# Antioxidant, antityrosinase and antibacterial properties of fresh and processed leaves of Anacardium occidentale and Piper betle

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# Antioxidant, antityrosinase and antibacterial properties of fresh and processed leaves of *Anacardium occidentale* and *Piper betle*

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ABSTRACT

In this study, the antioxidant, antityrosinase and antibacterial properties of fresh and processed leaves of *Anacardium occidentale* (cashew) and *Piper betle* (betel) were analysed and evaluated. For assessing antioxidant properties (AOP) of total phenolic content, total flavonoid content, caffeoylquinic acid content, free radical scavenging activity, ferric reducing power and ferrous ion chelating ability, the Folin–Ciocalteu, aluminium chloride, molybdate, DPPH radical scavenging, potassium ferricyanide and ferrozine assays were used, respectively. Antityrosinase and antibacterial properties were determined using the respective modified dopachrome method and disc-diffusion method. The outstanding AOP of fresh cashew leaves far exceeded those of betel leaves, including temperate culinary herbs of rosemary, thyme and marjoram. Blanching resulted in a significant decline in AOP of cashew and betel leaves with leaching of phenolic compounds into the blanching water. AOP of microwave-treated leaves of cashew remained unchanged but leaves of betel exhibited significant increase. Tyrosinase inhibition of fresh cashew leaves was high while betel leaves exhibited an enhancement of tyrosinase activities. Blanching did not affect the tyrosinase inhibition of cashew leaves but microwave treatment resulted in significant increase. For betel leaves, tyrosinase inhibition remained unchanged. Results showed that fresh cashew and betel leaves inhibited both Gram-positive and Gram-negative bacteria tested. Blanched and microwave-treated cashew leaves exhibited strong antibacterial activity with those of betel leaves showed variable effects. Blanching water of cashew leaves also possesses antibacterial activity. The enhancement of tyrosinase activities of betel leaves, and leaching of bioactive compounds with antioxidant and antibacterial properties into the blanching water warrant further investigations.



## 1. Introduction

Tropical culinary herbs or ulam are often consumed raw in Southeast Asian countries such as Malaysia, Thailand and Indonesia. Served as a side dish or as an ingredient in specialty dishes, it is believed that the regular intake of ulam can assist in preventing degenerative diseases, delaying aging and improving general health (Sulaiman, Sajak, Ooi, Supriatno, & Seow, 2011; Reihani & Azhar, 2012). Among the herbs consumed as ulam are cashew and betel leaves.

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*Anacardium occidentale* L. (family Anacardiaceae) or cashew is a small-sized tree with a dome-shaped crown (Lim, 2012). The bark is brown or grey with longitudinal fissures. Leaves are simple, alternate, narrowly to broadly obovate with a rounded apex. When young, they are pliable and reddish, and are dark green and leathery with prominent yellow veins when mature. Traditionally, cashew leaves have also been used to treat rheumatic disorders and hypertension (Andarwulan et al., 2012; Nugroho, Malik, & Pramono, 2013).

*Piper betle* L. (family Piperaceae) or betel is a woody, perennial and dioecious vine (Teo & Banka, 2000). Stems are swollen at the nodes. Leaves are alternate, simple and yellowish green to bright green with 2 or 3 pairs of secondary veins. Betel leaves are a specialised type of ulam, best known as an essential component of betel quid, consisting of areca nut slices wrapped in fresh betel leaves with slaked lime, tobacco or spices added for flavouring (Rai et al., 2011). Betel leaves are supposed to ameliorate bad breath, improve vocalisation, harden the gum, and prevent indigestion, bronchitis, constipation, congestion, cough and asthma. The species has been reported to possess antimicrobial, insecticidal, antioxidant, antinociceptive, antidiabetic, gastroprotective and anticoagulant properties (Arambewela, Arawwawala, & Rajapaksa, 2006).

Although the antioxidant properties of tropical culinary herbs are fairly well studied, little is known on the other bioactivities. The effects of different processing methods are also poorly studied. The objective of our study was to analyse and evaluate the antioxidant, antityrosinase and antibacterial properties of fresh and processed leaves of cashew and betel.

## 2. Materials and methods

### 2.1. Herbs

Fresh leaves of *A. occidentale* and *P. betle* (Fig. 1) were purchased from the Chow Kit market in Kuala Lumpur. Young cashew leaves are pliable and reddish in colour while betel leaves are bright green and heart-shaped with 2 or 3 pairs of secondary veins.

