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The antimicrobial and antioxidant properties of *Cassia alata* extraction under different temperature profiles

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Abstract

The current practice of using *Cassia alata* as a traditional medicine in Malay culture is to boil the plant before application. Therefore, this study was aimed to determine the effect of temperature (40°C, 60°C and 80°C) on antimicrobial and antioxidant of the different parts of *C. alata* (leaf, bark and root) using water and ethanol as extraction solvents. The antimicrobial study was performed using disc diffusion technique while the antioxidant properties were represented by total phenolic content (TPC) and DPPH. Results indicated that leaf extract with ethanol showed the highest antioxidant activities (455.75 GAE/100 g and 52.08 IC_{50}) but was inhibited by the increase of temperature (59.22% and 36% decrease in TPC and DPPH, respectively). For antimicrobial properties, the degree of inhibition to *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enterica serovar* Typhimurium was greater under the ethanol extract which was also enhanced by the increase of temperature. Root extract possessed the highest antimicrobial activities at under all conditions, especially towards *S. aureus* when extracted using ethanol at 80°C (14.70 mm inhibition zone diameter). In conclusion, the ethanolic root extract of *C. alata* may provide better potential for bacterial-related treatment, such as skin application, while the leaf was better for consumption for its antioxidant capacity especially when extracted under low-temperature condition.

1. Introduction

Cassia alata, also known locally as *Gelenggang*, belongs to the Fabaceae family. This plant can grow up to 12 m high and can be found mostly in tropical countries with warm and humid environment such as Africa and Southeast Asia (El-Mahmood and Doughari, 2003). This plant is famous to have various medicinal benefits, such as to treat skin infections, eczema, anti-parasitic, bronchitis, asthma and to relieve bites from poisonous insect (Palanichamy and Nagarajan, 1990; Rastogi and Mehrotra, 1998). In addition, the seeds of *C. alata* is also traditionally used as anthelminthic, the roots extract for the uterus disorder, and bark and crushed leaf for the skin infections caused by ringworms parasite (Ibrahim and Osman, 1995).

Apart from being medicinally useful, *C. alata* was shown to exhibit antimicrobial properties. The extraction of root of *C. alata* with methanol exhibit positive antimicrobial activity when compared with the synthetic antibiotic tested against *Aspergillus niger, Candida albicans, Candida tropicalis* and *Trichophyton mentagrophytes* (Khan *et al*., 2001). Root of *C. alata* is rich in secondary metabolite alkaloids, which possess antimicrobial activity against bacteria that can cause foodborne disease by inhibiting DNA topoisomerase (Bonjean *et al*., 1998). *C. alata* also possesses a potent antifungal activity many types of fungi, including *Aspergillus*, *Mucor*, *Rhizopus* and others (Palanichamy and Nagarajan, 1990).

The flower and leaf of *C. alata* extract with methanol have been shown to contain high amount of antioxidant activity, including Vitamin C, flavanoid compounds, DPPH radical scavenging activity and strong antioxidant activity against hydrogen peroxide and superoxide anion (Thambidurai *et al*., 2010; El-Mahmood and Doughari, 2007; Sagnia *et al*., 2014). One study shows that *C. alata* can act as an antioxidant in the blood plasma of rodents which have been exposed to carbon tetrachloride in a concentration-dependent environment to reduce the secretion of alanine aminotransferase and aspartate aminotransferase (Ehiowemwenguan and Yakubu, 2014).

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As *C. alata* is often consumed raw or by boiling in Malay and Indonesian traditional practice, this current study was aimed to determine the effect of temperature on antioxidant and antimicrobial of the different parts of *C.alata* (leaf, bark and root). The post-heat-treated extract from the different parts of *C. alata* was measured for its total phenolic content and radical scavenging activity, while its antimicrobial properties were determined against three different foodborne bacteria, which are *S. aureus*, *E. coli* and *S. enterica* serovar Typhimurium.

2. Materials and methods

2.1 Plant material preparation

Fresh and healthy leaf, stem and root of *C. alata* were collected from Kuantan, Pahang, Malaysia. This plant was collected after 6 months of cultivation, at about 1 m of height. The different parts of the plants were washed with water thoroughly to remove foreign particles, followed by freeze-drying the leaf, stems and roots within 24 hrs of collection. The dried parts of the plant were blended separately into fine powder before being kept in the airtight container.

