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Antimicrobial, antioxidant, anticancer property and chemical composition of different parts (corm, stem and leave) of Colocasia esculenta extract

INTRODUCTION

Colocasia esculenta or taro is a member of Araceae family and considered as aggressive due to its rapid growth along river, lake shores and humid tropics. The plant has arrow- shaped leaves with stem in green or red black colour and the height of this plant may reach a few meters (Nair et al, 2005). The leaves of the plant are reported to possess huge vitamin C content and the root is rich in starch and essential nutrient such as thiamine, riboflavin, niacin, oxalic acid, calcium oxalate and sapotoxin (Nair et al, 2005). This plant was also used to reduce fever and pain. Furthermore, Manisha et al. (2010) claimed that the leaf of *C. esculenta* exhibits neuropharmacological activity. However, taro is an important plant as a staple food for many countries such as Caribbean, Hawaii, Solomons, American Samoa, Philippines, Fiji, Sri Lanka, India, Indonesia and many more. As a matter of fact, this plant was also introduced to USA as substitute to potatoes in view of the fact that it is a significant source of protein. Moreover, this plant was cultivated for over 10000 years as long as wheat, barley and potatoes. In Japan, *C. esculenta* has been widely cultivated as it is traditional food in Okinawa (Aniya et al., 2002). The stem of *C. esculenta* was used as vegetable by serving it as element of salad, stew and soup. This is because Japanese in Okinawa believe that *C. esculenta* can contribute to maintain health. From the literature survey, very few studies were conducted to reveal antimicrobial, antioxidant and anticancer properties of this plant. Thus, this study was conducted to evaluate the medicinal properties of *C. esculenta*.

MATERIALS AND METHODS

Plant material. Plant extraction in the present study was prepared as described by Lee et al. (2009). The plant sample was collected from rural area located at Pasir Puteh, Kelantan, Malaysia. The fresh plant sample was oven dried at 37 °C for 4 days. Next, the plant sample was freeze-dried prior to extraction using 70% methanol and concentrated at 1 g/ml. Finally, the plant extraction was kept in -20 °C until further use.

Bacteria isolates. All bacterial isolates were provided by Universiti Malaysia Kelantan namely *Aeromonas hydrophila, Escherichia coli, Edwardsiella tarda, Flavobacterium sp., Klebsiella sp., Salmonella sp., Vibrio alginolyticus, V. parahaemolyticus, V. cholerae and Pseudomonas aeruginosa.* These bacteria were isolated from various aquatic animals and kept in tryptic soy agar (TSA) for further uses.

Minimum inhibitory concentration (MIC) determination. The values of minimum inhibitory concentration (MIC) of *C. esculenta* extracts against bacterial isolates were determined with a two-fold broth micro dilution method (Lee et al., 2009). The bacterial isolates were cultured in tryptic soy broth for 24 h at room temperature and the concentration of these cultures was set to 10^9 CFU mL^{-1} by using physiological saline. The concentration was cross checked with a Biophotometer (Eppendorf, Germany). The bacterial suspensions were then inoculated into a microtiter plate that contained a serial dilution of *C. esculenta* extracts. The microplate was then incubated at room temperature for 24 h. The MIC values were defined as the lowest concentration of the *C. esculenta* extracts in the wells of the microtiter plate that showed no visible turbidity after 24 h incubation.

Determination of antioxidant activity with α, α – diphenyl – β – picrylhydrazyl (DPPH) radical scavenging method. DPPH radical scavenging method was conducted as described by Blois (1958), Yen and Duh (1994), Brand-William et al. (1995) and Gadow et al. (1997) with some modifications. The assay was carried in a 96 wells of ELISA plate with three replicates. Five µl of the sample (0.5 mg/ml) solution was added into the well followed by 200 µl DPPH. The absorbance of the sample was recorded by using ELISA reader for ever interval 6 s. The percentage inhibition of DPPH radical was calculated based on the absorbance.

