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Ethnobotanical uses of neem (*Azadirachta indica* A.Juss.; *Meliaceae*) leaves in Bali (Indonesia) and the Indian subcontinent in relation with historical background and phytochemical properties

Wawan Sujarwo [a,c,*], Ary P. Keim [b], Giulia Caneva [c], Chiara Toniolo [d], Marcello Nicoletti [d]

[a] Bali Botanical Gardens, Indonesian Institute of Sciences (LIPI), Candikuning Baturiti, Tabanan 82191, Bali, Indonesia
[b] Botany Division (Herbarium Bogoriense), Research Center for Biology, Indonesian Institute of Sciences (LIPI), Cibinong Science Center, 16911 West Java, Indonesia
[c] Department of Science, University Roma Tre, Viale G. Marconi 446, 00146 Rome, Italy
[d] Department of Environmental Biology, La Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

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ABSTRACT

Ethnopharmacological relevance: Neem (*Azadirachta indica*; *Meliaceae*) is widely known for its cold pressed seed oil, mainly used as insecticide, but also for cosmetic, medicinal and agricultural uses. The seed oil is widely employed in the Indian subcontinent, and the leaves seem to have a lower relevance, but the ethnobotanical information of Bali (Indonesia) considers the utilisation of leaves for medicinal properties.
Aim of the study: We report ethnopharmacological information about current uses of neem, in particular of the leaves, besides the insecticidal one, we discuss on the historical background of their uses.
Materials and methods: Ethnobotanical data were collected using both literature and scientific references and semi-structured interviews with 50 informants (ages ranged between 14 and 76 years old) through the snowball method in thirteen *aga* (indigenous Balinese) villages, following Ethic code procedures. The informants were asked to specify: which part of the plant was used, and how that plant part was used. Plant specimens were collected, identified and made into herbarium voucher. In consideration of the high variability and complex chemical constituent of neem, a HPTLC analysis of neem leaves coming from both the Indonesian island of Bali and the Indian subcontinent was carried out.
Results: The data on the medical use of traditional preparations from leaves of neem display a wide spectrum of applications. In the Indian subcontinent, neem leaves are used to treat dental and gastro-intestinal disorders, malaria fevers, skin diseases, and as insects repellent, while the Balinese used neem leaves as a diuretic and for diabetes, headache, heartburn, and stimulating the appetite. Differences in utilisation cannot be related to chemical differences and other constituents besides limonoids must be investigated and related to the multipurpose activity of neem.
Conclusion: This study revealed that neem leaves are believed to treat diabetes in both Balinese and Indian communities. Limonoids can not be considered the only responsible of digestive properties. Further research would be the validation of this report by enzymatic tests and the identification of active constituents.

1. Introduction

The origin of neem (*Azadirachta indica* A. Juss.; syn. *Melia azadirachta* L.), which is a medium-sized tree of the *Meliaceae* family, is greatly believed in the dry inland forests of Myanmar (earlier Burma) or Upper Myanmar to be exact (Schmutterer, 1995; Puri, 1999). This species was later introduced into India and became naturalised (Puri, 1999; Förster and Moser, 2000; Ross, 2005; Khare, 2007; Hwee-ling et al., 2009). The introduction of neem into the Indian subcontinent is most likely to have occurred in *anti*quity, since remains of this species have been found at the Mohenjo-daro archeological site in Pakistan dates to as early as 2600 BCE (see Puri (1999)); so old that even the Persians (being the nearest ancient neighbours of the Indians) named the species "*Azad Darakth e Hind*", which means "bitter tree from India" (Puri,

* Corresponding author at: Bali Botanical Gardens, Indonesian Institute of Sciences (LIPI), Candikuning Baturiti, Tabanan 82191, Bali, Indonesia.
E-mail addresses: wawan.sujarwo@lipi.go.id (W. Sujarwo), ary.prihardhyanto@lipi.go.id (A.P. Keim), giulia.caneva@uniroma3.it (G. Caneva), chiara.toniolo@uniroma1.it (C. Toniolo), marcello.nicoletti@uniroma1.it (M. Nicoletti).

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1999). Nowadays, in the Indian subcontinent neem is considered a sacred tree and called “Divine Tree”, as well as considered useful in treatment of a series of illness, as tested by the names “Life giving tree”, “Nature's Drugstore”, “Village Pharmacy” and “Panacea for all diseases” (National Research Council, 1992; Brahmachari, 2004).