Two solvents were used for the preparation of the extracts which are distilled water, and 95% ethanol. The extract was prepared by weighing 20 g of the fine powdered *C. alata* in 100 mL solvent of choice (in this case, ethanol and water) at room temperature (30°C) in a conical flask and stirring vigorously with a glass rod for proper extraction. The mixture was allowed to settle in the water bath at 40°C, 60°C and 80°C temperature separately for 1 hr. The extracts were then filtered using Whatman no.1 filter paper. The solvent residue was further evaporated using the rotary evaporator at 45°C at the speed of 65 rpm to obtain crude extracts. The crude extracts were preserved in the refrigerator (4°C) for further use.

2.2 Determination of total phenolic content

Total phenolic content in extracts was determined using Folin-Ciocalteu assay method according to (Singleton and Rossi, 1965) with slight modifications, as follows; An aliquot (1 mL) of extracts or standard solution of Gallic acid were mixed with 5 mL of distilled water. 0.5 mL of Folin-Ciocalteu phenol reagent was added, vortexed and allowed to stand for 5 mins. Then, 1.5 mL of 20% sodium carbonate was added and the volume was made up to 10 mL using distilled water before incubated for 2 hrs at room temperature (30°C). The absorbance was measured with BIO-RAD 170-6930 Benchmark Plus Microplate Spectrophotometer at 765 nm and the results were analysed using the Microplate Manager Software.

2.3 DPPH radical scavenging activity assay

Free radical scavenging activity of different extracts of leaf, stems and roots of *C. alata* plant was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). 1 g of sample extracts (1.0, 2.0, 3.0, 5.0, and 10.0 mg/mL) were added to 2.0 mL of DPPH solution. Methanol solution was used as a control and gallic acid was used as a standard. The mixture was shaken vigorously and placed in the dark for 30 mins, followed by the absorbance reading 517 nm using spectrophotometer. The results are expressed as IC_{50} values, using the following equation:

DPPH radical scavenging activities = 100(Ac – As)/Ac

Where Ac is the absorbance of control solution and As is the absorbance of the sample solution.

2.4 Antibacterial activity assay

The antibacterial activity of distilled water and ethanolic extract of the different part of *C. alata* plants were determined by the disc diffusion method. The sterile Standard Methods Agar was used to incubate the three strains of foodborne bacteria, which were *S. aureus*, *E. coli* and *S. enterica* serovar Typhimurium. Then, the discs (10 mm in diameter) were cut from Whatman No.1 filter paper, and sterilised in the oven at 160°C for 2 hrs. The discs were then impregnated with the extract by soaking in the extract for 24 h. Each of the discs contained approximately 200 mg/mL of the distilled water (negative control), ethanol and methanol extract. Then, the discs were applied aseptically on the agar surface in a plate containing the test bacteria, after the solvents were evaporated. All the plates were incubated at 37°C for 24 h and the zone of inhibition was observed and measured.

2.5 Statistical analysis

All the data were reported using triplicate measurements. For each parameter considered, using two -way analysis of variance (ANOVA) and post hoc Tukey -HSD tests were performed to detect the differences of the samples by using Minitab 16.

3. Results and discussion

3.1 Extraction yield

Extraction is one of the most important steps involved for retrieving and separating bioactive compounds from sample. The crude extract of *C. alata*, obtained after the concentration process using rotary evaporator was in semi-solid form. Crude extract formed greenish colour for leaf extraction, yellowish colour for barks extraction and brown to light-dark colour for root extraction. Table 1 demonstrated that the extraction yield

Table 1. Extraction yield of *C. alata* using water and ethanol as solvents at different temperature