Cancer cell lines. The human breast adenocarcinoma (MCF-7) cell line was derived from Institute of Marine Biotechnology, Universiti Malaysia Terengganu. All the cells were grown in standard cell medium (RPMI 1640) supplemented with 5% foetal bovine serum in a 5% CO_2 atmosphere. The cells were then transferred into microplate at the concentration of 1 X 10^2 cells per well for cytotoxicity test of the plant extract. At 48 h, proliferation was measured by the MTT colorimetric assay. The IC 50 value was calculated from the following formula as described Adebayo et al. (2010):

$$\log_{10}(IC_{50}) = \frac{\log_{10} C_L (I_H - 50) + \log_{10} C_H (50 - I_L)}{I_H - I_L}$$

$IC_{50} = 10\log_{10}(IC_{50})$

Where:

I_H : I% above 50%

I_L : I% below 50%

C_H : High drug concentration

C_L : Low drug concentration

Colorimetric MTT (tetrazolium) assay. Colorimetric MTT (3-(4, 5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma, USA) assay was carried out as described by Mosmann (1983). Ten µl of MTT solution (5 mg/ml) was added to all of 96 wells of micro plate followed by 4 h incubation at 37^{O}C. Acid isopropanol was added to all wells for dissolving the dark blue crystals. The micro plate was then read on an ELISA reader at wavelength 570 nm within 1 h since adding isopropanol.

Bioactive compound characterization. The chromatographic procedure was carried out using a Shimadzu QP2010-GC-MS with autosampler. The sample was diluted 25 times with acetone and 1 µl of sample was injected into a column. A fused silica capillary column HP5-MS (30 m x 0.32 mm, film thickness 0.25 µm) was used. Helium was the carrier gas, and a split ratio of 1/100 was used. The oven temperature used was maintained at 60^{O}C for 8 min. The temperature was then gradually raised at a rate of 3^{O}C per min to 180^{O}C and maintained at 180^{O}C for 5 min. The temperature at the injection port was 250^{O}C. The components of the test solution were identified by comparing the spectra with those of known compounds stored in internal library.

RESULTS AND DISCUSSION

The minimum inhibitory concentration (MIC) values of different parts of *C. esculenta* extracts ranged from 7.81 to 500 mg/l in which the corm of *C. esculenta* extract was found to inhibit the growth of *Edwardsiella tarda Escherichia coli, Flavobacterium sp., Pseudomonas aeruginosa* and *Vibrio cholerae* at 15.6 mg/l, *Klebsiella* sp., *Aeromonas hydrophila* and *Vibrio alginolyticus* at 31.3 mg/l and it was able to control the growth of *Salmonella* sp. and *Vibrio parahaemolyticus* at 125 mg/l (Table 1). The MIC values of the stem of *C. esculenta* extract were found to inhibit the growth of *Edwardsiella tarda Escherichia coli, Flavobacterium sp., Pseudomonas aeruginosa* and *Vibrio cholerae* at 7.81 mg/l, *Klebsiella* sp., *Aeromonas hydrophila* and *Vibrio alginolyticus* at 15.6 mg/l and it was able to control the growth of *Salmonella* sp. and *Vibrio parahaemolyticus* at 62.5 mg/l. At the concentration of 62.5 mg/l, the leave extract of *C. esculenta* was found to inhibit the growth of *Edwardsiella tarda Escherichia coli*, *Flavobacterium* sp., *Pseudomonas aeruginosa* and *Vibrio cholera* whereas at the concentration of 125 mg/l of the extract, *Klebsiella* sp., *Aeromonas hydrophila* and *Vibrio alginolyticus* failed to grow. The plant extract was also found to control the growth of *Salmonella* spp. and *Vibrio parahaemolyticus* at 500 mg/l.

Table 1. The sensitivity of bacterial isolates against *Colocasia esculenta* extract

Bacterial isolates	Minimum Inhibitory Concentration (MIC) (mg/l)		
	Root	Strip	Leaf
Aeromonas hydrophila	31.3	15.6	125
Edwardsiella tarda	15.6	7.8	62.5
Escherichia coli	15.6	7.8	62.5
Flavobacterium sp.	15.6	7.8	62.5
Klebsiella sp.	31.3	15.6	125
Pseudomonas aeruginosa	15.6	7.8	62.5
Salmonella sp.	125.0	62.5	500
Vibrio alginolyticus	31.3	15.6	125
Vibrio cholerae	15.6	7.8	62.5
Vibrio parahaemolyticus	125.0	62.5	500

In the present study, the stem of *C. esculenta* showed the highest antioxidant activity compared to root and leaf of *C. esculenta* whereas antioxidant activity of *C. esculenta* root was the lowest in the present study. The inhibition concentration of stem extract of *C. esculenta* against 50% of DPPH (IC_{50}) was 0.125 ppt whereas IC_{50} of *C. esculenta* leaf extract was 0.28 ppt and the IC_{50} of corm extract was 4.8 ppt (Figure 1). At the concentration of 20 µg/ml and 30 µg/ml, root and stem extracts of *C. esculenta* were found to inhibit 30% of cancer cell, respectively. On the other hand, no anticancer activity was found in the leaf extract of *C. esculenta*.