Nowadays, neem is massively cultivated and planted in various areas in the tropics and subtropics, from numerous countries in Asia, Africa to the Caribbean (D'Arey, 1967; Lewis, 1983; Orwa et al., 2009), because of the ability of the species to withstand heat and dry climates, that makes it favourable to be planted against desertification and as shade trees along the sides of roads (Sara and Folorunso, 2002). The use of neem trees for shading in such grand scale can be seen in Saudi Arabia, where about 50,000 trees have been planted to shade Muslim pilgrims camping there annually for ‘Hajj’ rites (Ahmed et al., 1989; see also Mabberley et al., 1995).

The plant is commercially well known for its cold pressed oil from seeds widely used as insecticide (Ruskin, 1992; Jones et al., 1989; Pankaj et al., 2011). The neem's principal importance is based on three main aspects:

a. Efficacy, which has been reported against a broad spectrum of insects, mites, and nematodes, even also snails and fungi;
b. Seems not to generate resistance in the affected pests;
c. Neem has been considered as harmless to humans, birds, beneficial insects, and earthworms. The use of neem products has been approved by the US Environmental Protection Agency for use on food crops (EPA, 2012).

Furthermore, neem and its extracts are considered for medicinal and cosmetic activities (van der Nat et al., 1991; Saikia et al., 2006) and used in numerous herbal and allopathic medicines (Schmutterer, 1995; Bhowmik et al., 2010).

Reports have evidenced the high complexity of neem extracts, where more than 400 constituents have been identified and more are reported each year (Hatti et al., 2014), including the differences among raw materials of different origins. Concerning the insecticide activity of the seeds oil, a group of tetranortriterpenoids named limonoids are considered as the main active constituents of neem extracts with prevalence of azadirachtin A, salannin and nimbin, as confirmed by the HPLC analysis (Gallo et al., 2011; Nicoletti, 2011).

However, the multipurpose activity and uses of neem must be based on a quantity of substances as evidenced in case of other botanical materials. The main difficulty is in the relationship between activity and compounds, especially in the case of physiological effects. Recently, neem has been the object of extensive phytochemical studies, due to its strong biological effects (including antibacterial activities) based on its composition, which make it suitable to be implemented in agricultural and medicinal purposes (van der Nat et al., 1991; Pai et al., 2004; Khare, 2007; Thakurta et al., 2007; Del Serrone, 2015). Neem can be found also in Oman, where the plant is frequent in many towns. The chemical constituents and antioxidant activities of different extracts from leaves locally collected were studied and the leaves reported to contain limonoids, as nimbin, nimbanene and others, i.e. ascorbic acid, *n*-hexacosanol and aminoacids (Hossain et al., 2013; Siddiqui et al., 2003, 2004).

So far, there has been no scientific study on the leaf materials from Bali, including their connection with the Balinese culture (Keher and Nagi, 1949; Koul et al., 1990; Hossain et al., 2013; Benelli et al., 2014; Del Serrone et al., 2015). Therefore, the aim of the present study is to investigate the purposes of neem leaves in Bali and how the Balinese regard the species from an ethnobotanical point of view, especially for its medicinal purposes of various ailments. In consideration that the uses in Indonesia are mainly not present in the Indian subcontinent and that this could be derived from differences in chemical composition, a comparison of the leaves from the two environments was performed. In order to understand and validate the traditional uses, we studied the historical background and chemical composition of neem leaves both from the two environments. In consideration of this complexity, HPTLC (High Performance Thin Layer Chromatography) fingerprint method is used in order to evidence, as possible, the total metabolic production (Nicoletti et al., 2012; Benelli et al., 2014). HPTLC is the last evolution of planar chromatography (Gallo et al., 2011; Nicoletti, 2011; Gallo et al., 2012), which has been developed to increase separation and identification capacities of TLC. HPTLC amplifies ability to evidence natural products in complex extracts and mixtures, including constituents at very low concentrations.

