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COMAPARATIVE IN VITRO EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES ON STEMS AND LEAVES EXTRACTS OF *COSMOS CAUDATUS*

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Abstract:

Currently, people are looking forward to discover the beneficial content of natural resources such as marines and herbs that might have potential in enhancing healthy living. Naturally, Cosmos caudatus is one of the promoting herbs that had been introduced long time ago by the ancient communities, furthermore in Malaysia it is traditionally been used in the treatment of few ailments such as hypertension, osteoporosis, diabetic and inflammation. Even tough, there is a previous study that has been reported on its antimicrobial and antioxidant activity in leaves of C.caudatus, however there is no proven information on comparative evaluation of the plant parts for the above said activities. Thus, an effort was made in the present study to compare and evaluate the antimicrobial and antioxidant activities of methanolic extracts obtained from different parts of C.caudatus such as stems and leaves. The selected plant parts were extracted using maceration method using methanol as solvent. Methanolic extracts of stems and leaves were tested for its antimicrobial activity against Staphylococcus aureus and Escherichia coli using Mueller Hinton Agar plate. Antimicrobial activity of stems and leaves extracts were found with minimum zone of inhibition 7.16 mm ± 0.23 and 6.78 mm ± 0.57 respectively only against S.aureus with the concentration 100 mg/ml and 200 mg/ml independently.However, both extracts showed negative results for gram negative organism. Moreover, the extract was evaluated for its free radical scavenging activity using DPPH assay. Leaves methanolic extracts of C.caudatus showed free radical scavenging activity (IC_{50} value) at the concentration range of 31.35-62.5 µg/ml whereas stems extract recorded 125-250 µg/ml. Nevertheless, the standard ascorbic acid showed potential antioxidant activity compared to sample extracts. To conclude the study findings, antimicrobial activity was found more susceptible against gram positive microorganism like S.aureus with stems extract compared to leaves extract and in free radical scavenging activity the leaves methanolic extract recorded more potential antioxidant properties when compared to stems extract of C.caudatus.

Keywords: *Cosmos caudatus, Staphylococcus aures, Escherichia coli, Mueller Hinton Agar Plate, DPPH.*

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INTRODUCTION:

Cosmos caudatus (Kunth) was Malaysians well-known salad named 'ulam raja', eating raw habitual have been practiced by ancient people and was carried till today. Luckily, behind this unique creation, beneficial properties obtained from the plant would contribute good impact in healthy life, specifically in ailments such as bone mineral density problem, improving blood circulation, decreasing high blood pressure. The other few well-known ethno botanical uses of this herb was known to possess anti-infective and as antiaging agents [1]. The *Cosmos caudatus* was a shrub from the asteraceae family and was believed to have its native distribution from Mexico, Caribbean, Central and South America. The preferred climate zone for the plant was sub-tropical or monsoonal and the plant can grow upto 3 m tall with purple or pink flowers and has triangular to ovate leaves with 10-20 cm long which occur in pairs and are finely divided into many lance-shaped segments. The unique taste of shoots makes it flavourable to be consumed in raw salad-like form it also has a quite pleasent odour [2]. The plant was shown in Figure1. The plant was claimed for its potential antioxidant and antimicrobial activity which was reported by its pharmacological activity screening. However, majority of those studies were conducted by testing on the leaves of *C. caudatus.* Thus, the current study was attempted on the comparison of antimicrobial and antioxidant activities between stems and leaves parts of *C. caudatus* inorder to know their effectiveness.

According to World Health Organization (WHO), infectious disease was defined as a type of disease that caused by pathogenic microorganisms such as bacteria, viruses, fungi and parasites. Infectious is classified as communicable disease as it can be easily transmitted through various modes such as direct contact, droplets, airborne including contaminated food, water and medical equipment's. Food-borne illness mainly caused by direct infection from food-borne pathogens and microbial toxins that contaminated food. Although majority cases are mild and self-limiting, it may worsen and severe the condition. Some food-borne bacterial strain may produce toxin which causing mild symptoms of vomiting to severe condition of neuroparalytic. The pathogens are including *Staphylococcus aureus, Clostridium botulinum* and *Bacillus cereus* (Infectious disease).

