April 30, 2017

PharmacologyOnLine

Archives • 2017 • vol.1 • 113-123

ETHNOBOTANICAL USES OF 'CEMCEM' (SPONDIAS PINNATA (L. F.) KURZ; ANACARDIACEAE) LEAVES IN BALI (INDONESIA) AND ITS ANTIOXIDANT ACTIVITY

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Abstract

Spondias pinnata is commonly known in international fruit trade markets as wild mango or hog plum. The use of *S. pinnata* leaves is considered an integral part of the Balinese ethnobotanical tradition, and is used to make a herbal-medicinal beverage. This present study is not only to improve the historical and ethnobotanical knowledge of this important species, but also to analyse the antioxidant properties and polyphenolic content of the plant in order to confirm, if any, the traditionally well-known beneficial effects. Ethnobotanical data were collected using semi-structured interviews through the snowball method. Informants were asked to determine how familiar they were with *S. pinnata* and its ethnobotanical values. In order to confirm antioxidant activity, the DPPH and ABTS•+ radical scavenging assays method, together with the Folin-Ciocalteu analysis were carried out. The present study indicated that *S. pinnata* possesses strong antioxidant activities based on the ABTS•+ and DPPH assays. It was also found that 100% methanolic extracts of *S. pinnata* leaves contain large amounts of phenolic compounds mainly responsible for free radical scavenging activities. Further study is essential in order to better understand the therapeutic values of *S. pinnata* leaves and its role in the prevention and treatment of ailments and chronic diseases mentioned in this study, such as diabetes, heartburn, and urolithiasis.

Key words: Anthropology, antioxidant, botany, diabetes, traditional medicine Asia & Oceania

Introduction

Spondias pinnata is a deciduous tree usually with a height from 25 to 40 m, and native to Indian and Malesian regions, where it can grow in primary and secondary forests from lowland up to 500 m altitude [1]. It is also cultivated, but not as widely as its well-known closely related species, *Spondias dulcis*. In international fruit trade markets, *S. pinnata* is known as wild mango or hog plum.Within the Malesian region this species is widely known as 'kedondong' (from Malay, the lingua franca of West-Central Austronesia) with a string of varieties in pronunciations [1]. The species is one of 17 described species from the genus Spondias, in which 10 species are native to Tropical Asia (i.e. Indo-Pacific, including *S. pinnata*) and seven are confined to the Neotropics [1,2,3].

The use of *S. pinnata* leaves is considered an integral part of the Balinese ethnobotanical tradition, and is used in the making of herbal-medicinal beverages to treat diabetes, heartburn, and urolithiasis [4]. Leaves can be consumed fresh as vegetables or boiled first to make a kind of traditional herbal drink or 'loloh' (Fig. 1) [4,5,6,7]. As leaves are more commonly used in traditional medicine they were selected to be the main source of materials for analysis.

It is well known that Reactive Oxygen Species (ROS) are the major contributors to several clinical disorders [8] and many plants are an important source of antioxidant, the first exogenous defense against free radicals. Recently, many natural antioxidants have been isolated from different plant materials [9,10] and also from S. pinnata [11,12]. The antioxidant properties of natural extracts are influenced by many factors, which cannot be fully described by a single method of analysis. For this reason the DPPH and ABTS•+ radical scavenging assays, which are the most commonly used methods for assessment of the antioxidant properties of natural products are proposed in this study together with the Folin-Ciocalteu analysis, which is considered the best method for determination of total phenolic content [13,14].

Prior research on the usage of *S. pinnata* in Bali (known locally as 'cemcem' or 'kecemcem') by [15], concentrates more on the bioscience side of 'cemcem'. The aim of this present study is not only to improve the historical and ethnobotanical knowledge of this important species, but also to analyse the antioxidant properties and polyphenolic content of the plant in order to confirm, if any, the traditionally well-known beneficial effects.