2. Materials and methods

2.1. Study area and bioclimatic conditions

The direct ethnobotanical survey was conducted on the island of Bali, located at S 07°54′–08°50′ and E 114°26′–115°43′. We selected thirteen *aga* (indigenous Balinese) villages, i.e. villages inhabited by families whose ancestors have lived in Bali for many generations, and therefore considering them representative of the traditional ethnobotanical knowledge (TEK) present on the island (Fig. 1).

The thirteen *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 (Sujarwo et al., 2015). 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.

The total annual rainfall can vary across the island spanning from around 1200 to around 3700 mm. 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 20–25 °C. The soil is alluvial and dominated by latosol, regosol, and andosol (Badan Pusat Statistik, 2014).

The flora of Bali is constituted by 1595 species of Spermatophytes, 173 species of Pteridophytes (ferns), and 169 species of Bryophytes (Girmansyah et al., 2013). 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 composed by eucalyptus and mahogany) (Badan Pusat Statistik, 2014).

For the comparison with the Indian subcontinent, neem leaves were also collected from Coimbatore, which is located in an important area for the Indian ethnobotanical tradition Tamil Nadu, and can be considered the current neem's country. It lies at 11°1′6″ N 76°58′21″E in South India at 411 m above sea level, and has a tropical wet and dry climate, with wet season being from October to December. The mean minimum and maximum temperatures vary between 18 and 35 °C. The average annual rainfall is around 700 mm. The northern part of Coimbatore has a rich tropical evergreen forest (Natarajan et al., 1999).

2.2. Ethnobotanical and historical data collections

In Bali, 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 thirteen *aga* villages (Alexiades and Sheldon, 1996; Bernard, 2002; Sujarwo et al., 2015). Interviewees were