Free radicals are a type of a highly reactive metabolite that is naturally produced by body as a result of normal metabolism and energy production. It clearly determined the significance of aging process and act as natural biological response to environmental toxins like cigarette smoke, sunlight, chemicals, cosmic and manmade radiation. Thus, it is a key feature of pharmaceutical drugs development in the mean of antioxidant mechanism. Free radicals can severely affect DNA which may lead to several diseases such as cataract and cancer.

C. caudatus was a popular growing herb in many countries including Malaysia which gave beneficial effects on human health such as in treating ailment. Furthermore, the whole plant parts showed presence of terpenes, flavonoids, carbohydrates, amines and carboxylic acids. The leaf of *C. caudatus* showed the presence of phenolic acids and flavonoids while phenylpropane was found in the root parts [1].

The study on phytochemical screening of 14 different families of Malaysian ulam and fruits namely *Parkia speciosa* (petai), *Solanum torvum* (terung pipit), *Pithecellobium Bubalinum* (kerdas), *Moringa oleifera* (kacang kelor), *Dryobalanops oblongifolia* (keladan), *Cosmos caudatus* (ulam raja), *Mentha arvensis* (pudina), *Ocimum* sp. (selasih), *Cymbopogon nardus* (serai wangi), *Eugenia polyantha* (serai kayu), *Barringtonia scortechinii* (putat), *Musa* sp. (pisang), *Talinum paniculatum* (akar som) and *Phyllanthus acidus* (cermai) was selected and the samples were subjected to test the presence of alkaloid, saponin, steroid, phenolic, flavonoid and terpenoid content on stems, leaves, barks, fruits, seeds and seeds coat part. The results revealed that phytochemical substances were present in different part of the selected plant species. High content of alkaloids were found in *M. oleifera* leaf and fruit of *D. oblongifolia* and *P. bubalinum.* All the samples studied showed positive reactions of saponins except in bark and seed of *P. speciosa* and stem of *P. acidus.* Phytochemical screening on steroids, phenolics, flavonoids and terpenoids content showed these active compounds were present in the leaves of *C. caudatus, M. arvensis, Ocimum* sp., *M. oleifera* and *B. scortechinii.* Specifically, all the investigated screening bioactive constituents in this study were existed in *C. caudatus* leaves extract [3].

The effect of *Cosmos caudatus* (Kunth) extract on the number of microflora in chicken meat was studied.in Methanol extraction of *C. caudatus* leaves at different concentration (0.00%, 0.05%, 0.50%, 5.00%) by dilution method with exposure times (0, 5, 10, 15 min) were used to treat raw chicken meat that was obtained from wet market and supermarket. The results showed the number of microorganism in both chicken meat from different location has no significant difference. Total Plate Count of *Bacillus cereus, Escherichia coli* and *Staphylococcus aureus* of chicken meat source from wet market were 6.17 ± 0.02, 6.16 ± 0.02, 5.90 ± 0.05, 6.46 ± 0,09 respectively while 6.12 ±

0.01, 6.04 ± 0.06, 5.97± 0.04 and 6.46 ± 0.00 respectively for chicken meat from supermarket origin. Concentrations and exposure times of *C. caudatus* were effect on reduction number of microflora. *C. caudatus* extraction at 0.05% concentration with 10 min of exposure time gives the significant starting reduction number of microflora. This showed reasonable result for development *C. caudatus* extract as natural sanitizer for rinsing raw food materials such as chicken meat [4].

