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Original Research Article

Preliminary phytochemical analysis and *in vitro* antioxidant properties of Malaysian 'Kundang' (*Bouea macrophylla* Griffith)

KUMESHINI SUKALINGAM ✉

Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

ABSTRACT

The main objective of the present study was to evaluate preliminary phytochemical screening and *in vitro* antioxidant properties of leaves, ripe and unripe fruit extracts of Malaysian 'Kundang' (*Bouea macrophylla* Griffith; Family: Anacardiaceae), by using different solvents like water, ethanol, methanol, and hexane. Phytochemical analysis showed the presence of alkaloids, flavonoids, saponins, sterols and triterpenes, total phenol, tannins, and vitamin C. The prepared extracts from the leaves and fruits of the plant were further investigated for their potential antioxidant activity using radical scavenging DPPH (2,2'-diphenyl-2-picrylhydrazyl) technique, which was compared with ascorbic acid, as standard. This study showed that ripe, unripe fruits and leaves of *B. macrophylla* have higher amounts of various phytochemicals constituents in methanol and aqueous solvents and possess higher amounts of vitamin C. Moreover, aqueous and methanol extracts of leaves, unripe and ripe fruits of *B. macrophylla* exhibited a remarkable DPPH radical scavenging activity compared with that of the standard. The results revealed that fruit and leaves extracts are a potential source of antioxidants of natural origin.

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1. Introduction

Secondary metabolites are essential for plant growth and play a significant role in their defense mechanisms (Ibrahim et al., 2017). The consumption of these metabolites in the regular diet could be beneficial to human health by reducing the incidence of chronic diseases such as cancer, diabetes, cardiovascular and neurodegenerative diseases (Selim and Al Jaouni, 2016). Several biochemical reactions in the human body generate reactive oxygen species (ROS), which play significant functions in oxidative stress associated with the pathogenesis of various chronic diseases (Letha et al., 2016; Mohammadhosseini et al., 2019). In healthy individuals, the generation of ROS is balanced by antioxidative defense system (Ganesan et al., 2016; Mohammadhosseini, 2017; Mohammadhosseini et al., 2017). However, the intake of synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene,

tertiary butylated hydroquinone and gallic acid esters leads to negative health impacts (Nehete et al., 2010). Synthetic antioxidants exhibit lower soluble potential and moderate antioxidant activity (Nair et al., 2016). Several studies have suggested to reduce and restrict the usage of synthetic antioxidants due to their unpleasant side effects (Kahl and Kappus, 1993; Nahm et al., 2012; Schillacia et al., 2013). Natural antioxidants should be used as proper alternatives which are safe for consumption. The increasing demand of natural antioxidants leads to more production which makes them cheaper and more available (Ganesan et al., 2015). Recent studies showed the therapeutic potentials of medicinal plants used as antioxidants, which remarkably reduce free radical-induced tissue injury and protect human organs (Asif et al., 2017; Bhaskaran and Kannappan, 2017; Ganesan et al., 2017; Hu et al., 2017; Laamech et al., 2017; Yuan et al., 2017). Thus, more investigations are urgently required to study

✉ Corresponding authors: Kumeshini Sukalingam
Tel: +60 102624316; Fax: +60 102624316
E-mail address: meshni_anat@yahoo.com.my



the antioxidant potential of medicinal plants and their active compounds.

Bouea macrophylla Griffith ('Malaysian Kundang') belongs to the *Anacardiaceae* family and is related to mangoes (Rajan and Bhat, 2016, 2017). It is commonly known as the Mariam Plum or Plum Mango. It also has been shown as an evergreen and a perennial tree that grows up to 25 meters of height, highly seasonal and is widely distributed in South East Asia including Malaysia, Indonesia, Laos, Thailand, Java, Myanmar, and Sumatra (Rajan et al., 2014). The fruits and leaves are edible, popular in local communities of Malaysia, Sabah, and Sarawak (Ikram et al., 2009). The trees bearing Kundang fruits are widely cultivated in Malaysia for shade as well as for ornamental purpose in gardens (Rajan et al., 2014). Traditionally, almost all parts of Kundang plant involving leaves, unripe and ripe fruits, and seeds are edible and have been used for various culinary purposes (Rajan and Bhat, 2017). Ripe fruits with a sweet to sour taste have found various uses in preparing beverages, snacks, and jellies. On the other hand, unripe fruits (sour taste) are used as vegetable (Rajan and Bhat, 2016). In Malaysia and Indonesia, the tender leaves of the plant are used as a raw vegetable in the preparation of salads (Rajan et al., 2014). Hence, this study was undertaken to study the *in-vitro* antioxidant activity along with the preliminary phytochemical analysis of *B. macrophylla* Griffith.