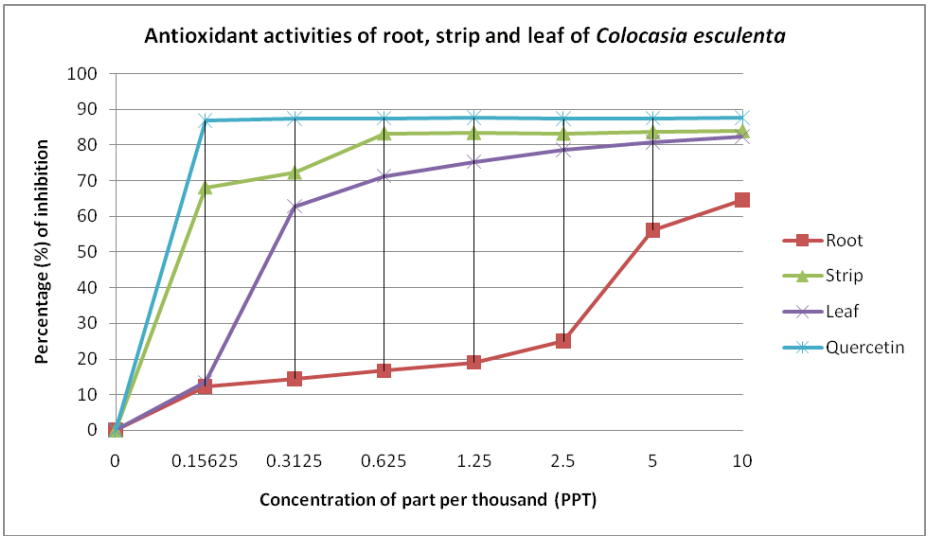

Fig. 1. Antioxidant activities of root, strip and leaf of *Colocasia esculenta* extract

A total of 6 compounds were identified in the *C. esculenta*'s corm extract namely 8, 11-Octadecadienoic acid, methyl ester (54.62%), Hexadecanoic acid, methyl ester (20.55%), 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (12.06%), 9-Octadecenoic acid, methyl ester, (E)- (6.42%), 3,5-Di-tert-butyl-4-trimethylsiloxytoluene

(1.96%), Cyclohexanol, 2-nethyl-5-(1-methylethenyl)-, (1 α, 2 β, 5α)- (1.88%) and 5 unidentified compounds (2.51%) (Table 2). Twelve compounds were successfully identified in *C. esculenta*'s stem extract in which Acetic acid 24.70% was the major compound. This was followed by 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (19.25%), Propanoic acid (11.83%), 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (6.82%), 9,12-Octadecadienoic acid, methyl ester, (E,E)- (6.34%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (5.37%), 2-Furanmethanol (4.74%), Butanoic acid, 2-methyl-3-oxo-, ethyl ester (4.18%), Cyclopentanol (2.69%), 2,2'-Bioxirane (2.33%), Propanoic acid, 2-oxo-, methyl ester (2.26%), Butanoic acid, 4-hydroxy- (2.11%), and 7 unidentified compounds (7.38%) (Table 3). Eight compounds were identified in the extract of *C. esculenta*'s leaf in which Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1S-(1 α., 2 β, 5 β)]- (54.73%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (9.00%), 3,5-Di-tert-butyl-4-trimethylsiloxytoluene (8.12%), 2-propanone, 1-hydroxy- (7.37%), 9,11-Octadecadienoic acid, methyl ester, (E,E)- (5.79%), Hexadecanoic acid, methyl ester (4.28%), Formic acid, 2-propenyl ester (3.21%), Megastigmatrienone (2.84%) and 5 unidentified compounds (4.66%) (Table 4).