Methods

Study area and climate

Bali is located at the westernmost end of the Lesser Sunda Islands (Indonesia), between Java to the west and Lombok to the east. Within Bali Island, we selected 13 aga (indigenous Balinese) villages, i.e. villages inhabited by families whose ancestors have lived in Bali for many generations, and therefore consider them representative of the traditional ethnobotanical knowledge (TEK) present on the island (Figure 1). The 13 aga villages belong to the Bali aga ethnic group, who are regarded as the indigenous Balinese people that already inhabited the island long before the coming of the Later Bali people, who are well known as Bali Majapahit [7]. These villages are located between 242 and 1187 m above sea level. Most villages are found in the higher altitudes of the island, and they are mainly concentrated in the north and east of the island, where the touristic pressure is lower.
In addition to cultural values and traditions, Bali has a rich biological diversity. Bali is home to 1595 species of Spermatophytes, 173 species of Pteridophytes (ferns), and 169 species of Bryophytes [16]. About 18.2% of its surface area is occupied by forests, of which 7.8%, 10.1%, and 0.3% are, respectively, primary, secondary, and plantation forests (mainly consisting of eucalyptus and mahogany) [17].
Bali has a tropical climate with a bimodal seasonality. The dry season is from May to October with temperatures sometimes exceeding 32 °C. In the rainy season (November to April), the temperature drops to about 20 to 25 °C. The total annual rainfall can vary across the island spanning from around 1200 mm to around 3700 mm. The soil is alluvial and dominated by latosol, regosol, and andosol [17].

Ethnobotanical data collection

Ethnobotanical data were collected through semi-structured interviews with 50 informants (ages ranged between 14 and 76 years old) through the snowball method between May and July 2013 in 13 aga villages [7,18,19). Interviewees were made aware of the scope of this study and Prior Informed Consent was requested verbally [20]. Interviews were conducted in both Balinese and Indonesian, and we provided vernacular names of the plant according to the information obtained from the local inhabitants. Informants were asked to determine how familiar they were with *S. pinnata* and its ethnobotanical values. Detailed information of the informants (e.g., total informants, age, gender, education level, occupation, monetary earning, geographical, informants' villages, and socioeconomic characteristics) is provided in [21].

The informants were also asked to specify: which part of the plant was used, and how that plant part was used. The plant was collected with the informants and then identified by the first author and professional botanists of the Bali Botanic Garden. Scientific names of the plant species was verified using online sources [22]. A voucher specimen was deposited at the Herbarium Hortus Botanicus Baliense (THBB) in the Bali Botanic Garden (Indonesian Institute of Sciences).

Methods

The leaves of *S. pinnata* were collected in June 2014, which was the dry season in Bali, from individuals found in Penglipuran and Tenganan villages, Bali (Figure 2). The two villages were selected due to their local knowledge. The plant identification was carried out in the Herbarium Bogoriense (BO) and the Herbarium of the Bali Botanic Garden (THBB).

ABTS•+, DPPH, Gallic acid, and potassium persulfate were purchased from SIGMA-Aldrich. Folin Ciocalteau reagent, KH_2PO_4, Na_2CO_3, and Na_2HPO_4 were purchased from Merck. Organic solvent and Whatman filter paper # 1 were of analytical grade.

Antioxidant analysis

The leaves of *S. pinnata* were dried at room temperature for seven days, finely powdered and used for extraction. 10 g powder was macerated with 100 ml methanol (100%) for 24 hours. The solution was filtered through Whatman filter paper # 1, and evaporated under vacuum using a Heidolph rotary evaporator at 500 °C [10].

ABTS•+ radical scavenging assay: The ABTS•+ radical solution was freshly prepared from the reaction of $K_2S_2O_8$ (13.2 mg) with 7.4 mM ABTS aqueous solution (20 ml) and kept for about 12 - 16 hours at room temperature in the dark, yielding a blue-green solution. The solution was diluted with PBS (pH 7.0) to attain an absorbance value of 0.7 ± 0.2 at 734 nm for the assay. For each analysis, 10 µl of sample was added to 200 µl of ABTS•+solution and the absorbance was measured at λ 734 nm. Trolox was used as a positive control. The inhibition ratio (%) was calculated according to the equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] x 100. The antioxidant capacities were reported as TEAC (Trolox equivalent antioxidant capacity), defined as the concentration (µmol/ml) of Trolox having the antioxidant capacity equivalent to that of a 1.0 g/ml solution of the substance [10,23]. Statistical analyses were performed by applying Student's test. The level of significance was $p< 0.005$ for all data.