2. Experimental

2.1. Chemicals

Dragendorff reagent, copper sulphate, sodium hydroxide, 2,2'-diphenyl-1-picrylhydrazyl, ascorbic acid, ferric chloride, lead acetate, gelatin, HCl, chloroform, H₂SO₄, glacial acetic acid, hexane, methanol, and ethanol were all purchased from chemical company of Malaysia Bhd, Malaysia. All the chemicals used in the present study were of analytical reagent grade.

2.2. Collection and authentication of the plant material

The leaves, ripe and unripe fruits of *Bouea macrophylla* Griffith were collected from Market of Alor Setar, the capital city of Kedah, Malaysia during the fruiting season in the month of October-2014. The plant was taxonomically identified and authenticated by the Dr. Sujit Kumar Sarker, Department of Pharmacognosy, Management and Science University, Shah Alam, Malaysia and was kept at the Herbarium (voucher specimen number SKS 2014/16) in Centre for Molecular Systematics, Shah Alam for future references. The ripe and unripe fruits devoid of seeds were cut and freeze dried and then powdered by using the blender. The leaves were dried in the shade and powdered coarsely.

2.3. Extraction of plant material

About 100 g of freeze-dried fruit powder (ripe and unripe) and coarse leaves powder of *Bouea macrophylla* Griffith were first soaked in adequate water and the subsequent extraction was conducted using 250 mL of respective solvents including ethanol, methanol, aqueous and hexane. The obtained extracts were kept in an orbital shaker for 24 h and then stored in the dark for 12 h followed by centrifugation (1000 x g for 10 min) at a room temperature. The collected supernatants were used to analyze antioxidant properties and preliminary phytochemical studies.

2.4. Preliminary phytochemical screening

Phytochemical screening of the extract was carried out to identify the secondary metabolites such as alkaloids (Mayer and Dragendorff test), flavonoids (Shinoda test and Con. H₂SO₄ test), terpenoids (Salkowski test), carbohydrate (Anthrone and Benedict's), glycosides (Keller Killiani Test and Bromine water test), proteins and free amino acids (Ninhydrin and Biuret test), gums and mucilage (95% alcohol test), tannins (ferric chloride test), phenolic compounds (gelatin containing sodium chloride, ferric chloride test and lead acetate solution), saponins (foam test), and anthraquinones (Borntrager test), sterols and triterpenoids (Liebermann-Buchard's and Salkowski reaction), fixed oils (spot test), and vitamin C (DNPH test) according to standard phytochemical methods as described by Paech and Tracey (1955) and Ganesan et al. (2016).

2.5. *In vitro* DPPH radical scavenging activity

The antioxidant activity of the fruits and leaves extracts were analyzed based on the percentage inhibition of free radical scavenging activity of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical, as described by De Ancos et al. (2002). Briefly, serial dilutions (10-50 µL) of the fruit and leaves extracts were added to every test tube and made up to a final volume of 100 µL using distilled water. In the next step, 3.9 mL of DPPH methanolic solution (25 mM) was added. The obtained mixture was vortexed for 5 min and kept in the dark for 30 min. After this incubation period, the absorbance was measured at 515 nm using UV spectrophotometer against methanol as blank. Ascorbic acid was used as standard, as well. The percent decrease in the absorbance was recorded for each concentration and percent quenching of DPPH was calculated on the basis of the observed decrease in absorbance of the free radicals in the reaction medium. The radical scavenging activities were then expressed as the inhibition percentage and calculated using the formula:

$$\text{Radical scavenging activity is based (\%)} = \left[\frac{A_0 - A_1}{A_0} \right] \times 100 \quad (\text{Eqn. 1})$$

Table 1

Preliminary phytochemical analysis of leaves, unripe and ripe fruits of *Bouea macrophylla* Griffith using aqueous, ethanol, methanol, and hexane solvents.

Analysis	<i>Bouea macrophylla</i> Griffith											
	Aqueous			Ethanol			Methanol			Hexane		
	Leaves	Unripe fruits	Ripe fruits	Leaves	Unripe fruits	Ripe fruits	Leaves	Unripe fruits	Ripe fruits	Leaves	Unripe fruits	Ripe fruits
Alkaloids	+	++	+	++	+++	++	+++	+++	+++	+	+	+
Protein and amino acids	+	-	-	+	+	-	-	+	+	-	+	-
Anthraquinones	+	+	++	++	+++	+++	++	+++	+++	+	+	+
Flavonoids	+	++	++	+++	+++	+++	+	++	+	+	++	+
Glycosides	-	+	+	-	+	+	-	+	-	-	-	-
Saponins	+	+++	+	++	+++	++	++	+++	+++	+	++	+
Total phenol	+	++	++	++	+++	++	+++	+++	+++	+	++	+
Tannins	+	++	++	++	+++	++	+++	+++	+++	+	++	+
Sterols and Triterpenes	++	+++	+++	++	+++	+++	++	+++	+++	++	+++	+++
Carbohydrates	+	++	+	+	++	++	++	++	++	+	+	+
Gums and mucilage	++	++	++	++	++	++	-	++	+	+	+	+
Fixed oils	+	+	+	+	+	+	+	+	+	-	+	+
Vitamin C	++	++	+++	++	+++	+++	+	+++	+++	++	++	++

+++ = Appreciable amount (positive within 2 min.); ++ = Moderate amount (positive after 2 min. but within 5 min.); + = Trace amount (positive after 5 min. but within 10 min.); - = Completely absent.