The antibacterial activities of ethanol and water extracts of leaves from *Cosmos caudatus, Murayya koenigii, Melicope lunu, Pereskia bleo, Jasminum sambac* and *Centella asiatica* against bacteria identified from a spoilage of yellow alkaline noodle was attempted to extend its shelf life by applying local plants extracts. The isolated and identified spoilage bacteria of yellow alkaline noodle were *Bacillus pumilus, Clavibacter agropyri, Corynebacterium urealyticum, Corynebacterium jelkelum, Enterobacter cloaceae, Pseudomonas aeruginosa, Serratia marcescens* and *Staphylococcus sciuri*. Ethanol extracts of *M.koenigii* was the most effective against all the bacteria studied as it possess the highest antibacterial activity represented by zone of inhibition ranging from 15.47 to 20.63 mm. *C.caudatus* extracts showed no antibacterial activity against *C.agropyri* and *C.jelkelum* but with low degree of inhibition against other bacteria strains [5].

The antimicrobial potential of *C. caudatus* leaves against two gram positive bacteria; *Bacillus subtilis* and *Staphylococcus aureus*, two gram negative bacteria; *Escherichia coli* and *Pseudomonas aeruginosa*, and one fungi; *Candida albicans* by disc diffusion method was stuided. The crude extractions were obtained from n-hexane, diethyl ether, ethanol and phosphate buffered saline. Inhibition of n-hexane, diethyl ether and ethanol extracts against all tested microbes were proven except phosphate buffered saline extract has inactive against *B. subtilis* and *S. aureus* in the preliminary antimicrobial screening. By using microtiter plate method, minimum inhibition concentration (MIC) values for test crude extracts were observed from 6.25-25 mg/ml. Potential source of crude extracts of leaves *C. caudatus* as new antimicrobial agents to treat infections caused by tested microbial strain was proven in this study. Furthermore, the results gave strong evidence of its utility in folk medicine [6]. The study was conducted by selection of 32 medicinal plants used in Malaysian traditional medicine for gastrointestinal disorder and wounds including *C. caudatus*. Disc diffusion and agar dilution methods was used in tested extracts against *H. pylori* by using petroleum ether, chloroform and methanol extractions of *C. caudatus* leaves, the inhibition zone diameter were 16.0 ± 0.6 (0.4), 11.7 ± 0.5 (29.2) and 23.0 ± 0.9 (2.1) respectively. However, the highest inhibition zone diameter was presented by *Derris trifoliata* Lour stem; 42.0 ± 0.9 (87.5), 47.0 ± 1.7 (117.5) and 47.0 ± 0.9 (5.0) respectively with the same extraction [7].

A study was conducted on the different maturity of *C. caudatus* leaves as raw materials in herbal tea to test effect of antioxidant capacities. The study was conducted at different maturity of young, mature and old stages. The method used to analyse the properties are Total Phenolic Content (TPC), FerricRreducing/Antioxidant Power (FRAP), Ferric thiocyanate (FTC) and Thiobarbituric acid (TBA) and DPPH radical scavenging assays. The results showed significantly strong antioxidant activity of young leaves for all assays tested compared to mature and old leaves. Strong positively correlation with reducing power exhibited by TPC and TFC while inversely correlated with DPPH scavenging activity indicates that these compounds are major contributors to antioxidant activity. Therefore, it was recommended to use young leaves for herbal tea preparation since it possessed good antioxidant activity as proven the properties reduced along with increase maturity [8]. A study conducted on investigating the antioxidant, antibacterial and cytotoxic activities of essential oils and ethanol extracts of *Cosmos caudatus, Artemisia argyi, Centella asiatica* and *Polygonum hydropiper*. Gas chromatography mass spectrometry was used to analyze the essential oils with a total of 57 types of volatile organics were identified. *C.caudatus* contained only 19 types of volatiles which the lowest amount among these four traditional herbs. Ethanol extracts of *C.caudatus* and *P.hydropiper* exhibited stronger antioxidant activities in comparison to *A.argyi* and *C.siatica*. TPC of ethanol extracts analyzed by Folin-Ciocalteu method were in the range of 31.58 ± 3.08 to 84.03 ± 8.15 mg GAE/100 g. FRAP of the four ethanol extracts were in the range of 26.58 ± 110 to 50.08 ± 0.71 mg AAE/g while free radical scavenging activity were in the range of 654.43 ± 17.22 to 5857. 54 ± 164.13 mg AA/100 g. However both these assays showed negative results by essential oils on all four species. Only ethanol extracts of *C.caudatus* and *A.argyi* showed activity against P388 murine leukemia cell line while essential oils were inactive. 60 to 80% and 20 to 50% of ten strains of human pathogenic bacteria tested were inhibited by ethanol extracts and essential oils of these herbs [9].