DPPH radical scavenging activity: 50 µl of the sample at variable concentration was added to 200 µl of DPPH solution. The reaction was incubated at room temperature for 30 minutes, and then the absorbance was measured at λ 517 nm. Ascorbic acid was used as a positive control. The inhibition ratio (%) was calculated according to the equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] x 100. The data were plotted as the percentage of DPPH inhibition versus antioxidant concentration. Linear regressions were elaborated using Graphpad Prism 4 software. Statistical analyses were performed applying Student's test. The level of significance was $p< 0.005$ for all data. IC50 were extrapolated from each graph as the concentration rate that caused quenching of 50% of DPPH radical [23]. The antiradical activity (ARA) was determined as 1/IC50.

Determination of total phenolic content: 20 µl of sample was added with 100 µl of Folin Ciocalteu (10% v/v) and Na_2CO_3 (7.5% w/v) solution in methanol. The reaction was incubated at 500 °C for 15 minutes and the absorbance was measured at λ 760 nm. A similar procedure was also performed with different concentrations of Gallic acid taken as standard. Total phenolic content was expressed as gallic acid equivalent (GAE) [10,23].

Results and Discussion

Historical backgrounds of S. pinnata in Bali

The origin of *S. pinnata* itself is still unclear and has been the subject of long debates [1,3]. The first scientific record of the presence of *S. pinnata* in the wild of Java was by [24]; however, whether this means native is uncertain as the trees are planted in clearings (especially in West Java), which may have been abandoned later [1]. In other words, the origin of this species is difficult to ascertain due to its wide cultivation and tendency to naturalise. [3] suggested that *S. pinnata* is not native to Malesia and has long been confused with *S. malayana* and *S. novoguineensis*. According to [3] this species is found growing spontaneously in the deciduous dry forests of India, Burma, and perhaps in Thailand and Indochinese, and had probably been introduced in Sri Lanka. Furthermore, he argued againts earlier claim by [25] and [1] that the species was widely cultivated in Malesia. Despite the still on-going debates of the origin of *S. pinnata*, until the result of DNA analysis has become available, *S. pinnata* is regarded here as "naturally existed" in Malesia, including in various places in Indonesia, such as Java and Bali

The earliest scientific information of the presence of *S. pinnata* in Bali is apparently provided by [26], where he described 'catsjemtsjem' (pronounced: 'kecemcem') as the vernacular Balinese name for his 'condondum.' Thus, Rumphius somehow knew and recognised the presence of 'condondum' in Bali, where it was also cultivated. As 'condondum' has been regarded as equal to the Linnaean *S. pinnata* [1], it thus marks the first scientific report of the existence of the species in Bali. Thus, *S. pinnata* is now proven to be a native to Bali or at least Bali is included within its natural distribution area. This is quite possible as *S. pinnata* was reported to be native of Java [24]; then still as *S. mangifera* var. *javanica*) or already a cultivated plant at least around the 9th century as it is celebrated in the carving of the Borobudur temple (Figure 3) [27]. By then *S. pinnata* might have been a common plant in Javanese rural areas.

Unlike in Java, *S. pinnata* has never been celebrated in Indian culture and is not known to have been depicted in any Indian Hindu or Buddhist temples. In other words, the species is apparently less ethnobotanically important in India than in Java and Bali. We suggest, therefore, that the celebrated status of *S. pinnata* in Java and Bali must have more to do with Austronesian civilisations than with the Aryan-Sanskrit based civilisation of India.

The possibility that *S. pinnata* was brought to Bali from Java by the Javanese Hindu Majapahit that took refuge in Bali following the advancing of Islam in Java is also widely open to debate. As has been previously mentioned, *S. pinnata* might have been a cultivated species in Javanese rural areas as early as the 9th century during the time of the Syailendra dynasty of the Medang Kingdom, who built the magnificent Borobudur temple, which was listed as a UNESCO world heritage site, and was completed in around 825 AD; about 470 years prior to Majapahit. The island of Bali itself is separated from the neighboring Java only by a narrow strait.