Where A_0 was the control absorbance (blank, without extracts) and A_1 showed the absorbance of solvent extracts.

2.6. Statistical Analysis

All data are expressed as mean \pm S.D. Fifty percentage and above inhibition of DPPH radical was considered as significant for scavenging activity (Omisore et al., 2005).

3. Results and Discussion

3.1. Phytochemical screening

The present study dealt with the preliminary phytochemical investigation of the four different extracts (methanol, ethanol, aqueous and hexane) from *B. macrophylla* Griffith leaves, unripe and ripe fruits. Maximum extractions of phytochemicals were found to be present in methanolic extract when compared with ethanol, hexane and aqueous extracts of *B. macrophylla* Griffith (Table 1). The phytochemical screening of methanolic extract showed that the leaves and fruits were rich in alkaloids, flavonoids, anthraquinones, saponins, total phenol, tannins, sterols and triterpenes and vitamin C (Table 1). These compounds may be responsible for pharmacological potential as well as the physiological activity of *B. macrophylla* Griffith.

Phenolic compounds are recognized as powerful antioxidants and dominant constituent components of the plant materials (Ganesan and Xu, 2017; Hussein et al., 2017). Dietary polyphenols are the subject of prime interest due to their possible beneficial effects on human health. They exert inhibitory effects on mutagenesis, tumorigenesis and carcinogenesis in humans, when ingested up to 1.0 g/day in the diet rich in fruits and vegetables (Patil et al., 2009; Clementino et al., 2017; Syed and Ganapasam, 2017). Phenols are used in the preparation of some antimicrobial agents

such as dettol and cresol. In addition, polyphenols have been proved to be as antidiabetic (Mohib et al., 2017), antiobesity (Suk et al., 2017), antihypertensive (Dib et al., 2017) and prevent neurodegenerative disorders (Fernando et al., 2017). It has been recognized that flavonoids also have known to be potential antioxidants and their impacts on human nutrition and health are notable (Baby et al., 2017). The mechanisms of action of flavonoids are through scavenging or chelating process (Amir et al., 2012; Xiong, 2017; Kolar et al., 2017). Saponins have been proved as hypotensive agents and cardio-depressant properties (Zhu et al., 2017), which are helpful for the management of heart failure and cardiac myopathy (Hu et al., 2017). The occurrence of saponins in *B. macrophylla* Griffith might play a role in the cardioprotective potential. Alkaloids have the potential of anti-hyperglycaemic (Kwon et al., 2017) and anti-inflammatory activities (Liu et al., 2017). These phytochemicals of medicinal plants have primarily reported for their medicinal value, which can be valuable folkloric remedies in the treatment of various ailments.

3.2. *In vitro* DPPH radical scavenging activity

Fig. 1, Fig. 2 and Fig. 3 illustrated *in vitro* DPPH radical scavenging assay of ripe, unripe fruit and leaves extracts of *B. macrophylla* Griffith, which have significant antioxidant potentials compared with standard ascorbic acid. The highest scavenging activities on DPPH radicals showed in aqueous extracts of ripe fruit (83%), unripe fruit (82%) and leaves (76%) at the higher concentration of 50 μ g/mL. In addition, methanolic extracts of ripe (82%), unripe fruit (70%) and leaves (75%) extracts of *B. macrophylla* Griffith had also significant radical scavenging effects, respectively. The antioxidant capability of all plant extracts were compared to that of ascorbic acid standard (85%). The highest scavenging activities were observed in aqueous extract of the ripe, unripe fruits and leaves followed by methanol, ethanol

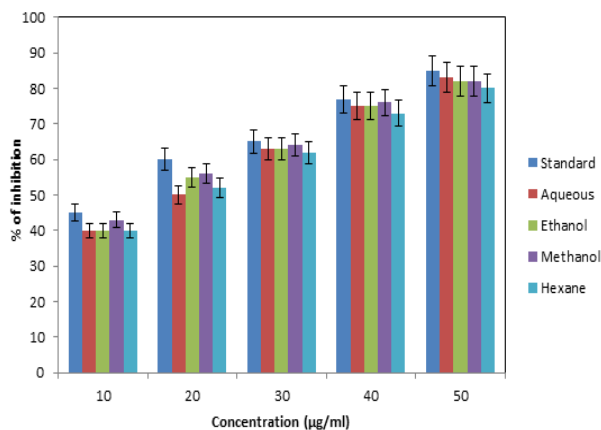


Fig. 1. DPPH radical scavenging activity of different solvent ripe fruit extract of *Bouea macrophylla* Griffith.