The free radical activity of methanolic plant extracts from *C. caudatus* (ulam raja), *Polygonum minus* (kesum), *Oenanthe javanica* (selom),

Centella asiatica (pegaga) and *Murraya koenigii* (curry leaf) was assessed by using Total Phenolic Content(TPC), FerricRreducing/Antioxidant Power (FRAP), Ferric thiocyanate (FTC)and Thiobarbituric acid (TBA) tests on the selected plants. *M.koenigii* had the highest yield extraction (1.65%), highest TPC (38.60 mg TAE/100 g fresh weight) and antioxidant activity (70.60%) using FTC method. Increased in FRAP for all methanolic extracts tested were affected by increasing in concentration of extracts. *C.caudatus* exhibited the highest antioxidant effect in TBA analysis [10]. *Cosmos caudatus* is a promising natural source that is rich in therapeutic agents such as antimicrobial and antioxidant properties. Thus, the findings of this study may contribute to prove ascertain of folk claims regarding treatment of infectious disease and its strong antioxidant properties on the comparison parts of *Cosmos caudatus*.

MATERIALS AND METHODS:

Collection and Identification

Five kilograms of *C. caudatus* plant was collected from local market of Parit, Perak, Malaysia. The plant was authenticated by the botanist Mr. Suhaimi at plant biosecurity division Pulau Pinang, Malaysia and the herbarium was recorded and stored for future reference. The herbarium was shown in Figure 2. Leaves and stems were separated and was washed under running tap water to remove the dirt or residues if any, upon partial drying the stems were cut into small pieces. The collected leaves and stems was left air-dry for overnight at room temperature. Furthermore, to achieve maximum drying the samples were subjected to hot air oven at 40°C for 2-3 days. Dried leaves and stems was grounded into coarse powder using dry blender, packed and stored respectively in an air tight container for further study.

Fig 1:*C.caudatus* plant

Fig 2: Herbarium of *C.caudatus*

Extraction

Each 400 g of coarsely powdered dried leaves and stems were macerated in 2000 ml of methanol as solvent in a closed glass container and allowed to stand for 7 days at room temperature, with frequent agitation. Upon the desired days of extraction was achieved, the mixture was filtered through muslin cloth, the marc was pressed for complete extraction, the collected filtrate was further filtered to remove the residues using whatman filter paper. The collected filtrate of both extracts was subjected to rotary evaporator under control pressure and temperature to evaporate the excess solvent. The obtained crude extracts from leaves and stem extracts was weighed, packed in a glass bottle and stored respectively in refrigerator until further study.

Antimicrobial Assay

The test extracts for the study was diluted with the mother solvent inorder to obtain the desired concentration for the study. Agar disc diffusion method was carried out as qualitative analysis to determine the antibacterial effect of *C. caudatus* extracts by determining the zone of inhibition. The empty disc was impregnated aseptically with 15 µl concentrations of each extracts and allowed to dry before placed on inoculated commercial Mueller-Hinton agar (MHA) plates. The bacterial strains used in this study was *S.aureus* and *E.coli*, which was inoculated in Petri dishes containing selective nutrient agar and incubated overnight for seeding at 37°C. The positive control to determine sensitivity of isolated strains used was tetracycline while negative control was methanol. The resulting diameters of clear zone of inhibition reflect to the

antibacterial activities and were recorded. All the assays were carried out in duplicate.