### 2.2. Extraction

For antioxidant properties, fresh herbs (1 g) and processed herbs (0.3 g) were powdered with liquid nitrogen in a mortar and extracted with 50 mL of methanol with continuous shaking for 1 h at room temperature. Extracts were filtered under suction and stored at 4 °C for further analysis.

For antityrosinase and antibacterial activity, fresh herbs (10 g) and processed herbs (3 g) were powdered with liquid nitrogen in a mortar and extracted with 100 mL of methanol, three times for 1 h each time. After swirling continuously in an orbital shaker, the extracts were filtered and stored at 4 °C for further analysis.

### 2.3. Processing of herbs

Herbs were blanched by immersing 1 g of sample in 50 mL of boiling water for 30 s. The blanched samples retained on the sieve were wiped dry and extracted while the blanching water was kept for analysis of phenolic content. Microwave treatment of herbs involved placing 1 g of sample in a microwave oven (230–240 V, 50 Hz) at the centre position for 30 s.

### 2.4. Antioxidant assays

Fresh and processed herbs were analysed for phenolic content (total phenolic content, total flavonoid content and caffeoylquinic acid content) using the Folin–Ciocalteu, aluminium chloride and molybdate assays as described by Chan, Kong, Yee, Chua, and Loo (2012a, 2012b). Antioxidant activity (free radical scavenging activity, ferric reducing power and ferrous ion chelating ability) was measured using the DPPH radical scavenging, potassium ferricyanide and ferrozine assays following the procedures of Chan et al. (2012).

Total phenolic content (TPC) was assessed using the Folin–Ciocalteu (FC) assay. Extracts (300 μL) were introduced into test tubes wrapped with aluminium foil, followed by 1.5 mL of FC reagent (10 times dilution) and 1.2 mL of sodium carbonate (7.5%, w/v). After incubating for 30 min in the dark, absorbance was measured at 765 nm. TPC was expressed as gallic acid equivalent (GAE) in mg per 100 g of sample.

Total flavonoid content (TFC) was evaluated using the aluminium chloride assay. Extract (1 mL) is added into test tubes containing 4 mL of water. Subsequently, 0.3 mL of 5% sodium nitrite was added, followed by 0.3 mL of 10% aluminium chloride. Sodium hydroxide solution (2 mL, 1 M) was then added, followed by 2.4 mL of water to make up to

**Fig. 1 – Fresh leaves of *Anacardium occidentale* (left) and *Piper betle* (right).**

10 mL. The mixtures were mixed well and incubated at room temperature for 10 min. Absorbance was determined at 415 nm against a sample blank of 1 mL of the respective extracts with 9 mL of water. TFC was expressed as quercetin equivalent (QE) in mg/100 g of sample.

Caffeoylquinic acid content (CQAC) was quantified using the molybdate assay. Molybdate reagent was prepared by dissolving 16.5 g sodium molybdate, 8.0 g dipotassium hydrogen phosphate, and 7.9 g potassium dihydrogen phosphate in 1 l of water. The reagent (2.7 mL) was added to the plant extract (0.3 mL), mixed and incubated at room temperature for 10 min. Absorbance was measured at 370 nm against a sample blank of 0.3 mL of the respective extracts with 2.7 mL of water. CQAC was expressed as mg chlorogenic acid equivalent (CGAE)/100 g of sample.

Free radical scavenging (FRS) activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Different dilutions of extracts (1 mL) were added to 2 mL of DPPH (5.9 mg per 100 mL methanol). Absorbance was measured at 517 nm after 30 min. $IC_{50}$ was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg ascorbic acid (AA)/100 g of sample. AEAC was calculated as $IC_{50}$ (a)/$IC_{50}$ (s) $\times 10^5$ (a=ascorbic acid, s=sample) where $IC_{50}$ of ascorbic acid was 0.00387 mg/mL.

Ferric reducing power (FRP) was measured using the potassium ferricyanide assay. Different dilutions of extracts (1 mL) were added to 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%, w/v). The mixture was incubated at 50 °C for 20 min. After adding trichloroacetic acid solution (2.5 mL, 10%, w/v), the mixture was separated into aliquots of 2.5 mL, and diluted with 2.5 mL of water. To each diluted aliquot, 500 mL of ferric chloride solution (0.1%, w/v) was added. After 30 min, absorbance was measured at 700 nm. FRP was expressed as mg GAE/100 g. The calibration equation for gallic acid was $y=16.767x$ ($R^2=0.9974$), where y is the absorbance and x is the GA concentration in mg/mL.

