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Ulam herbs: A review on the medicinal properties of *Anacardium occidentale* and *Barringtonia racemosa*

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ABSTRACT

In Southeast Asia, the young leaves of ulam herbs are consumed raw as condiment. The regular intake of ulam is believed to assist in preventing degenerative diseases, delaying aging and improving overall health. In this review, the current knowledge on the phytochemistry and pharmacology of two ulam herbs of *Anacardium occidentale* (cashew) and *Barringtonia racemosa* (putat) is updated with some description of their botany and uses. Flavonoids and phenolic acids are the major metabolites of leaves in both species. Leaves of *A. occidentale* possess antioxidant, antibacterial, antiviral, antifungal, anti-tyrosinase and anti-quorum sensing activities. Methyl gallate isolated from cashew leaves showed potent anti-quorum sensing properties. Other pharmacological properties of cashew leaves include cytotoxic, hypoglycaemic, hypolipidemic, anti-cholesterolemic, anti-ulcerogenic, anti-hypertensive, analgesic and anti-inflammatory activities. These bioactivities affirm that cashew leaves have medicinal values, and confer their traditional uses as food and medicine. For *B. racemosa*, only pharmacological properties of antioxidant, antimicrobial, cytotoxic, cytoprotective and anti-inflammatory activities have been reported in the leaves unlike other plant parts that have many more bioactivities. However, excessive consumption of these two ulam herbs may be detrimental to human health due to their toxic effects.

INTRODUCTION

In Southeast Asian countries, particularly Malaysia, Thailand and Indonesia, some herbs are consumed raw as condiment (ulam), and they form an important component of the traditional diet. Dipped in hot and spicy sauce made from shrimp or fish paste, these herbs would whet the appetite during meals. These herbs are believed to have health-promoting properties, and their regular intake can assist in preventing degenerative diseases, delaying aging and improving overall health (Sulaiman *et al*., 2011; Reihani and Azhar, 2012). Young leaves of herbs commonly consumed as ulam include *Anacardium occidentale* (cashew), *Barringtonia racemosa* (common putat), *Centella asiatica* (pennywort), *Cosmos caudatus* (wild cosmos), *Murraya koenigii* (curry leaf), *Oenanthe javanica* (water dropwort), *Persicaria hydropiper* (water pepper) and *Piper sarmentosum* (wild pepper). Major chemical constituents reported in ulam herbs are flavonoids and phenolic acids. These compounds are known to possess various health benefits with a multitude of bioactivities including antioxidant, antimicrobial, anti-proliferative, anti-allergic, anti-inflammatory, anti-hypertensive and vasodilating properties (Pietta *et al*., 2003; Yao *et al*., 2004). Herbs with multiple pharmacological functions mediated by complex interactions of phytochemicals are effective in treating multiple diseases and age-related disorders (Satoh, 2014). Their anti-aging effects and improvement of blood circulation have also been reported. Unlike Labiatae herbs which are widely used in western cuisines, herbs consumed as ulam in eastern cuisines are not well reviewed. This review updates the current knowledge on the phytochemistry and pharmacology of leaves of *A. occidentale* (cashew) and *B. racemosa* (putat) with some description of their botany and uses.

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Currently, there is only one review on *A. occidentale* with focus on the nuts (Leite *et al*., 2016), and two reviews on all plant parts of *B. racemosa* (Kabir *et al*., 2013; Osman *et al*., 2015). However, this review is deemed relevant and appropriate as it is focused on the leaves of *A. occidentale* and *B. racemosa*.

ANACARDIUM OCCIDENTALE

Botany and uses

Anacardium occidentale L. (cashew) of the family Anacardiaceae is native to Brazil (Lim, 2012a). It is a small-sized tree with a dome-shaped crown. The bark is brown or grey, and smooth to rough with longitudinal fissures. Leaves are leathery and obovate with a rounded apex. Borne on terminal clusters, leaves are pliable, lustrous and reddish when young, and dark green when mature with prominent yellow veins. Occurring as terminal panicles, flowers are whitish turning pinkish-red. Fruits are a kidney-shaped nut attached to the distal end of an enlarged pear-shaped receptacle called the cashew apple.