Free radical scavenging assay using DPPH

Antioxidant was proven to have an inhibitory or delaying effect during oxidation process. This in vitro method is based on inhibition whereby samples were added to the generating system and the antioxidant activity is measured indirectly by measuring the inhibition of the free radical action. Both the extracts and standard ascorbic acid were tested for its in vitro antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) using free radical scavenging assay. The final concentration of the extract and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25 and 15.625 µg/ml. The absorbance was measured using spectrophotometer against the corresponding blank solution.

The percentage of free radical scavenging was calculated using the following formula.

Scavenging activity % = $(A_{control} - A_{sample}) / A_{control} \times 100$

$A_{control}$: absorbance of control reaction

A_{sample} : absorbance in present extract

IC_{50}, which is the concentration of the sample required to inhibit 50% of free radicals was calculated.

DPPH Assay

The present study on estimation of free radical scavenging activity of leaves and stem extracts of C. *caudatus* on 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical was analyzed using standard assay method.

Preparation of Reagents

2, 2-Diphenyl-1-picryl hydrazyl solution (DPPH, 100 µg):

22 mg of DPPH was weighted accurately and added into100 ml of methanol. 18 ml from the stock solution prepared was pipetted out using 10ml pipette and diluted to 100 ml with methanol to obtain 1000 µg DPPH solution.

Preparation of Extract Solutions

21 mg of each extracts was weighed and dissolved in 1 ml of freshly distilled $DMSO_4$ to obtain solutions of 21 mg/ml concentration. These solutions were serially diluted separately to obtain the lower concentrations.

Preparation of Standard Solutions

21 mg of ascorbic acid was weighed and dissolved in 1 ml of freshly distilled $DMSO_4$ to get 21 mg/ml concentration. These solutions were serially diluted separately to obtain the lower concentrations.

To 2 mL of DPPH solutions was added separately to 100 µl of each test and standard concentration solution. The solutions were incubated at 37 ^{o} C for 30 min and the absorbance of each solution was measured at 490 nm using UV spectrophotometer.

RESULTS AND DISCUSSION:

The nature and percentage yield of both the extracts were shown in Table 1.

Antimicrobial Activity

Leaves and stems extracts of *C.caudatus* showed different degree of antimicrobial activity against both *S.aureus* and *E.coli* bacterial strains. The antimicrobial activity against *S.aureus* can be observed from stem extract at the concentration of 100 mg/ml with minimum zone of inhibition of 7.16 mm ± 0.23 while for leaf extract, minimum zone of inhibition is 6.78mm ± 0.57 at the concentration of 200 mg/ml. The gram positive bacteria *S.aureus* is more susceptible compared to gram negative of *E.coli.* The negative results were dedected against *E.coli* at all test concentrations with both stem and leaf extracts of *C.caudatus.* The figure 3-6 represents the activity of the test extracts on the *S.aureus* with different concentrations. The readings on zone of inhibition against both tested organisms against the extracts were shown in Table 2.

Table 1: Nature and Percentage Yield of the Extract

Extract	Nature	Percentage Yield w/w
Leaves extract of *C.caudatus*	Darkish green	13.5%
Stem extract of *C.caudatus*	Yellowish green	4.8%

