the use of ultrasound in which the mechanic effect of acoustic cavitation from the ultrasound increases the surface contact between solvents and samples and permeability of cell walls. Physical and chemical properties of the materials subjected to ultrasound are altered and disrupt the plant cell wall which will facilitate the release of compounds and enhance mass transport of the solvents into the plant cells. This was followed by the Soxhlet extraction technique and maceration extraction technique. Among three extraction methods, maceration was reported to have the lowest scavenging activity for both aqueous and ethanol extracts which it required 286.71 µg/ml and 170.54 µg/ml to scavenging 50 % of DPPH radical as shown in Table 3.

Table 3: IC_{50} for aqueous and ethanol extract of *C. caudatus* using three different extraction methods

Extract	IC_{50} value (µg/ml)		
	Maceration	Soxhlet	Ultrasonic
Water	286.71	199.04	83.85
Ethanol	170.54	81.84	42.49

Similar DPPH scavenging activity was noted for the standard, aqueous and ethanolic extract of the plant from 0.7815 µg/ml to 400 µg/ml concentration. At the highest concentration (400µg/ml) used for the study, the DPPH scavenging activity of the standard was 94.22 µg/ml with IC_{50} value of 19.974 µg/ml. A lower IC_{50} value denotes a higher antioxidant activity. The results demonstrated that all extracts required only low extract concentration (< 1mg/ml) to decrease 50 % of the DPPH [5].

3.4 Cytotoxicity activity of C. caudatus extracts

The cytotoxic effects of *C. caudatus* extracts were analyzed on HCT 116 cells and Vero cell act as control. The cytotoxic effects of them were evidenced by MTT cell proliferation assay which measures cell viability based on mitochondrial enzymes in living cells. the percentage of viable cells was calculated based on MTT test. The result showed that ethanolic extract through ultrasonic extraction is the most potent cytotoxic extract with lowest percentage of viable cells with 68.12 % (Table 4) and hence, it has been selected for further studies.

Table 4: Percentage of viable cells after treatment of *C. caudatus* extracts

Extracts	% cell viability
Water (maceration extraction)	87.52
Water (Soxhlet extraction)	80.83
Water (ultrasonic extraction)	78.57
Ethanol (maceration extraction)	101.45
Ethanol (Soxhlet extraction)	96.81
Ethanol (ultrasonic extraction)	68.12

The ethanolic extract was then fractionated into 10 fractions (Table 5) and fractions with highest cytotoxicity were evaluated for apoptosis and anti-tumorgenicity on HCT 116 human colorectal carcinoma cells.

Table 5: Yield collected for each fraction from the fractionation of *C. caudatus*

Fraction	Mobile phase	Ratio	Total solvent used (mL)	Yield (mg)
F1	Hexane	100 %	500	6.3
F2	Hex:$CHCl_3$	3:1	300	2.7
F3	Hex:$CHCl_3$	1:1	300	51.1
F4	Hex:$CHCl_3$	1:1	300	271.2
F5	Hex:$CHCl_3$	1:3	300	51.2
F6	$CHCl_3$	100 %	300	142.1
F7	Hex:EA	1:1	300	92.1
F8	Hex:EA	1:3	300	90.5
F9	EA	100 %	300	118.1
F10	Methanol	100 %	300	1307.0

Cell viability was measured following 48 hours at five different concentration points (6.25, 125, 25, 50,100 µg/mL). Analysis of the response curves indicated that IC_{50} values for F2, F7 and F8 were 18.53 ± 0.4, 32.72 ± 0.3 and 34.16 ± 1.4 µg/mL and higher than 50 µg/mL for the other 7 fractions (n = 3) (Figure 6). Betulinic acid was used as positive control. Figure F1-F10 showed that fractions 2, 7 and 8 exhibited strong cytotoxic activity in a dose dependent manner (Figure 5). The apoptotic effects of fraction 2, 7 and 8 were studied on early and late markers of apoptosis including loss of mitochondria and chromatin and nuclear condensation. During the early process of apoptosis, it can be observed that

the cell shrinkage and pyknosis were visible through light microscopy. This caused the cells are smaller in size, the cytoplasm is dense, and the organelles are more tightly packed. Pyknosis is the most characteristic feature of apoptosis result from chromatin condensation. The apoptotic cell exists as a round or oval mass with dark eosinophilic cytoplasm and dense purple nuclear chromatin fragments. The apoptotic cells produced loss of cellular adhesion to the substrate and most cells even detached from the surface of the tissue culture dishes plate that appeared floating in the culture medium [21]. Visualization of the control (untreated) cells showed that the cells maintained their original morphology from containing several nucleoli. Most of the control cells were adherent to the tissue culture dishes.