Table 2. Compound composition of the root of Colocasia esculent

Compound	Compound Composition (%)
8, 11-Octadecadienoic acid, methyl ester	54.62
Hexadecanoic acid, methyl ester	20.55
9, 12, 15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	12.06
9-Octadecenoic acid, methyl ester, (E)-	6.42
3,5-Di-tert-butyl-4-trimethylsiloxytoluene	1.96
Cyclohexanol, 2-nethyl-5-(1-methylethenyl)-, (1.alpha., 2.beta.,5.alpha.)-	1.88
Unidentified compounds	2.51
Total	100

Table 3. Compound composition of *Colocasia esculenta* strip

Compound	Compound Composition (%)
Acetic acid	24.70
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	19.25
Propanoic acid	11.83
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	6.82
9,12-Octadecadienoic acid, methyl ester, (E,E)-	6.34
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	5.37
2-Furanmethanol	4.74
Butanoic acid, 2-methyl-3-oxo-, ethyl ester	4.18
Cyclopentanol	2.69
2,2'-Bioxirane	2.33
Propanoic acid, 2-oxo-, methyl ester	2.26
Butanoic acid, 4-hydroxy-	2.11
Unidentified compounds	7.38
Total	100

Table 4. Compound composition of *Colocasia esculenta*'s leaf

Compound	Compound Composition (%)
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1S-(1.alpha.,2.beta.,5. beta.)]-	54.73
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.00
3,5-Di-tert-butyl-4-trimethylsiloxytoluene	8.12
2-propanone, 1-hydroxy-	7.37
9,11-Octadecadienoic acid, methyl ester, (E,E)-	5.79
Hexadecanoic acid, methyl ester	4.28
Formic acid, 2-propenyl ester	3.21
Megastigmatrienone	2.84
Unidentified compounds	4.66
Total	100

Up to date, no study has been conducted in as much detail as the present study in terms of antimicrobial property of *C. esculenta*. The studies of Lee et al (2008) and Lee et al. (2010) were only screening the antimicrobial property of *C. esculenta* leaf against various bacterial species. Lee et al. (2008) claimed that aqueous extract of *C. esculenta* leaf can inhibit the growth of *Vibrio* spp. with the inhibition zone 7 mm whereas Lee et al (2010) reported that methanol extract of *C. esculenta* leaf exhibits antimicrobial property against various species of bacteria isolated from aquaculture sites. Other study of Nair et al. (2005) claimed that methanol extract of *C. esculenta* leaf can only inhibit the growth of *Klebsiella pneumonia*. The antimicrobial test results in the present study provide valuable information on determination of the concentration of *C. esculenta* extracts that need to eliminate the tested bacteria. Therefore, we suggest that these plant extracts can be used as antimicrobial agent in the treatment of food borne diseases due to bacterial infection such as *A. hydrophila, E. tarda, E. coli, V. cholerae* and many more. Furthermore, several bioactive compounds that have been reported as responsible for the antimicrobial activity of the *C. esculenta* extracts such as Octadecadienoic acid, Acetic acid; Propanoic acid and Formic acid were identified as the major compound in the plant extracts.

Antioxidant is a molecule that is used to eliminate free radicals in the animal body. Antioxidants can be obtained from fresh plants. The antioxidant was reported to protect liver from inflammation and preventing diabetes complication (Aniya et al., 2002). In the literature survey, very few studies have been conducted to characterize antioxidant property of *C. esculenta*. The study of Aniya et al. (2002), only characterizes the antioxidant of *C. esculenta* stem and leaf but not its root. However, a different finding has been observed in the present study compared to the study of Aniya et al. (2002) in which *C. esculenta* stem had higher antioxidant activity than *C. esculenta* leaf in the present study and contrary to the study of Aniya et al. (2002). This may be due to different strain of *C. esculenta* that was applied in the present study and the study of Aniya et al. (2002) in which *C. esculenta* in the present study was collected from naturally growing plants whereas the plant sample in the study Aniya et al. (2002) was derived from cultivation. However, further study should be conducted before we can come to a conclusion.

This study is the first report on the anti-breast cancer activity of *C. esculenta*. However, Brown et al. (2005) claimed that *C. esculenta* can be used in colorectal cancer treatment. In the present study,

only root and stem of *C. esculenta* were found to possess anti-breast cancer activity. Although, the plant extracts did not show a huge anti-cancer activity potential, however, we can improve the plant extraction method to enhance in yielding higher anti-cancer properties of the plant extracts.