Solvent system	Different parts	Temperature (°C)		
		40°C	60°C	80°C
Distilled water	Leaf	28.27 ± 1.20^{A}	28.27 ± 1.20^{A}	27.52 ± 1.11^{A}
	Bark	28.10 ± 1.35^{A}	27.57 ± 1.38^{A}	27.38 ± 1.62^{A}
	Root	29.20 ± 1.21^{A}	28.87 ± 0.82^{A}	28.37 ± 1.43^{A}
95% Ethanol	Leaf	32.17 ± 1.75^{B}	29.87 ± 1.18^{C}	19.77 ± 1.50^{D}
	Bark	30.53 ± 1.53^{C}	28.42 ± 1.83^{A}	18.30 ± 1.36^{E}
	Root	30.05 ± 1.44^{C}	28.96 ± 1.54^{A}	20.00 ± 1.29^{D}

Different subscript letters indicate significance ($p<0.05$).

using 95% ethanol is higher compared to extraction using distilled water at 40°C, due to the higher polarity of ethanol than water. However, as the temperature increased, the extraction yield of *C. alata* at 95% of ethanol was also significantly decreased. In contrast, only smaller decreased was observed when distilled water was used as solvent. This larger decrease in the extraction yield of ethanol may due to the fact that the boiling point of ethanol is lower than water, which can cause degradation of phenolic compounds (Dent *et al*., 2013).

3.2 Antioxidant and antimicrobial activity of C. alata under different extraction solvents and temperatures

Phenolic compounds can have different properties, but they are usually linked with antioxidant activities such as preservation of other antioxidant species, and binding of pro-oxidant metal ions (Sroka and Cisowski, 2003). Additionally, DPPH could complement TPC well, as it measures the ability of compounds to scavenge free radicals or hydrogen donors, thus giving information on the antioxidant state of the samples (Kedare and Singh, 2011). In this study, total phenolic content (TPC) and scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) were selected to demonstrate the antioxidant properties of *C. alata* affected by different extraction solvents and heat treatment (Figure 1). All TPC values of *C. alata* extract decreased as the temperature was increased from 40°C to 80°C. This is because the increase in temperature can destroy the phenolic compound, therefore causing the less phenolic compound to be present in the sample extract at high temperature (López-Vidaña *et al*., 2017). The leaf, in particular, showed high TPC compared to the other parts of the plant in the water extract. The presence of high phenolic compounds in the leaf is especially important for the plant defense system against pathogens and pests (War *et al*., 2012). Interestingly, the highest TPC in the bark was recorded with ethanol extraction, not water. This observation indicated that high amount of polar phenolic compounds is available within the bark. Although the ability of water and ethanol to draw out the phenolic compounds is similar, the higher TPC of the bark indicated that ethanol is the better solvent for *C. alata* extraction process under low temperature.

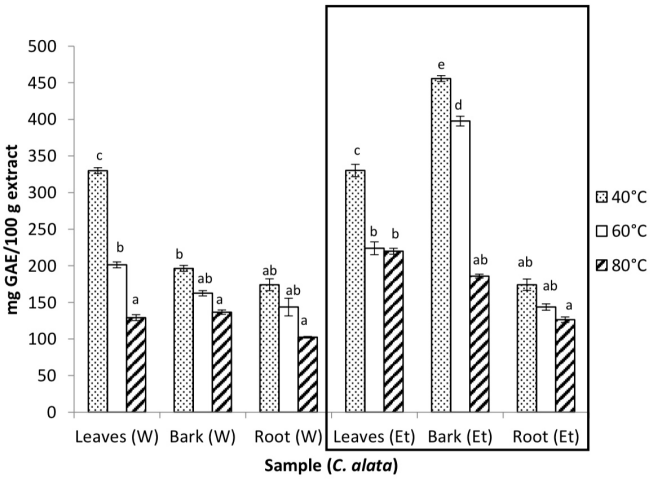

Figure 1. Total phenolic content of different part of *C. alata* extract with water (W) and ethanol (Et) at different temperatures. The box represents the ethanolic extracts of C. *alata.* Values are Mean ±SD of three separate experiments. Different subscript letters indicate significance ($p<0.05$)

DPPH is a stable substance which can scavenge unstable aggressive free radical with absorbance around 517 to 519 nm (Hseu *et al*., 2008). It is defined as the amount of antioxidant needed to reduce the concentration of DPPH by 50%, which can be performed by interpolation from a linear regression analysis. The IC_{50} value of the extract is inversely and linked to its antioxidant activity. Based on Figure 2, both water and ethanol plant extracts produced similar or non-significant IC_{50} capacity. This is different from TPC, as certain significance could be seen when exposed under different parameters such as extraction solvents and plant parts, which further demonstrated the importance of conducting more than one antioxidant assays. All the extracts at 40°C (the lowest temperature used in this study) showed the highest DPPH antiradical activity (Figure 2). In contrast, all the extracts at 80°C showed the lowest DPPH antiradical activity, as the increase in temperature is likely contributed to the degradation of the heat-sensitive phenolic compounds (López-Vidaña *et al*., 2017).