The young and tender leaves of *A. occidentale* (Figure 1) are a popular herb consumed raw as ulam and sometimes blanched to reduce their stringent taste. In traditional medicine, leaves are used for treating dysentery, diarrhoea and piles, and an infusion of bark and leaves are applied to relief toothache and sore gums (Akinpelu, 2001). Other uses of leaves include remedy for rheumatism and hypertension (Andarwulan *et al*., 2012; Nugroho *et al*., 2013).

Fig. 1: Young leaves of *Anacardium occidentale*.

Phytochemistry of leaves

Flavonoids of quercetin, quercetin-3-*O*-rhamnoside, myricetin, myricetin-3-*O*-rhamnoside and amentoflavone have been isolated from *A. occidentale* leaves (Arya *et al*., 1989; Konan and Bacchi, 2007). Phenolic acids such as gallic, cinnamic, *p*-coumaric, ferulic, protocatechuic and *p*-hydroxybenzoic acids (Kogël and Zech, 1985), and flavonoids such as agathisflavone, quercetin 3-*O*-rutinoside and quercetin 3-*O*-rhamnoside (Ajileye *et al*., 2015), have also been reported. From the leaf shoots of two varieties of *A. occidentale*, 15 flavonol glycosides have been identified by Shukri and Alan (2010). The major components were kaempferol 3-*O*-glucoside, kaempferol 3-*O*-arabinofuranoside, quercetin 3-*O*-glucoside and quercetin 3-*O*-galactoside. Overall, the total phenolic content of leaf shoots of the red variety was almost two times that of the yellow variety. According to Andarwulan *et al*. (2012), quercetin (125 mg/100 g) is the dominant flavonoid and chlorogenic acid (13.5 mg/100 g) is the dominant phenolic acid in cashew leaves. Other phenolic compounds reported in the leaves include anthocyanidins of cyanidin and peonidin (Kongkachuichai *et al*., 2015). The chemical composition of essential oils from leaves and other plant parts of *A. occidentale* has been analysed. The leaf oil contains (*E*)-*β*-ocimene (29%), *α*-copaene (14%) and *δ*-cadinene (9%), the fruit oil contains palmitic acid (20%) and oleic acid (20%), and the flower oil contains β-caryophyllene (26%), methyl salicylate (13%) and benzyl tiglate (11%) as major components (Maia *et al*., 2000).

Pharmacological properties of leaves

Antioxidant

Among the leaves of 10 species of ulam herbs assessed for antioxidant properties, values of *A. occidentale* leaves were by far the highest (Chan *et al*., 2014). The total phenolic content (3890 mg GAE/100 g) and free radical scavenging ability (6620 mg AA/100 g) of *A. occidentale* leaves were 1.7 and 2.6 times those of *Persicaria hydropiper*, which ranked second, and 16 and 30 times those of *Centella asiatica*, which ranked last. Antioxidant properties of cashew leaves were significantly stronger than western culinary herbs such as rosemary, thyme and marjoram (Chan *et al*., 2012a, 2012b). Similarly, the total phenolic content and antioxidant activities of *A. occidentale* leaves were the strongest amongst herbs and vegetables studied (Sulaiman *et al*., 2011; Huda-Faujan *et al*., 2015).

The methanol leaf extract of *A. occidentale* yielded higher total phenolic content, free radical scavenging and ferric reducing properties than ethyl acetate and hexane extracts (Razali *et al*., 2008). Based on DPPH radical and NO scavenging activities, the ethanol leaf extract of *A. occidentale* possessed the strongest activities followed by aqueous and petroleum ether extracts (Jaiswal *et al*., 2010). In Thailand, the phenolic content and antioxidant activities of young leaves of cashew ranked second out of 13 indigenous vegetables screened by Kongkachuichai *et al*. (2015). The phenolic content of young cashew leaves was 10 times higher than that of fruits.