Fig 3: Zone of inhibition 50 and 100 mg/ml of leaf extract against *S.aureus*

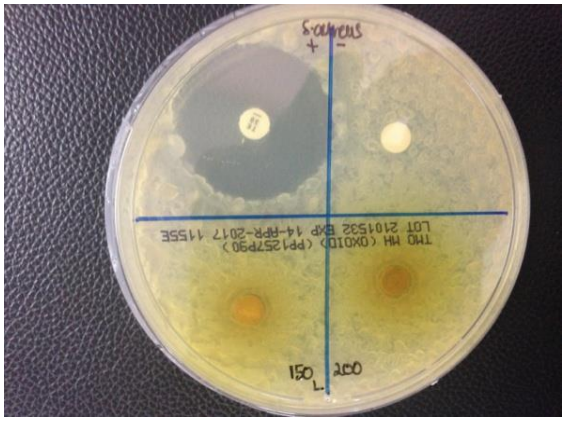

Fig 4: Zone of inhibition 150 and 200 mg/ml of leaf extract against *S.aureus*

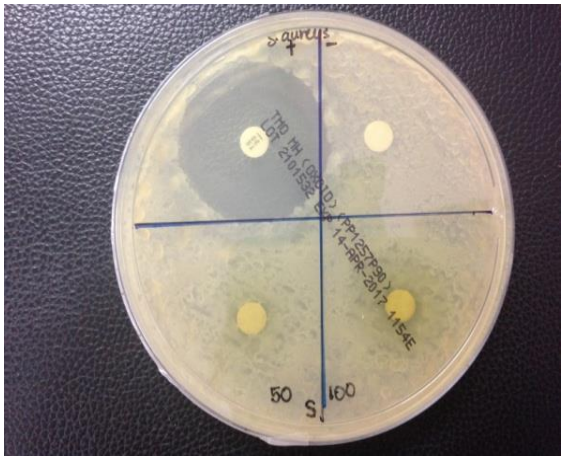

Figure 5: Zone of inhibition 50 and 100 mg/ml of stem extract against *S.aureus*

Fig :6 Zone of inhibition 150 and 200 mg/ml of stem extract against *S.aureus*

L= leaves extract of *C.caudatus*

S= stem extract of *C.caudatus*

Table 2: Zone of inhibition for stems and leaves extracts of *C.caudatus* against *S.aureus* and *E.coli* at different concentrations.

Microorganism	Part of plant	Diameter of zone of inhibition (mm)					
		Control		Concentration of extracts (mg/ml)			
		Tetracycline (positive)	Methanol (negative)	50	100	150	200
S.aureus	Stem	31.89 ± 0.69	6.00 ± 0.00	6.00 ± 0.00	7.16 ± 0.23	-	-
		32.68 ± 0.02	6.00 ± 0.00	-	-	7.38 ± 0.11	9.55 ± 0.64
	Leaf	31.64 ± 0.20	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	-	-
		30.31 ± 1.26	6.00 ± 0.00	-	-	6.00 ± 0.00	6.78 ± 0.57
E.coli	Stem	24.64 ± 1.92	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	-	-
		24.80 ± 0.28	6.00 ± 0.00	-	-	6.00 ± 0.00	6.00 ± 0.00
	Leaf	25.62 ± 1.44	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	-	-
		24.75 ± 2.47	6.00 ± 0.00	-	-	6.00 ± 0.00	6.00 ± 0.00

FREE RADICAL SCAVENGING ASSAY

The antioxidant activity was compared on both methanolic leaves and stems extract of *C.caudatus* using DPPH free radical scavenging assay. The absorbance value of extracts and standard was shown in Table 3. Meanwhile, the calculated inhibition concentration was shown in Table 4. The results showed that free radical scavenging properties in methanolic leaves extracts recorded potential inhibition concentration (IC_{50}) range of 31.35-62.5 µg/ml against methanolic stems extract with inhibition concentration (IC_{50}) range of 125-250 µg/ml. However, standard ascorbic acid showed the most powerful antioxidant activity compared to the tested extracts. The lower the IC_{50} value, the greater its free radical scavenging capability of extracts [11]. The free radical Scavenging ativities of methanolic extracts of leaves and stems of *C.caudatus* and standard ascorbic acid on DPPH was shown in Figure 7.

Table 3: UV Absorbance of stems and leaves methanolic extracts of *C.caudatus* and standard ascorbic acid.