Ferrous ion chelating (FIC) ability was determined using the ferrozine assay. Different dilutions of extracts (1 mL) were mixed with $FeSO_4$ (0.1 mM, 1 mL), followed by ferrozine (0.25 mM, 1 mL). Absorbance was measured at 562 nm after 10 min. The ability of extracts to chelate ferrous ions was calculated as chelating effect (%)=$(1-A_{sample}/A_{control})\times 100$. FIC ability was expressed as chelating efficiency concentration ($CEC_{50}$) in mg/mL, i.e., the effective concentration of extract to chelate ferrous ions by 50%.

### 2.5. Tyrosinase inhibition

Tyrosinase inhibition of herbs was determined using the modified dopachrome method with L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate following the procedures of Masuda, Yamashita, Takeda, and Yonemori (2005) and Chan et al. (2008). Assays were conducted in a 96-well microtiter plate and a plate reader was used measure absorbance at 475 nm, with 700 nm as reference. Samples were dissolved in dimethyl sulphoxide (DMSO). Each well contained 40 μL of sample with 80 μL of phosphate buffer (0.1 M, pH 6.8), 40 μL of tyrosinase (31 units/mL) and 40 μL of L-DOPA (2.5 mM). Each sample was accompanied by a blank that had all the components except L-DOPA. Results were compared with a control consisting of 50% DMSO in place of sample. Tyrosinase inhibition was calculated as $(A_{control}-A_{sample})/A_{control}\times 100\%$.

### 2.6. Antibacterial activity

Antibacterial activity of herb extracts was assessed using the disc-diffusion method as described by Chan, Kong, Yee, Chua, and Loo (2012a, 2012b). Agar cultures of Gram-positive bacteria of *Brevibacillus brevis*, *Micrococcus luteus* and *Staphylococcus cohnii*, and Gram-negative bacteria of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* were prepared. Inoculums (100 μL) were spread evenly onto 20 mL Mueller–Hinton agar set in 90 mm Petri dishes using a sterile cotton swab. Sterilised paper discs (6 mm diameter) were impregnated with two-fold decrease in the amount of plant extract, starting from 2.0 mg/disc, using a micropipette and firmly placed onto the inoculated agar ensuring even distribution to avoid overlapping of zones. After incubation overnight at 37 °C, the minimum inhibitory dose (MID) or minimum concentration of extract in mg/disc required to show a zone of inhibition was noted.

### 2.7. Statistical analysis

All experiments were done in triplicate ($n=3$) and results were expressed as means±standard deviation (SD). Analysis of variance (ANOVA) was analysed using the Tukey Honestly Significant Difference (HSD) test, based on significant difference of $p<0.05$.

## 3. Results and discussion

### 3.1. Antioxidant properties

Antioxidant properties (AOP) based on phenolic content and antioxidant activity of fresh leaves of *A. occidentale* far exceeded those of *P. betle* (Table 1). Phenolic content of cashew leaves based on TPC (3890±336 mg GAE/100 g), TFC (347±48 mg QE/100 g) and CQAC (1090±70 mg CGAE/100 g) were 5.8, 5.1 and 5.7 times higher than those of *P. betle*. Antioxidant activity based on AEAC (6620±513 mg AA/100 g), FRP (3260±235 mg GAE/100 g) and $CEC_{50}$ (1.9±0.2 mg/mL) were 7.1, 7.2 and 2.6 times higher than those of *P. betle*. AOP values of *A. occidentale* leaves were significantly higher than those of temperate culinary herbs such as *Rosmarinus officinalis* (rosemary), *Thymus vulgaris* (thyme) and *Origanum majorana* (marjoram) reported by Chan, Kong, Yee, Chua, and Loo (2012a, 2012b).