Antibacterial and antiviral

When tested against Gram-positive bacteria of *Brevibacillus brevis*, *Micrococcus luteus* and *Staphylococcus cohnii*, and Gram-negative bacteria of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* using the disc-diffusion method, leaves of *A. occidentale* inhibited all bacterial species except *S. enterica* (Tan and Chan, 2014). Minimum inhibitory dose (MID) ranged from 0.13−0.50 mg/disc. Against *E. coli*, *P. aeruginosa*, *Bacillus cereus*, *Bacillus megaterium* and *Cryptococcus neoformans*, cashew leaves inhibited all bacterial species except *E. coli* (Mackeen *et al*., 1997). Other studies by Anand *et al*. (2015) and Thomas *et al*. (2015) also reported on the

antibacterial properties of *A. occidentale* leaves. Inhibition of the leaf extract of *A. occidentale* was stronger than that of the bark extract (Manasa *et al*., 2013) while the flower extract displayed the strongest inhibition (da Silva *et al*., 2016). The essential oil extracted from leaf shoots of cashew also possessed antimicrobial activity (Nor Ayshah Alia *et al*., 2016). Among 12 medicinal plant species screened for simian (SA-11) and human (HCR3) rotavirus inhibition in Brazil, the aqueous leaf extract of *A. occidentale* inhibited the growth of SA-11 by 85% at non-cytotoxic concentration of 4.0 µg/ml (Gonçalves et al., 2005). Rotaviruses are known to be major agents of diarrhoea in infants and young children.

Anti-quorum sensing

Using the violacein inhibition assay with *Chromobacterium violaceum* as the test organism, Tan *et al*. (2015) reported that *A. occidentale* leaves exhibited strong anti-quorum sensing (QS) activity. Diameters of inhibition zones (DIZ) of violacein production and of *C. violaceum* growth were 22 mm and 15 mm, respectively. Minimum inhibitory dose (MID) of violacein production and *C. violaceum* growth were 0.013 and 0.25 mg/disc. From the methanol leaf extract of cashew, methyl gallate (methyl ester of gallic acid) was isolated using column chromatography, and identified by NMR and MS analyses. Violacein production inhibition of methyl gallate at 0.05 mg/disc was 14.5 mm for DIZ, 0.006 mg/disc for MID and 0.10 mg/ml for minimum inhibition concentration (MIC). The closely-related gallic acid, however, did not display any anti-QS activity, suggesting that the methyl moiety in methyl gallate may be responsible for the QS inhibition. Using reversed-phase HPLC, the content of methyl gallate was quantified as 1.83 mg/g. Tan *et al*. (2015) was the first report on the systematic isolation and quantification of methyl gallate with potent QS inhibition from leaves of *A. occidentale*.

Antifungal

Cashew leaves have been shown to have antifungal activity based on an interesting study conducted on the microbiology of dentures of 50 elderly people from Mangalore in Karnataka, India (Shetty *et al*., 2014). Total *Candida* counts were conducted on swabs collected from their dentures before and after application of cleansing agents. Results showed that cashew leaves can be used as a natural cleansing agent although their antifungal activity was not as effective as denture cleansing tablets of triphala.

Tyrosinase inhibition

The anti-tyrosinase activity of *A. occidentale* leaves (40%) was comparable to that of *Psidium guajava* (41%) and *Hibiscus tiliaceus* (42%) used as positive controls (Tan and Chan, 2014). Compared to fresh cashew leaves, inhibition was not affected by blanching (44%) but was significantly enhanced by microwave treatment (49%). The leaf extract of *A. occidentale* inhibited tyrosinase and reduced melanin in human epidermal melanocytes for more than 24 h, suggesting their potential use as therapy for pigmentation problem (Gaffar *et al*., 2008).

Cytotoxic

Screening of 42 families of edible plants from Malaysia for Epstein-Barr virus (EBV) activation in Raji cells showed that the methanol leaf extract of *A. occidentale* (8 µg/ml) was strongly active (Murakami *et al*., 2000). The ethanol leaf extract of cashew was reported to be cytotoxic to Jurkat cells by inducing apoptosis (Konan *et al*., 2012). Agathisflavone, a biflavonoid isolated, displayed high anti-proliferative effect on Jurkat cells with IC_{50} value of 2.4 µg/ml. When tested against human gingival fibroblast and Chinese hamster lung fibroblast (V79) cell lines, the ethanol leaf extract of *A. occidentale* resulted in 26% and 22% mortality, respectively (Anand *et al*., 2015). These values were significantly weaker than the commercial mouth rinse of rexidin (41% and 49%) and betadine (46% and 50%) indicating that the extract was much less toxic.