However, the existence of a relief depicting a plant bearing numerous fruits that it is thought are highly likely to be identifiable in both 'kedondong' and 'cemcem' (possiblly *S. dulcis* and *S. pinnata*) in Borobudur temple indicates the ethnobotanical importance of both species in the Javanese –and later Balinese– culture long before the establishment of the great empire of Majapahit, which was in the 13th century. Thus, it is possibile that 'cemcem' was already being both cultivated and used in medicinal beverages in Java and Bali as early as the 9th century, long before the end of the Majapahit era, which was in the 16th century. It is quite possible that the Balinese sacred book of medicine or Usada Bali had been written long before the final era of Majapahit [28].

Ethnobotanical aspects

Among 50 informants interviewed only seven informants, all male, mentioned 'cemcem' medicinal uses. Indeed, among the 13 aga villages, the uses came only from two villages, i.e. Penglipuran and Tenganan, where the plant grows, and often appears as a home garden plant. In fact, the plant can be found in lowland areas up to 500 metres above sea level. However, the ethnobotanical values are only obtained from two such villages.

The informants also declared that local people consume herbal drinks made from 'cemcem' leaves at least once a week. According to our previous study, came from the same informants' set, 'cemcem' availability index (the availability of the plant) is rare to middle, and its use value (the number of uses) and relative importance (the local importance of species) are 0.14 and 0.67 respectively [29]. In addition to some ethnobotanical indices, the taste score appreciation obtained from the local inhabitants is fair.

The modality of preparation is to produce juice by extracting leaves using traditional simple techniques which involve grinding the leaf with a flate stone or pestle; the juice is then filtered by twisting the leaves in a clean cloth. Leaves are used fresh, after collecting from the surrounding area. In the Usada Bali (the book of Balinese Traditional Medicine), *S. pinnata* leaves have been described as the remedy to cure various ailments and diseases [4,5]. In spite of lacking direct evidence, in the present study *S. pinnata* leaves can prevent or cure heartburn, urolithiasis, and diabetes. The anti-diabetic effect might be enhanced by using the traditional method of serving the 'cemcem' leaves as a herbal drink intended to treat high sugar levels. This herbal drink is a kind of juice, which has been traditionally used by the Balinese for a thousand years. It is believed that *S. pinnata* leaves have been used since the 11th century on the Indonesian island of Bali [30]. On the contrary, the earliest text on the medicinal purposes of *S. pinnata* was in the ancient Sanskrit book of medicine known as Ayurveda [31]. The first scientific report on the medicinal use of *S. pinnata* was in 'Hortus Malabaricus' [32] see also [33], where it was described as having medicinal purposes in curing uncontrolled menstruation, dysentery, and gonorrhea. [26] described that the Ambonese used to have baths with water that had been boiled with the leaves of 'condondum' (i.e. *S. pinnata*) to clean their bodies, both when they were ill or healthy. The tartness of the fruit of *Condondum malaccense* (i.e. Moluccan variety of *S. pinnata*) is believed to be good for the stomach.

Antioxidant activity

The ABTS•+ and DPPH assays are widely used methods for assessment of the antioxidant capacities of natural products, they both are spectrophotometric techniques based on quenching of stable colored radicals (ABTS•+ or DPPH) and show the radical scavenging ability of antioxidants even when present in complex biological mixtures such as plant or food extracts. *Spondias pinnata* extracts were capable of scavenging ABTS•+ and DPPH in a concentration-dependent manner. The DPPH radical assay performed on both extracts of *S. pinnata*, SPP and SPT, gave a low value of IC50 (Table 1). To show values directly dependent on antioxidant activity, antiradical activity (ARA) was calculated as 1/IC50. These results of scavenging activities indicate that *S. pinnata* leaves of both sites have good antioxidant capacity even if lower than pure ascorbic acid (IC50 = 2.5 ± 0.2; ARA = 0.40 ± 0.02). The results of ABTS•+ radical assays were presented as Trolox Equivalent Antioxidant Capacity (TEAC) using Trolox as reference standard. The TEAC values were 0.61 ± 0.01 and 0.310 ± 0.004 µmol Trolox/g for the two extracts of SPP and SPT respectively (Table 1). TEAC data confirm that both *S. pinnata* leaves are active even on ABTS radical cation and show good antioxidant capacity.