The outstanding AOP of *A. occidentale* leaves reported in this study may be attributed to its phenolic constituents. Major flavonoids in leaf shoots of two varieties of *A. occidentale* are kaempferol 3-*O*-glucoside, kaempferol 3-*O*-arabinofuranoside, quercetin 3-*O*-glucoside and quercetin 3-*O*-galactoside (Mohd Shukri & Alan, 2010). Anacardic acids, cardanols and cardols are the major alkyl phenols of cashew fruits (Trevisan et al., 2006). The presence of phytyl side-chains in anacardic acids confers them greater antioxidant capacity. It has been reported that besides being a potent scavenger of reactive oxygen species, anacardic acids also inhibit generation of superoxide

**Table 1 – Phenolic content and antioxidant activity of fresh and processed leaves of *Anacardium occidentale* and *Piper betle* (fresh weight equivalent).**

| Herb | Fresh and processed | Phenolic content | | | Antioxidant activity | | |
|---|---|---|---|---|---|---|---|
| | | TPC | TFC | CQAC | AEAC | FRP | $CEC_{50}$ |
| Cashew | Fresh | $3890 \pm 336^{a}$ | $347 \pm 48^{a}$ | $1090 \pm 70^{a}$ | $6620 \pm 513^{a}$ | $3260 \pm 235^{a}$ | $1.9 \pm 0.2^{a}$ |
| | Blanching | $3100 \pm 151^{b}$ | $261 \pm 16^{b}$ | $749 \pm 33^{b}$ | $4810 \pm 169^{b}$ | $2280 \pm 58^{b}$ | $4.8 \pm 0.3^{b}$ |
| | | (203 ± 84) | (69 ± 8) | (112 ± 21) | | | |
| | Microwave | $3920 \pm 333^{a}$ | $318 \pm 47^{a,b}$ | $1130 \pm 137^{a}$ | $7290 \pm 1010^{a}$ | $3490 \pm 387^{a}$ | $4.1 \pm 0.3^{b}$ |
| Betel | Fresh | $676 \pm 136^{b}$ | $68 \pm 32^{a,b}$ | $190 \pm 49^{a}$ | $928 \pm 289^{b}$ | $454 \pm 100^{b}$ | $4.9 \pm 0.9^{b}$ |
| | Blanching | $387 \pm 67^{c}$ | $36 \pm 25^{b}$ | $109 \pm 13^{b}$ | $373 \pm 88^{c}$ | $318 \pm 101^{b}$ | $5.2 \pm 0.2^{b}$ |
| | | (14 ± 2) | (6 ± 1) | (90 ± 12) | | | |
| | Microwave | $1180 \pm 296^{a}$ | $81 \pm 8^{a}$ | $247 \pm 67^{a}$ | $1560 \pm 369^{a}$ | $838 \pm 210^{a}$ | $1.2 \pm 0.4^{a}$ |

Data on phenolic content and antioxidant activity are means ± standard deviations. Figures in brackets are the phenolic content of blanching water. Within the same column, different superscripts (a–c) are significantly different at $p < 0.05$, as measured by the Tukey HSD test. ANOVA does not apply between herbs. *Abbreviations and units*: TPC = total phenolic content (mg GAE/100 g), TFC = total flavonoid content (mg QE/100 g), CQAC = caffeoylquinic acid content (mg CGAE/100 g), AEAC = ascorbic acid equivalent antioxidant capacity (mg AA/100 g), FRP = ferric reducing power (mg GAE/100 g), $CEC_{50}$ = median chelating efficiency concentration (mg/mL), GAE = gallic acid equivalent, QE = quercetin equivalent, CGAE = chlorogenic acid equivalent, AA = ascorbic acid and HSD = honestly significant difference. Lower $CEC_{50}$ values mean stronger ferrous ion chelating ability.

anion and uric acid by xanthine oxidase (Masuoka & Kubo, 2004).

Blanching in boiling water for 30 s resulted in a significant decrease in the phenolic content and antioxidant activity of *A. occidentale* and *P. betle* compared to fresh samples. Declines in values of the blanched leaves ranged from 20% to 31% and 30% to 60%, respectively. Leaching of flavonoids and caffeoylquinic acids into the boiling water were 26% and 15% of the blanched cashew leaves, and 17% and 83% of the blanched betel leaves.

Microwave treatment did not exhibit any effect on the AOP of *A. occidentale* with TPC, TFC, CQAC, AEAC and FRP values comparable to fresh samples. However, *P. betle* exhibited significant increase in values ranging from 19% to 85%.

Findings on the effects of blanching in this study are consistent with those of earlier research, which reported that blanching of vegetables generally caused declines in AOP. Declines of 20–30% in the antioxidant capacity have been reported for cauliflower (Puupponen-Pimiä et al., 2003), 9–40% in total antioxidant activity for cruciferous vegetables (Ismail & Lee, 2004) and 70% in TPC for *Amaranthus* vegetables (Ismail, Norazaidah, & Emmy Hainida, 2006).