Hypoglycaemic

Leaves of *A. occidentale* were found to have hypoglycaemic properties. The blood glucose level of rats administered with the aqueous leaf extract of cashew increased by 48% compared with 208% increase in streptozotocin (STZ)-induced diabetic rats (Kamtchouing *et al*., 1998). Results indicated that cashew leaves have a protective effect against STZ-induced diabetes in rats. A related study reported that oral administration of the methanol leaf extract of *A. occidentale* at doses of 35, 175 and 250 mg/kg significantly reduced blood glucose levels in diabetic rats after 3 h (Sokeng *et al*., 2007). Maximum reduction of 37% and 35% in blood glucose levels was observed with doses of 175 and 250 mg/kg, respectively. When administered repeatedly with 175 mg/kg of extract, the decline in blood glucose (48%) was more pronounced. The hexane leaf extract of cashew (300 mg/kg) had no nephrotoxic effect in normal rats, and effectively reduced diabetes-induced functional and histological alterations in the kidney (Tedong *et al*., 2006). The leaf extract of *A. occidentale* has been reported to significantly lower blood glucose levels in normoglycaemic and hyperglycaemic rabbits (Esimone *et al*., 2001), in normoglycaemic rats (Saidu *et al*., 2012), and in alloxan-induced diabetic rats (Fagbohun and Odufuwa, 2010). The lowering of blood glucose levels is accompanied by hypoglycaemic and hypolipidemic effects in alloxan-induced diabetic rats (Elekofehinti *et al*., 2016). Another related study showed that the cashew leaf extract ameliorated the level of thiobarbituric acid reactive substances, and improved the activities of glucose-6-phosphate dehydrogenase, superoxide dismutase and glutathione peroxidase in the testicular homogenate of STZ-induced diabetic rats (Ukwenya *et al*., 2013).

Anti-cholesterolemic

The anti-cholesterolemic activity of the aqueous leaf extract of *A. occidentale* has been reported in male rabbits supplemented with a high cholesterol diet (Fazil *et al*., 2011). *In*

vitro toxicity screening of the extract demonstrated very low LC_{50} values and no IC_{50} value was detected. In cholesterolemic rabbits, the extract was able to inhibit the increment of liver enzymes.

Anti-ulcerogenic

The aqueous ethanol leaf extract of *A. occidentale* exhibited anti-ulcerogenic effect in rats (Konan and Bacchi, 2007). The extract inhibited gastric lesions induced by HCl/ethanol in female rats, with an ED_{50} value of 150 mg/kg. Extract doses higher than 100 mg/kg were more effective than 30 mg/kg of lansoprazol in preventing gastric lesions. No signs of acute toxicity were observed when the rats were treated with extract doses up to 2000 mg/kg.

Anti-hypertensive

A purified *A. occidentale* leaf extract has shown to have *in vitro* anti-hypertensive effects using the isolated organ technique (Nugroho *et al*., 2013). At 0.5 and 1.0 mg/ml, the extract reduced the contraction of isolated rat aorta induced by phenylephrine by 26% and 40%, respectively. This finding was complemented by reports that the aglycones and glycosides of quercetin (major constituents of cashew leaves) have the ability to reduce hypertension (Duarte *et al*., 2001), to stimulate vasorelaxation of aortic vessels (Khoo *et al*., 2010), and to lower blood pressure (Edwards *et al*., 2007; Larson *et al*., 2012) in animal models and human subjects.

Analgesic and anti-inflammatory

The analgesic and anti-inflammatory effects of cashew leaf extracts have also been reported. Leaves successively extracted with petroleum ether, chloroform and methanol were screened for analgesic and anti-inflammatory activity using the carrageenan-induced rat paw oedema assay by Pawar *et al*. (2000). Results showed that the petroleum ether and chloroform leaf extracts, and the acetone soluble fraction of the methanol extract exhibited 57%, 48% and 62% inhibition of paw oedema, respectively. The aqueous, hexane, dichloromethane and methanol leaf extracts of *A. occidentale* investigated for analgesic effects on acetic acid-induced pain in mice showed that the extracts significantly reduced the number of writhing and the highest analgesic effect was seen in the dichloromethane extract (Onasanwo *et al*., 2012). A recent study on the anti-inflammatory properties of cashew leaves reported stronger activity in the ethanol extract than the aqueous extract (Thomas *et al*., 2015).