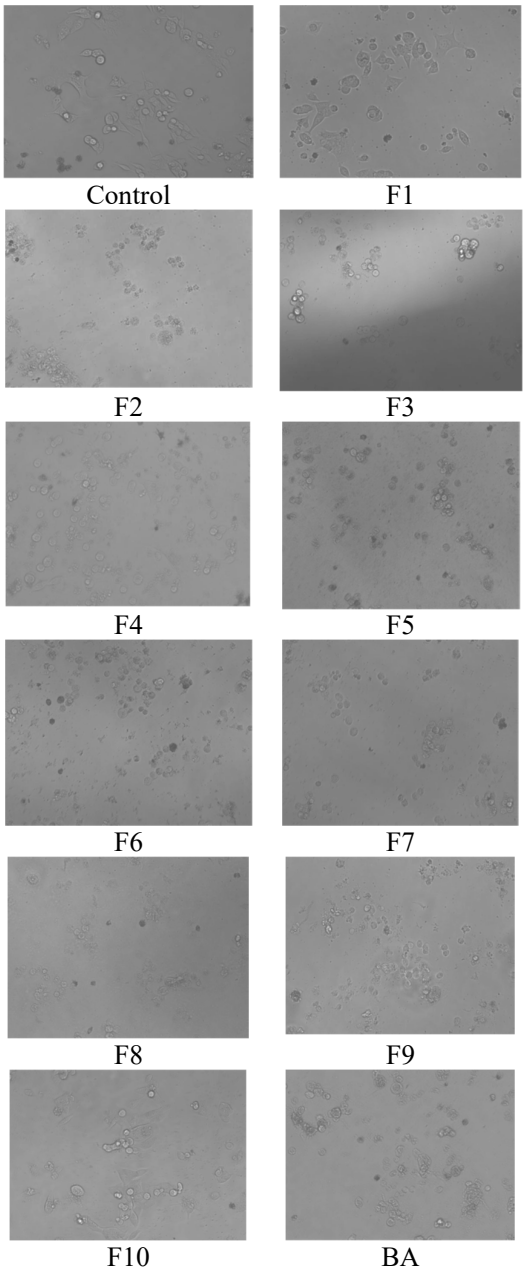

Figure 5: Cell viability of HCT 116 after treated with Control, F1, F2, F3, F4, F5, F6, F7, F8, F9, F10 and BA fraction of *C. caudatus* extract.

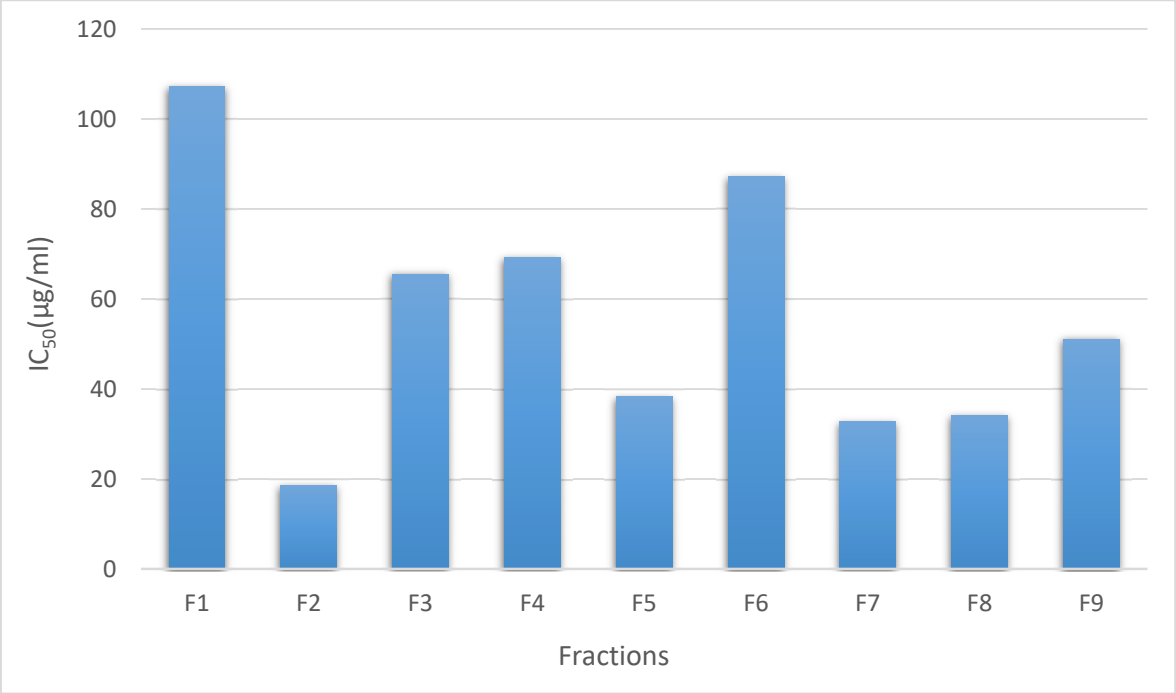

Figure 6: IC_{50} of *C. caudatus* fractions on HCT 116 cells

IV. CONCLUSION

Ethanol extracts of *C. caudatus* exhibited stronger antioxidant activities in comparison to that of water extracts. It is suggestive that the consumption of *C. caudatus* leaves plays a vital role in preventing the formation of free radicals and thus reduce the damages led by these radicals. Both the water and ethanol extracts showed no antimicrobial activity against the studied pathogenic bacteria. For cytotoxicity activity, the most active extract which was ethanol extract and F2, F7 and F8 showed apoptosis on HCT 116. Further studies on anticancer using different cancer cell line and the isolation and characterization of active principles of *C. caudatus* by HPTLC fingerprint should be carried out in order to provide more information on this plant and its bioactivity for future utilization.