On the contrary, some studies have also reported gains and/or loss in AOP of vegetables following blanching. Asparagus, burdock, carrot, eggplant and green chilli blanched for 5 min resulted in 10–120% increase in FRS activity (Yamaguchi et al., 2001). Onion, radish and spinach showed declines of 56–80%. Green leafy vegetables blanched for 5 min showed up to 200% increase in phenolic content, but up to 52% and 80% loss in FRS activity and ferric reducing ability, respectively (Oboh, 2005). FRP and quercetin content of blanched ginger rhizomes of *Curcuma mangga* were significantly higher than those of fresh rhizomes (Pujimulyani, Raharjo, Marsono, & Santoso, 2012). Loss in AOP during blanching can be due to solubilisation of phenolic compounds and their leaching into the boiling water (Howard, Wong, Perry, & Klein, 1999; Yamaguchi et al., 2001).

In this study, declines in TPC, TFC and CQAC of the blanched cashew and betel leaves ranged from 20% to 31% and 30% to 60%, respectively. Leaching of flavonoids and caffeoylquinic acids into the boiling water were 26% and 15% of the blanched cashew leaves, and 17% and 83% of the blanched betel leaves, suggesting that the amount of antioxidants leached varies with the species.

The gain in FRS activity during blanching may be attributed to thermal destruction of cells releasing antioxidative compounds and to the inactivation of polyphenol oxidase, which inhibits polyphenol degradation (Yamaguchi et al., 2001). The increment may also be due to the hydrolysis of flavonol glycosides to their respective aglycones and the breakdown of tannins to simple phenolic compounds, which are expected to possess more potent antioxidant activity (Oboh, 2005; Pujimulyani et al., 2012).

Data on the effects of microwave in this study are supported by earlier findings. Microwave drying resulted in a significant increase in the AOP of leaves of *Morus alba* and *Thunbergia laurifolia* (Chan, Lye, Eng, & Tan, 2013). TPC, AEAC and FRP of microwave-dried leaves of *M. alba* were 24%, 91% and 30% higher than those of fresh leaves, respectively. TPC and AEAC of microwave-dried *T. laurifolia* leaves were 38% and 84% higher. The enhancement effects of microwave treatment on the AOP of plant samples have also been reported in green tea of *Camellia sinensis* (Gulati, Rawat, Singh, & Ravindranath, 2003) and in citrus pomace (Khizar et al., 2010).

The enhanced AOP of microwave-dried herbs has been attributed to the release of bound phenolic compounds, brought about by the breakdown of cellular constituents (Chan et al., 2009). Microwave energy could have increased the solubility of polyphenols by preventing them from binding to the leaf matrix (Gulati et al., 2003). Other contributing factors included rapid heat transfer and thermal inactivation of polyphenol oxidase activity in samples due to microwave irradiation (Rodriquez-Lopez et al., 1999). Another likely cause for the increase in the antioxidant activity following microwave drying is the production of additional phenolic compounds from precursors already present in the samples (Chan & Lim, 2006).

### 3.2. Tyrosinase inhibition

Tyrosinase inhibition of fresh leaves of *A. occidentale* (40±2.2%) was the highest with negative inhibition (−20±5.5%) displayed by fresh leaves of *P. betle* (Table 2). Compared to fresh cashew leaves, tyrosinase inhibition was not affected by blanching. On the other hand, microwave treatment, resulted in significant increase in tyrosinase inhibition (49±4.0%). For betel leaves, tyrosinase inhibition remained unchanged. Values after blanching (−13±3.8%) and microwave (−23±7.3%) were comparable to that of fresh leaves. This is the first report on the effects of blanching and microwave on the tyrosinase inhibition of cashew and betel leaves. Previous studies on the tyrosinase inhibition or enhancement have been based on fresh plant samples.

The tyrosinase inhibition value of cashew leaves was comparable to that of *Psidium guajava* (41%) and *Hibiscus tiliaceus* (42%) as reported by Wong, Lim, and Chan (2010), which were used as positive controls. Leaves of *H. tiliaceus* displayed the highest tyrosinase inhibition among 39 tropical coastal plant species screened (Masuda et al., 2005).