Toxicity studies of leaves

Studies on the toxicity of cashew leaves have been conducted by Tedong *et al*. (2007). In the acute toxicity study, oral administration of a single dose of hexane leaf extract of *A. occidentale* in mice showed no toxic symptoms at 6 g/kg and no mortality up to 14 g/kg after a week. At doses higher than 6 g/kg, signs of toxicity included asthenia, anorexia, diarrhoea and syncope. The LD_{50} of the extract was found to be 16 g/kg. In the sub-chronic study, oral administration to mice at doses of 6, 10 and 14 g/kg for eight weeks resulted in reduction in food intake and weight gain. Histopathological examinations revealed evidence of microscopic lesions in the liver or kidney caused by changes in biochemical parameters of blood samples.

BARRINGTONIA RACEMOSA

Botany and uses

Barringtonia racemosa (L.) Spreng (putat) of the family Lecythidaceae is a small tree with greyish-brown bark (Giesen *et al*., 2007; Yaplito, 2001). Leaves, clustered at the end of branches, are oblong-obovate and have serrated margins. Inflorescences are long and pendulous, bearing many fragrant pink flowers with conspicuous stamens (Figure 2). Fruits are oblong or pear-shaped and bear single seeds. Occurring in association with mangrove and other coastal forests, the natural distribution of *B. racemosa* stretches from East and South Africa eastwards though South, Southeast and East Asia to Polynesia (Giesen *et al*., 2007).

In Southeast Asia, leaf shoots and young leaves of *B. racemosa* are consumed raw as ulam or cooked as vegetables (Yaplito, 2001; Lim, 2012b). As ethnomedicine, leaves have been used to treat high blood pressure, itchiness and chicken pox (Kabir *et al*., 2013; Osman *et al*., 2015). Known to be rich in saponins, the bark and fruits of *B. racemosa* are used by coastal communities as fish poison (Giesen *et al*., 2007).

Fig. 2: Flowers of *Barringtonia racemosa*.

Phytochemistry of leaves

Using HPLC analysis, phenolic compounds of gallic acid, protocatechuic acid, ellagic acid, quercetin and kaempferol have been identified in sequential leaf extracts of *B. racemosa* (Kong *et al*., 2012). In a follow-up study, the contents were of the order: gallic acid > ellagic acid > quercetin > protocatechuic acid > rutin > kaempferol (Kong *et al*., 2014). The contents of gallic acid and ellagic acid were 2200 and 1450 µg/g in freeze-dried leaves, and 2270 and 1140 µg/g in air-dried leaves, respectively. Earlier, gallic acid, ferulic acid, naringin, rutin, luteolin and kaempferol have been reported in the methanol leaf extract of *B. racemosa* (Hussin *et al*., 2009).

Pharmacological properties of leaves

Antioxidant

Antioxidant activities of leaf extracts of *B. racemosa*, measured using the ferric thiocyanate, thiobarbituric acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assays, showed strongest activities in the chloroform extract followed by extracts of hexane and ethanol (Behbahani *et al*., 2007). The phenolic contents and antioxidant activities of different plant parts of *B. racemosa* were studied by Mariam *et al*. (2008). Results showed that the methanol extracts of twigs and barks yielded higher total phenolic and flavonoid contents, respectively. A study on the total phenolic content and antioxidant properties of different stages of young leaves of *B. racemosa* showed variable results depending on the solvent used for extraction and the type of assays employed (Dalilah *et al*., 2015).

The antioxidant properties of water, ethanol, ethyl acetate and hexane extracts of leaf shoots of *B. racemosa* were assessed by Kong *et al*. (2012). The water extract had the highest polyphenol and ascorbic acid contents while the contents of flavonoids and carotenoids were highest in the leaf ethyl acetate extract. The water extract had the highest ferric reducing activities and scavenging activities against DPPH, ABTS and superoxide anion radicals.