Concentration (µg/mL)	Absorbance at 490 nm		
	Stem	Leaf	Ascorbic acid
15.625	0.648	0.621	0.262
31.250	0.610	0.443	0.152
62.500	0.513	0.282	0.132
125.000	0.467	0.245	0.135
250.000	0.248	0.200	0.138
500.000	0.191	0.185	0.128
1000.000	0.164	0.159	0.140

Table 4: Inhibition concentration (IC_{50}) of stems and leaves extracts of *C.caudatus* and standard ascorbic acid.

Concentration (µg/mL)	IC_{50} Value		
	Stem	Leaf	Ascorbic acid
15.625	5.677	10.628	49.320
31.250	11.208	35.590	70.660
62.500	25.400	59.025	74.470
125.000	32.096	64.338	73.820
250.000	63.974	70.961	73.310
500.000	72.198	73.071	74.180
1000.000	76.201	76.856	72.990

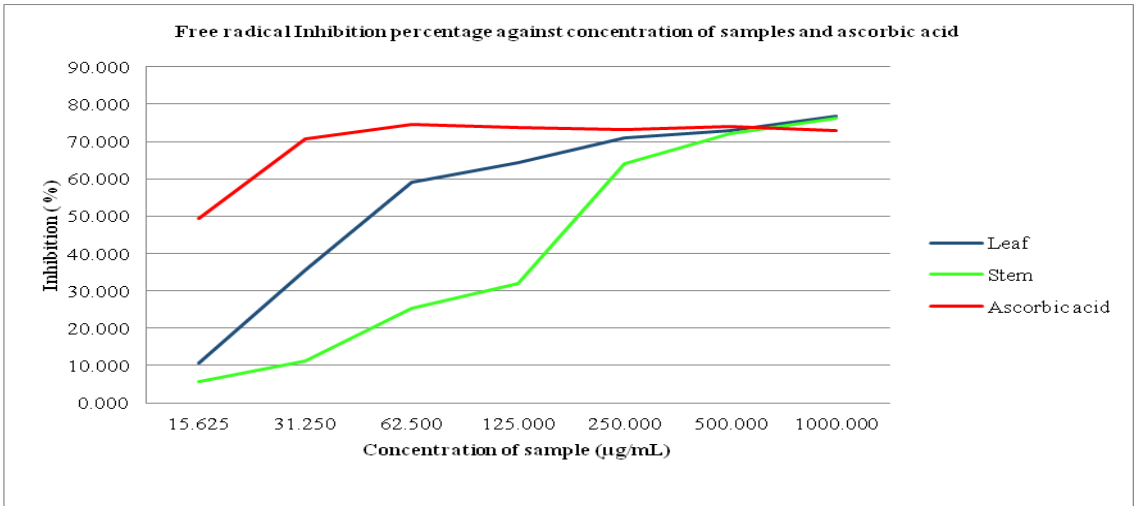

Fig 7: Free radical scavenging ativities of stems and leaves *C.caudatus* extracts and standard ascorbic acid

CONCLUSION:

C.caudatus shows various traditional uses including enhancing blood circulation, strengthening bones and reducing body heat. However, the current study was attempted to compare and evaluate the antimicrobial and antioxidant activity of methanolic extracts of leaves and stems of *C.caudatus* . The results of the study prove that the antimicrobial activity was found to be higher in stems extract against leaves extract with gram positive microorganism like *S.aureus.* Both the extracts were failed against the gram negative organism like *E.coli*. Furthermore, on the comparison evaluation of free radical scavenging activity the leaves extract showed potential antioxidant activity when compared to stem extract of *C.caudatus*. In conclusion, antimicrobial and antioxidant potential of leaves and stem extract were comparable. Meanwhile, this study had limitation on microbial strain tested for antimicrobial activity. Thus, it is recommended to vary the microbial strains in future study to identify most susceptible microorganism between stems and leaves extracts of C.caudatus. The selected plant can be focused more for its antioxidant activity whereby its leaves can be considered as one of the potential source of antioxidants supplement in nutraceutical and cosmeceutical formulations.

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