Compounds responsible for the potent tyrosinase inhibition in cashew leaves remain to be studied. Various mechanisms of tyrosinase inhibition have been proposed. Chelating agents are able to sequester the cupric ion at the active centre of the enzyme (Alam, Yoon, Lee, Lee, & Lee, 2011). Other tyrosinase inhibitors share structural similarities with the natural substrate, which allow them to compete with the substrate when binding to the active centre of the enzyme (Jeong et al., 2009). Anacardic acids, 2-methyl cardols, and cardols isolated from cashew fruits have been found to exhibit tyrosinase inhibitory activity (Kubo, Kinst-Hori, & Yokokawa, 1994; Kubo, 1997). Anacardic acids act as a direct inhibitor of tyrosinase by chelating with $Cu^{+}$ of the enzyme.

This study is the first to report on the enhancement of tyrosinase activities of betel leaves. The tyrosinase enhancement effect suggests their melanogenic or skin-darkening properties, which have been reported in some plants such as *Salvia miltiorrhiza* (Chiang, Chen, Hung, Lee, & Lin, 2012), and in compounds such as apigenin, hyperosid and icariin (Ye, Chou, Wang, Chu, & Yu, 2010). Parkinson's disease in humans is partly caused by dopamine deficiency (Meiser, Weindl, & Hiller, 2013). In the nervous system, tyrosinase catalyses L-tyrosine to L-DOPA which is converted to dopamine by DOPA decarboxylase. Since, tyrosinase is one of the essential enzymes to produce dopamine, *P. betle* might be a potential drug that can be used to treat Parkinson's disease.

This is the first report on the effects of blanching and microwave on the tyrosinase inhibition of cashew and betel leaves. Previous studies on the tyrosinase inhibition or enhancement were based on fresh plant samples.

**Table 2 – Tyrosinase inhibition activity of fresh and processed leaves of *Anacardium occidentale* and *Piper betle* (fresh weight equivalent).**

| Herb | Fresh and processed | Tyrosinase inhibition (%) |
|---|---|---|
| Cashew | Fresh | $40\pm2.2^{b}$ |
| | Blanching | $44\pm3.5^{a,b}$ |
| | Microwave | $49\pm4.0^{a}$ |
| Betel | Fresh | $-20\pm5.5^{a,b}$ |
| | Blanching | $-13\pm3.8^{b}$ |
| | Microwave | $-23\pm7.3^{a}$ |

Data on tyrosinase inhibition (%) are means±standard deviations. Concentration of extracts used for determining tyrosinase inhibition was 0.25 mg/mL. Within the same column, different superscripts (a and b) are significantly different at $p<0.05$, as measured by the Tukey HSD test. ANOVA does not apply between herbs. The enhancement of tyrosinase activities (negative inhibition) of betel leaves denotes melanogenic or skin-darkening properties.

### 3.3. Antibacterial activity

Results showed that fresh leaves of *A. occidentale* and *P. betle* inhibited both Gram-positive and Gram-negative bacteria (Table 3). Based on minimum inhibitory dose (MID), *B. brevis* was most susceptible to cashew leaves (0.13 mg/disc), followed by *M. luteus* (0.25 mg/disc) with no activity against *S. enterica*. Betel leaves showed broad-spectrum activity by inhibiting all six bacterial species with MID ranging from 0.5 mg/disc for *M. luteus* and *P. aeruginosa* to 2.0 mg/disc for *B. brevis*, *S. cohnii* and *E. coli*.

Blanched cashew leaves exhibited stronger antibacterial activity with MID of 0.13 mg/disc against Gram-positive bacteria of *M. luteus* and *S. cohnii*, and Gram-negative bacteria of *E. coli* and *P. aeruginosa* than fresh leaves. Microwave-treated cashew leaves similarly displayed strong antibacterial activity with MID of 0.06 mg/disc against *B. brevis* and *M. luteus*, and MID of 0.13 mg/disc against *S. cohnii*, *E. coli* and *P. aeruginosa*. Like fresh cashew, inhibition of *S. enterica* was not detected.

The effects of blanching and microwave on the antibacterial activity of betel leaves were somewhat variable. Blanching enhanced inhibition against *E. coli* and *S. enterica* with no inhibition detected against *B. brevis*, *M. luteus* and *P. aeruginosa*. Antibacterial activity of microwave-treated leaves was stronger against *B. brevis*, *E. coli* and *S. enterica*, weaker against *M. luteus* and *P. aeruginosa*, and unchanged against *S. cohnii*.