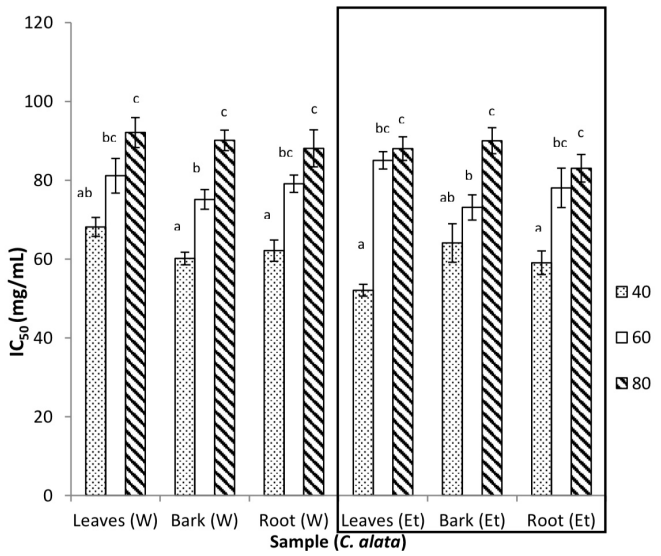

Figure 2. IC_{50} of different part of *C. alata* extract with water at different temperatures. The box represents the ethanolic extracts of *C. alata*. Values are mean±SD of three separate experiments. Different subscript letters indicate significance ($p<0.05$)

In this study, Kirby–Bauer test is among the most basic test to estimate the antibiotic potential of the intended sample. If the sample halts the indicator bacteria from growing or kills the bacteria, there will a clear area known as the "inhibition zone". In this study, the inhibition zone for each different parts of *C. alata* extraction against *S. aureus, E. coli,* and *S. enterica* serovar Typhimurium were measured and tabulated in Table 2. It was shown that all *C. alata* extracts produced inhibition zone against *S. aureus, E. coli* and *S. enterica* serovar Typhimurium, which indicated that *C. alata* possessed certain antibacterial properties. In particular, root extracts consistently showed the highest inhibition zones in all the extracts. This observation is in line with the TPC and DPPH results, which also demonstrated the high phenolic and antioxidative capacity of the root. These properties may be caused by the presence of antraquinones in the plant root (Yadav and Kalidhar, 1994). Antraquinones, an emodine-type compound, is a potent antimicrobial substance (Kemegne *et al*., 2017).

In term of effectiveness, it was demonstrated that *S.*

Table 2. In vitro screening antimicrobial activity of *C. alata* extracts on *S. aureus*, *E. coli* and *S. enterica* serovar Typhimurium (determined by diameter of inhibition zones)