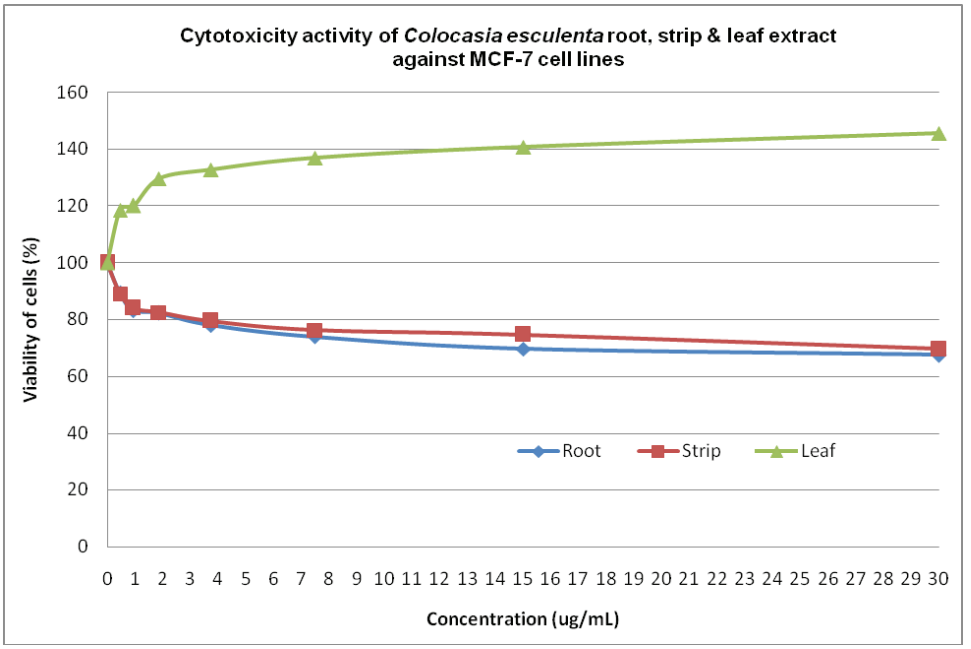

Fig. 2. Cytotoxicity activities of root, strip & leaf of *Colocasia esculenta* extracts

In conclusion, this study revealed a huge potential of *C. esculenta* properties to be used as antimicrobial, antioxidant and anticancer agents. However, this study is only at the stage of onset of drug discovery. Clinical tests on the *C. esculenta* extracts should be carried out in the near future for development of new drugs that address hitherto unmet pharmaceutical needs.

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SUMMARY

This study was carried out to reveal antimicrobial, antioxidant and anti-cancer activities of extracts from different parts of *Colocasia esculenta* namely the corm, stem and leaf. The chemical composition of each part of *C. esculenta* was also characterized. Antimicrobial property of *C. esculenta* extracts against *Aeromonas hydrophila, Escherichia coli, Edwardsiella tarda, Flavobacterium sp., Klebsiella sp., Salmonella sp., Vibrio alginolyticus, V. parahaemolyticus, V. cholerae* and *Pseudomonas aeruginosa* was revealed by using broth micro-dilution method whereas antioxidant activity of the plant extracts was characterized by using α, α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging method. Anticancer

activity of the extracts was determined with Colorimetric MTT (tetrazolium) assay against human breast adenocarcinoma (MCF-7). Compounds of the plant extracts were screened and identified by using gas chromatography - mass spectrometry (GC-MS). The minimum inhibitory concentration (MIC) values of different parts of *C. esculenta* extracts ranged from 7.81 to 500 mg/l against the tested bacteria. In terms of antioxidant activity, the inhibition concentration of stem extract of *C. esculenta* against 50% of DPPH (IC_{50}) was 0.125 ppt whereas IC_{50} of *C. esculenta* leaf extract was 0.28 ppt and the IC_{50} of corm extract was 4.8 ppt. At the concentration of 20 µg/ml and onward, root extract of *C. esculenta* was found to inhibit 30% of cancer cell growth whereas stem extract of *C. esculenta* at 30 µg/ml can inhibit 30% of cancer cell growth. On the other hand, no anticancer activity was found in the leaf extract of *C. esculenta*. A total of 6 compounds were identified in the *C. esculenta* corm extract with the 8 major compounds, 11-Octadecadienoic acid, methyl ester (54.62%) and Hexadecanoic acid, methyl ester (20.55%) whereas, 12 compounds were successfully identified in *C. esculenta* stem extract in which Acetic acid 24.70% was the major compound. Eight compounds were identified in the extract of *C. esculenta* leaf in which Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1S-(1 α., 2 β, 5 β)]- (54.73%) was the major compound. This study revealed that the potential of *C. esculenta* to be used as medicinal drug is promising.

Keywords: antioxidant, anticancer, antimicrobial, chemical compound, *Colocasia esculenta*

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