It is interesting to note that the blanching water of cashew leaves also possesses antibacterial activity. Its inhibitory effect was stronger against Gram-positive bacteria (0.25–0.50 mg/disc) than that of Gram-negative bacteria (2.0 mg/disc) with no inhibition detected against *E. coli* and *S. enterica*. In general, the MID of the blanching water was weaker that the blanched leaves. Loss of antibacterial activity was observed in the blanching water of betel leaves with no activity detected against *E. coli* and *S. enterica*.

The potent antibacterial properties of cashew leaves have yet to be studied although those of cashew fruits have been attributed to polyphenols such as anacardic acids and cardols, which possess a side chain with double bonds (Himejima & Kubo, 1991; Kubo, Muroi, & Kubo, 1995). An increase in the number of double bonds in the side chain increases the antibacterial activity against Gram-positive bacteria.

Despite the trend that extracts from most plants are more effective against Gram-positive bacteria (Alzoreky & Nakahara, 2003; Chung, Chung, Ngeow, Goh, & Imiyabir, 2004), cashew and betel leaves inhibited both Gram-positive and Gram-negative bacteria. Compounds responsible for the antibacterial properties of cashew and betel leaves have yet to be studied.

**Table 3 – Antibacterial activity of fresh and processed leaves of *Anacardium occidentale* and *Piper betle* based on minimum inhibitory dose (mg/disc).**

| Herb | Fresh and processed | Gram-positive bacteria | | | Gram-negative bacteria | | |
|---|---|---|---|---|---|---|---|
| | | *Brevibacillus brevis* | *Micrococcus luteus* | *Staphylococcus cohnii* | *Escherichia coli* | *Pseudomonas aeruginosa* | *Salmonella enterica* |
| Cashew | Fresh | 0.13 | 0.25 | 0.50 | 0.50 | 0.50 | ND |
| | Blanching | 0.13 (0.25) | 0.13 (0.25) | 0.13 (0.50) | 0.13 (ND) | 0.13 (2.00) | ND (ND) |
| | Microwave | 0.06 | 0.06 | 0.13 | 0.13 | 0.13 | ND |
| Betel | Fresh | 2.00 | 0.50 | 2.00 | 2.00 | 0.50 | 1.00 |
| | Blanching | ND (ND) | ND (ND) | 2.00 (2.00) | 0.50 (ND) | ND (ND) | 0.50 (ND) |
| | Microwave | 1.00 | 1.00 | 2.00 | 0.25 | 2.00 | 0.25 |

For the analysis of minimum inhibitory dose (MID), a two-fold decrease in the amount of plant extract was used, starting from 2.0 mg/disc. Figures in brackets are the MID of blanching water. *Abbreviation*: ND=not detected.

Although blanching caused a decline in the AOP of cashew leaves, antibacterial activity was enhanced. Microwave enhanced both the antioxidant and antibacterial properties of cashew leaves. It is likely that blanching could have reduced enzyme-mediated degradation and microwave irradiation might have led to the formation of bioactive phenolic compounds at high temperature.

The finding that blanching water of cashew leaves possesses antibacterial activity is most interesting as we often pour away the water after blanching vegetables and herbs without even realising its bioactivity. It also reminds us that blanching water can be reused for cooking.

## 4. Conclusion

Values of phenolic content and antioxidant activity of fresh cashew leaves far exceeded those of betel leaves. Blanching resulted in a significant decline in AOP of both herbs cashew and betel leaves with leaching of phenolic compounds into the blanching water. AOP of microwave-treated leaves of cashew remained unchanged but leaves of betel exhibited significant increase. Tyrosinase inhibition of fresh cashew leaves was comparable to positive controls with negative inhibition displayed by fresh betel leaves. Blanching did not affect the tyrosinase inhibition of cashew leaves but microwave treatment resulted in significant increase. For betel leaves, tyrosinase inhibition remained unchanged. Fresh leaves of both herbs inhibited Gram-positive and Gram-negative bacteria tested. Blanched and microwave-treated cashew leaves exhibited strong antibacterial activity with those of betel leaves showed variable effects. Blanching water of cashew leaves also possessed antibacterial activity. The enhancement of tyrosinase activities of betel leaves, and the antioxidant and antibacterial properties of blanching water warrant further studies.

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