Test bacteria	Extract	Temperature (°C)		
		40°C	60°C	80°C
	Control	-	-	-
S. aureus	**Water**			
	Leaf	8.33 ± 0.15^{aA}	9.10 ± 0.10^{bA}	10.77 ± 0.21^{cA}
	Bark	7.93 ± 0.06^{aB}	8.73 ± 0.25^{bB}	10.67 ± 0.21^{cB}
	Root	9.27 ± 0.25^{aC}	10.90 ± 0.26^{bC}	13.13 ± 0.40^{cC}
	Ethanol			
	Leaf	9.27 ± 0.25^{aA}	10.53 ± 0.25^{bA}	12.03 ± 0.25^{cA}
	Bark	9.11 ± 0.30^{aB}	9.70 ± 0.20^{bB}	10.83 ± 0.15^{cB}
	Root	10.47 ± 0.15^{aC}	11.97 ± 0.25^{bC}	14.70 ± 0.30^{cC}
E. coli	**Water**			
	Leaf	8.27 ± 0.25^{aA}	8.97 ± 0.25^{aA}	9.37 ± 0.11^{bA}
	Bark	8.10 ± 0.46^{aB}	9.47 ± 0.15^{bB}	9.50 ± 0.20^{cB}
	Root	9.20 ± 0.20^{aB}	10.87 ± 0.15^{aC}	11.37 ± 0.32^{bB}
	Ethanol			
	Leaf	9.17 ± 0.15^{aA}	9.87 ± 0.15^{aA}	10.77 ± 0.25^{bA}
	Bark	8.70 ± 0.20^{aB}	9.17 ± 0.18^{aA}	10.30 ± 0.26^{bA}
	Root	10.03 ± 0.25^{aC}	11.47 ± 0.15^{bB}	12.00 ± 0.20^{cB}
S. enterica serovar Typhimurium	**Water**			
	Leaf	7.03 ± 0.25^{aA}	8.43 ± 0.12^{aA}	9.00 ± 0.20^{cA}
	Bark	7.83 ± 0.21^{aB}	8.47 ± 0.12^{bB}	9.87 ± 0.15^{cB}
	Root	8.87 ± 0.15^{aC}	10.27 ± 0.25^{aB}	10.63 ± 0.15^{bC}
	Ethanol			
	Leaf	9.00 ± 0.20^{aA}	9.67 ± 0.15^{bA}	10.4 ± 0.17^{cA}
	Bark	9.17 ± 0.15^{aB}	10.2 ± 0.20^{bB}	10.83 ± 0.15^{cB}
	Root	9.67 ± 0.21^{aB}	10.73 ± 0.15^{bC}	11.27 ± 0.20^{cB}

No inhibition zone "-".Values are Mean ±SD of three separate experiments. Superscripts 'a-c' are means within a row where no common superscripts differ significantly ($P<0.05$). Superscripts 'A-C' within a column mean no common superscripts differ significantly ($P<0.05$).

aureus was more sensitive towards both water and ethanol extract compared to *S. enterica* serovar Typhimurium and *E. coli* due to bigger inhibition zones. This difference may be contributed to by their difference in the cell wall structure. *S. enterica* serovar Typhimurium and *E. coli* are Gram-negative bacterium, which is comprised of unique cell membrane capable of resisting foreign compounds, including antibiotics (Delcour, 2009). Gram-positive bacteria that consist of the peptidoglycan membrane layer is more prone to the penetration of extract into the cell (Delcour, 2009). As previously mentioned, the increase in temperature caused lower amount of phenolic and antioxidant compounds to be available which might contribute to the greater antibacterial activity of the extract (Lucchini *et al*, 1990). Furthermore, the removal of free radicals from the environment could disrupt microbial survival, as certain microorganisms require these nascent oxygens for their growth. However, from Table 2, it is evident that all extracts produce greater inhibition zone when the temperature is increased. It is possible that the heating can result in the leaching of other undetected bioactive compounds from plants into the extract (Lima *et al*., 2017), therefore increasing its antibacterial activity. Furthermore, ethanolic extracts of *C. alata* produced bigger inhibition zone, due to its polarity that is able to draw more phytochemical out from the plants. Overall, the antimicrobial activity in *C. alata* is due to the action of plant phytochemicals such as alkaloids, saponins, flavonoids, tannins, glycosides and diterpenes (Adelowo and Oladeji, 2017). Flavanoids and saponins bioactive compound can damage cell structure of bacteria and disrupt the permeability of cell membrane which can alter the cell function (Cushnie and Lamb, 2005; Korchowiec *et al*., 2015), while tannins can prevent the formation of bacterial cell wall by inactivation of essential enzyme and genes of the bacteria (Scalbert, 1991).

4. Conclusion

C. alata is always traditionally known to have useful properties. Based on the results, the practise of boiling the plant components before usage is shown to reduce its antioxidant and phenolic compounds, which is unfavourable as it could reduce the nutritional qualities of *C. alata*. However, the high-temperature extraction of this plant enhances its antimicrobial properties, which could be useful for bacterial-related treatment such as skin infection.

Conflict of Interest

The authors declare no conflict of interest.

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