The TPC determined for *S. pinnata* extracts show high levels of polyphenols in both *S. pinnata* leaves under examination. It was 168 ± 2 mg GAE/g dw for SPP, and 101 ± 1 mg GAE/g for SPT (Table 1). Previous data reported by [34] referred to phenolic contents of *S. pinnata* at 47.2 and 42.6 mg GAE/g dry weight, in fruit flesh and seed extracts, respectively. [14] also found phenolic content of 42.6 mg GAE/g dry weight in *S. pinnata* leaf extracts of plants purchased from marked places in Thailand, which is lower with respect to the data obtained in the present study. The high TPC observed might be due to various causes either derived by the different site of plants or storage, drying procedure, processing, polarities of solvent (MeOH instead of EtOH), and the contribution of carbohydrates in the extracts [14,35,36].

There are significant differences in antioxidant capacities and total phenolic content among *S. pinnata* extracts from the two different sites/villages. *Spondias pinnata* extracts derived from Penglipuran village were two-times higher in antioxidant capacity (ABTS•+), 1.2 times in antiradical activity (DPPH), and 1.7 times in polyphenolic content (TPC) respectively. This may be attributed to the different elevations between the two villages, which reflect on the ecological conditions for the species. Tenganan is located close to the coast; whereas, Penglipuran is situated at 700 m altitude, and this condition might be more suitable for the growth of S. pinnata.

Furthermore, the results of this present study can be regarded as supporting evidence for a direct correlation between TPC and antiradical activity derived from the DPPH assay. Moreover, it is possible to evaluate by mathematical extrapolation that the radical scavenging properties of S. pinnata are not due to polyphenolic antioxidant for around 30% in SPT and 13% in SPP.

People living in the traditional villages in Bali have been using antioxidants and polyphenols as a part of their traditional medicine for centuries. The present study indicates that *S. pinnata* possesses strong antioxidant activities based on ABTS•+ and DPPH assays. It is also concluded that 100% methanolic extracts of *S. pinnata* leaves contain large amounts of phenolic compounds mainly responsible for free radical scavenging activities. Further study is essential in order to better understand the therapeutic values of *S. pinnata* leaves and its role in the prevention and treatment of ailments and chronic diseases such diabetes, heartburn, and urolithiasis.

Acknowledgments

The authors would like to express their gratitude to all of the Herbarium of Bali Botanic Garden staff for their help, particularly in the plant identification. The study was financially supported (for the field investigations) by the Italian Ministry of Education, University and Research (MIUR) through University Roma Tre (Rome, Italy). Deep appreciation is also sent to Dr. Tjandrawati of the Research Center for Chemistry, LIPI for providing essential laboratory facilities so this present study could be completed sufficiently.

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Table 1. Total phenolic content, $ABTS^{\bullet+}$ assay as Trolox equivalent antioxidant capacity (TEAC) and DPPH assay as IC_{50} and antiradical activity (ARA, $1/IC_{50}$) of two different extracts of SPP and SPT.

Extract	Total phenolic content (mg GAE/g dw)	$ABTS^{\bullet+}$ assay TEAC (µmol Trolox/g)	DPPH assay	
			IC_{50} (µg/ml)	ARA (ml/µg)
Spondias pinnata collected in Penglipuran village (SPP)	168 ± 2	0.61 ± 0.01	7.72 ± 0.03	0.108 ± 0.005
Spondias pinnata collected in Tenganan village (SPT)	101 ± 1	0.310 ± 0.004	9.30 ± 0.4	0.1295 ± 0.0004

Figure 1. Herbal traditional beverage, made from leaves decoction of *Spondias pinnata*.

Figure 2. The leaves of *Spondias pinnata* collected in Penglipuran village (SPP).

Figure 3. The relief of *Spondias pinnata* in Borobudur temple.