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REFERENCES

[1] Grusak, M. A., & DellaPenna, D. (1999). Improving the Nutrient Composition of Plants To Enhance Human Nutrition and Health. *Annual Review of Plant Physiology and Plant Molecular Biology*, *50*(1), 133–161.
[2] Mitra, D. R., Orbell, P. J., & Muralithran, D. M. S. (2007). Medicinal Plants of Malaysia. *Agriculture*, *11*(2), 105–110.
[3] Bunawan, N., Baharum, S. N., Bunawan, S. N., Amin, N. M., & Noor, N. M. (2014). Cosmos Caudatus Kunth: A Traditional Medicinal Herb. *Global Journal of Pharmacology*, *8*(3), 420–426.
[4] Sultana, N., Alsarhan, A., Al-Khatib, A., & Kadir, M. (2014). Review on Some Malaysian Traditional Medicinal Plants with Therapeutic Properties. *Journal of Basic & Applied Sciences*, *10*(April), 149–159.

[5] Cheng, S.H., Barakatun-Nisak, M.Y., Anthony, J. & ismail, A. (2015). Potential medicinal benefits of Cosmos caudatus (Ulam raja): A scoping review. Journal of Research in Medicinal sciences, 20(10), 1000-1006.
[6] Moshawih, S., Cheema, M. S., Ahmad, Z., & Amiruddin Zakaria, Zainul Amiruddin Zakaria, M. N. H. (2017). A Comprehensive Review on Cosmos caudatus (Ulam Raja): Pharmacology , Ethnopharmacology , and Phytochemistry. *International Research Journal of Education and Sciences (IRJES)*, *1*(1), 14–31.
[7] Mediani, A., Abas, F., Khatib, A., & Tan, C. P. (2013). Cosmos Caudatus as a potential source of polyphenolic compounds: Optimisation of oven drying conditions and characterisation of its functional properties. *Molecules*, *18*(9), 10452–10464.
[8] Ramadhan, F., Mukarramah, L., Risma, F. A., Yulian, R., Annisyah, N. H., & Asyiah, I. N. (2018). Flavonoids From Endophytic Bacteria of Cosmos Caudatus Kunth. Leaf As Anticancer and Antimicrobial. *Asian Journal of Pharmaceutical and Clinical Research*, *11*(1), 200.
[9] Ren, W., Qiao, Z., Wang, H., Zhu, L. & Zhang, L. (2003). Flavonoids: Promising anticancer agents. *Medicinal Research Review*, 23(4), 519-534.
[10] Victor Kuete. (2017). Medical spices and vegetables from Africa: therapeutic potential against metabolic, inflammatory, infectious and systemic diseases. Academic Press
[11] Tan, M. C., Tan, & Ho, C. W. (2013). Effect of Extraction Solvent System, Time, and Temperature on Total Phenolic Content of Henna Stems. *International Food Research Journal*, *20*(6), 3117–3123.
[12] Fornari, T., Vicente, G., Vázquez, E., García-Risco, M. R., & Reglero, G. (2012). Isolation of essential oil from different plants and herbs by supercritical fluid extraction. *Journal of Chromatography A*, *1250*, 34–48.
[13] da Silva, R. P. F. F., Rocha-Santos, T. A. P., & Duarte, A. C. (2016). Supercritical fluid extraction of bioactive compounds. *TrAC - Trends in Analytical Chemistry*, *76*, 40–51.
[14] Dent, M., Dragović-Uzelac, V., Penić, M., Brñić, M., Bosiljkov, T., & Levaj, B. (2013). The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in dalmatian wild sage (Salvia officinalis L.) extracts. *Food Technology and Biotechnology*, *51*(1), 84–91.
[15] Yang, Y., & Zhang, F. (2008). Ultrasound-assisted extraction of rutin and quercetin from Euonymus alatus. *Journal of Science, 15*, 308–313.
[16] Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79.
[17] Chan, E. W. C., Lim, Y. Y., & Omar, M. (2007). Antioxidant and antibacterial activity of leaves of Etlingera species (Zingiberaceae) in Peninsular Malaysia. *Food Chemistry*, *104*(4), 1586–1593.
[18] Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*, 55-63.
[19] Bali, E. B., Acik, L., Akca, G., Sarper, M., Elci, M. P., Avcu, F., & Vural, M. (2015). antimicrobial activity against periodontopathogenic bacteria, antioxidant and cytotoxic effects of various extracts from endemic Thermopsis turcica. *Asian Pasific Journal of Tropical Biomedicine*, *4*(7), 505–514.
[20] Albert, J., Kevers, C., Pincemail, J., Olivier, J., & Dommes, J. (2012). Extraction conditions can greatly influence antioxidant capacity assays in plant food matrices. *Food Chemistry*, *130*(4), 986–993.
[21] Elmore, S. (2007). Apoptosis: A review of programmed cell death. *National Institute Health*, 35(4), 495-516.