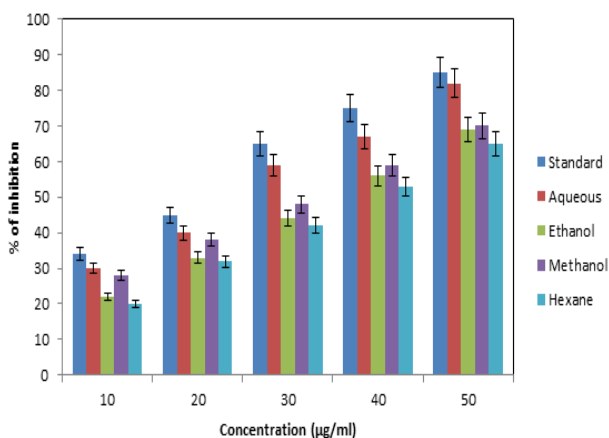


Fig. 2. DPPH radical scavenging activity of different solvent unripe fruit extract of *Bouea macrophylla* Griffith.

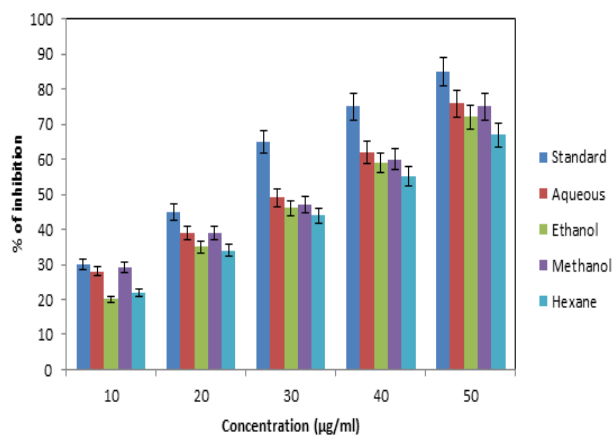


Fig. 3. DPPH radical scavenging activity of different solvent leaves extract of *Bouea macrophylla* Griffith.

and hexane extracts of the plant.

DPPH radicals are widely used as antiradicals to investigate the scavenging activities of several natural compounds and plant materials. To evaluate the scavenging effect of the extract in this study, DPPH reduction was investigated against positive control

ascorbic acid (Koleva et al., 2002; Suresh et al., 2008). The result of DPPH radical scavenging activity analysis indicates that ripe, unripe fruits and leaves of *B. macrophylla* Griffith displayed remarkable antioxidant properties. The DPPH contains an odd electron which gives a strong absorption maximum at 515 nm (Sarla et al., 2011). The purple color of DPPH turns into yellow when the odd electron of DPPH radical becomes paired with hydrogen from scavenging antioxidant to form a reduced DPPH. The greater reduction of DPPH is directly proportional to the presence of more antioxidant capability in the corresponding extract. This result indicates that the plant extract (*B. macrophylla* Griffith) contains compounds, which have the potential to donate a hydrogen atom to a free radical, converting the free radical to an unstable form. In addition, the capacity of these plant extracts to scavenge DPPH may possibly reoccur and prevent the generation of free radicals. The radical scavenging activities of these herbal extracts were found to be significant and may be useful for the treatment of radical associated pathological tissue injury (Andina and Musfirah, 2017). The presence of phenolic and flavonoids in the plant extracts are likely to be responsible for the antioxidant activity. These compounds are reported to be potent antioxidant or free radical scavengers (Andina and Musfirah, 2017).

4. Concluding remarks

Based on the solvent extraction, the type of antioxidant compound from the leaves, unripe and ripe fruits of *B. macrophylla* Griffith have been varied. Aqueous and methanolic extracts of fruits and leaves were found to be effective solvents for extracting antioxidant compounds from *B. macrophylla* Griffith. However, the aqueous extract can be a better free radical scavenging candidate since it has been accepted by human beings as an appropriate ready to be used or consumed as a raw fruit or in the juices. Overall, both fruits and leaves had higher amounts of antioxidant compounds and possessed higher amounts of vitamin C, flavonoid, polyphenolic and other phytochemicals constituents. The results obtained in the present investigation provides further insights on the presence of various antioxidant compounds both in fruits and leaves of *B. macrophylla* Griffith and may promote the commercialization trend of its production. Furthermore, these findings confirm that *B. macrophylla* Griffith may be considered among the best therapeutic agents in preventing various diseases associated with free radical damage.

Conflict of interest

The author declares that there is no conflict of interest.

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