The protective effects of leaves of *B. racemosa* against low-density lipid (LDL), serum and haemoglobin oxidation have been reported by Kong *et al*. (2014). In all three oxidation assays, the leaf extract was stronger than the stem extract.

Antimicrobial

Dried leaves of 15 medicinal plants in South Africa were sequentially extracted (hexane, dichloromethane, acetone and methanol) and tested for inhibitory activity against *Mycobacterium smegmatis* which causes tuberculosis (Mmushi *et al*., 2010). Results showed that the leaf extracts of *B. racemosa* were among the most effective with minimum inhibitory concentration (MIC) ranging from 0.84−0.11 mg/ml. The antifungal activity of methanol, ethanol and hot water extracts of *B. racemosa* leaves, twigs and barks has been tested against seven fungal species (Hussin *et al*., 2009). Results showed that the methanol extracts showed strongest inhibitory activity, particularly against *Fusarium* (53%), *Ganoderma* (35%) and *Aspergillus* (32%).

Cytotoxic

The ethanol leaf extract of *B. racemosa* displayed cytotoxic activity against HeLa human cervical carcinoma cells with a 50% cytotoxic dose of 10 µg/ml (Mackeen *et al*., 1997). However, in another study, the methanol leaf extract of *B. racemosa* was found to show weak anti-proliferative activity (IC_{50} value of 3.5 mg/ml) against cancer cells tested (Emylia *et al*., 2008).

Cytoprotective

The aqueous extract of the leaf shoots of *B. racemosa* was found to protect HepG2 cells against oxidative damage (Kong *et al*., 2016). Results of the cell viability assay revealed that the extract was non-cytotoxic at concentration less than 250 mg/ml. The extract improved the cellular antioxidant status and protected HepG2 cells against H_2O_2-induced cytotoxicity, and also inhibited lipid peroxidation and the production of reactive oxygen species.

Anti-inflammatory

Leaves of *B. racemosa*, successively extracted with chloroform, hexane and ethanol, were tested for anti-inflammatory activity using the Griess assay based on the inhibition of nitric oxide (NO) in RAW 264.7 cells (Behbahani *et al*., 2007). At concentrations of 25, 50, 100 and 200 µg/ml, non-polar extracts of chloroform (23−74% inhibition) and hexane (26−61% inhibition) were found to be strong inhibitors of NO. Using the xanthine oxidase (XO) and albumin denaturation inhibition assays, the leaf extract of *B. racemosa* (1.0 mg/ml) yielded inhibition of 59% and 65%, respectively (Osman *et al*., 2016). The results indicated that the leaves of *B. racemosa* have anti-inflammatory activities that can be used in alleviating gouty arthritis and XO-related diseases.

CONCLUSION

Leaves of *A. occidentale* possess a wide array of pharmacological properties, which reflect their health benefits, and confer their traditional uses as food and medicine. They include antioxidant, antibacterial, antiviral, antifungal, anti-tyrosinase and anti-quorum sensing, cytotoxic, hypoglycaemic, hypolipidemic, hypocholesterolemic, anti-ulcerogenic, anti-hypertensive, analgesic and anti-inflammatory activities. On the contrary, only pharmacological properties of antioxidant, antimicrobial, cytotoxic, cytoprotective and anti-inflammatory activities have been reported in leaves of *B. racemosa*, unlike other plant parts which have many more bioactivities. The oral administration of high dosage of *A. occidentale* leaves has toxic effects on mice, and the bark and fruits of *B. racemosa* are used as fish poison. One would therefore assume that the excessive consumption of these two ulam herbs may be detrimental to the human health, contrary to the belief that the regular intake of ulam herbs has health-promoting properties and can assist in preventing degenerative diseases, delaying aging and improving overall health. Further studies can be conducted on the pharmacological properties of these ulam herbs.

They include isolating and identifying novel bioactive compounds; assessing the properties and elucidating the mechanisms of action of the isolated compounds; analysing the effects of different processing methods on these herbs; evaluating their toxic effects; and exploring their potentials of developing herbal and pharmaceutical products. Notwithstanding, the prospects of studies on new pharmacological properties are equally promising.

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