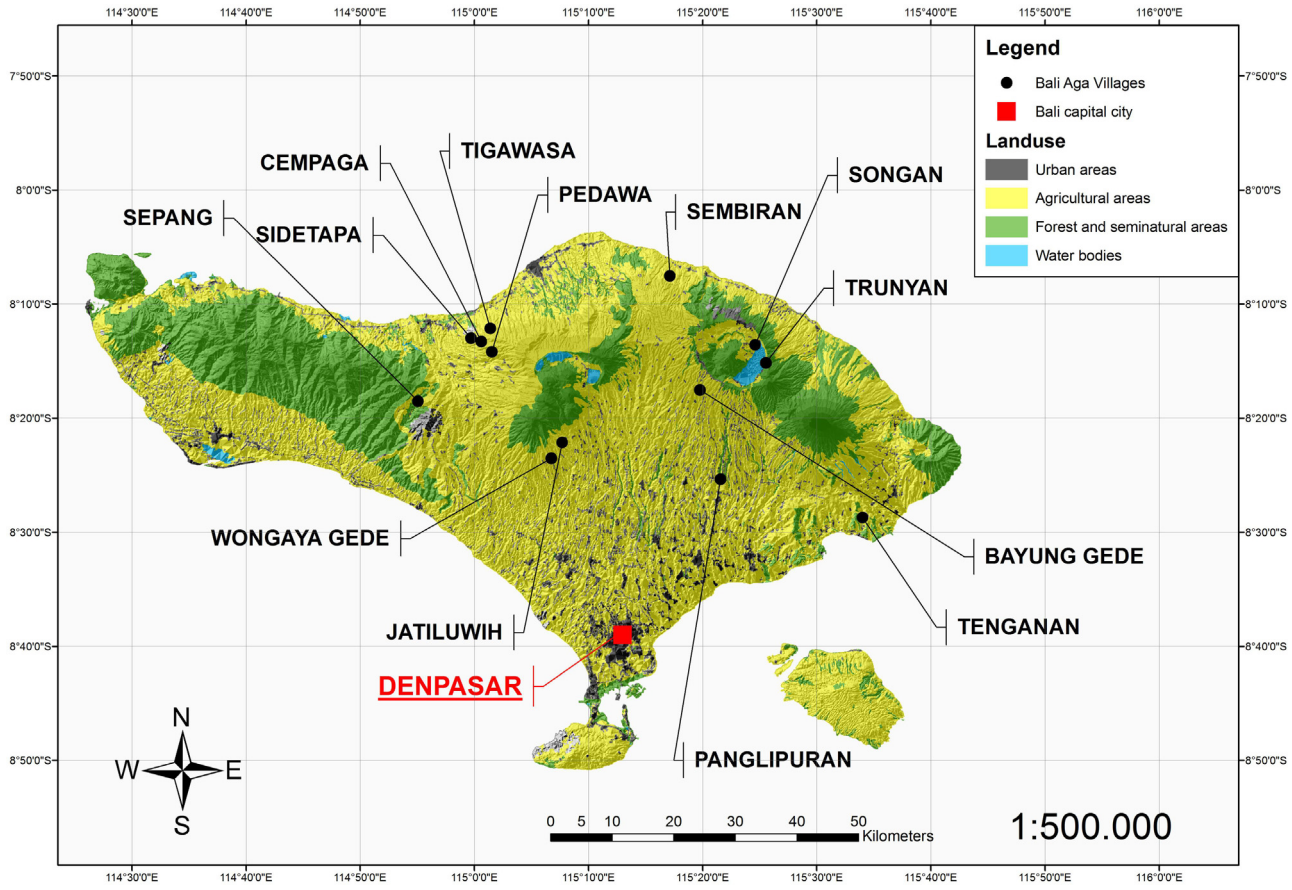

Fig. 1. The island of Bali and 13 surveyed *aga* (indigenous Balinese) villages.

Fig. 2. Hundreds-years Neem tree growing well in dry area of northern part of Bali Island.

made aware of the scope of this study and Prior Informed Consent was requested verbally (Rosenthal, 2006). 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 neem and its respective value. The informants were also asked to specify: which part of the plant was used, and how that plant part was used. 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 our previous contribution (Sujarwo et al., 2014). Ethnobotanical and historical data on the Indian subcontinent were collected from both literature and scientific references (Pruthi, 1937; Jain, 1991; Husain et al., 1992; Chattopadhyay, 1999; Puri, 1999; Siddiqui et al., 2003; Kala et al., 2004; Pai et al., 2004; Siddiqui et al., 2004; Khare, 2007; Thakurta et al., 2007; Bhowmik et al., 2010).

2.3. Plant material

Neem is an evergreen plant, which renews the leaves throughout the year (Heyne, 1927; Backer and Bakhuizen van den Brink Jr., 1965). Neem leaves were collected, during dry season from outer layer of canopy, by the first author from local areas in Sembiran Village, Buleleng Regency, Northern part of Bali (Fig. 2). The leaves from Coimbatore (Tamil Nadu, India) were collected in a similar way by the fifth author in the large park inside the local university. The botanical identification of the leaves (from individuals with flowers) both from Bali and Coimbatore was done using *Hortus Indicus Malabaricus* and Flora of Java Vol. II (Rheede tot Drakenstein, 1673; Backer and Bakhuizen van den Brink Jr., 1965). Voucher specimens were deposited at *Herbarium Hortus Botanicus Baliense* (THBB) in Bali Botanical Gardens and *Erbario Museo di Botanica* (RO) in Sapienza University of Rome. The updated scientific name of the plant species was verified using online sources (e.g., The Plantlist, 2015).

Table 1
Neem uses (with their frequencies of citation) recorded in Bali (Indonesia), and the comparison with previous ethnobotanical research in the Indian subcontinent.

Plant family and species (life form)	Bali (Indonesia)				The Indian subcontinent
	Vernacular names	Medicinal uses	Number of informants	Villages	Medicinal uses
Meliaceae					
Azadirachta indica A. Juss. (**Tree**)	Intaran, mimba, mimbo	Diabetes	5	Tg, Tw	Diabetes[1,2,6]
		Diuretic	1	Sm	Dental care[3,7]
		Headache	1	Sm	Gastrointestinal disorders[4,5,12]
		Heartburn	2	Sm	Insect repellant[8]
		Stimulating the appetite	2	Sm	Malaria[10,11]
					Various skin diseases[1,9]

Abbreviations. Villages: Sm=Sembiran; Tg=Tenganan; Tw=Tigawasa. Notes. [1] Bhowmik et al., 2010; [2] Chattopadhyay, 1999; [3] Husain et al., 1992; [4] Jain, 1991; [5] Kala et al., 2004; [6] Khare, 2007; [7] Pai et al., 2004; [8] Pruthi, 1937; [9] Puri, 1999; [10] Siddiqui et al., 2003; [11] Siddiqui et al., 2004; [12] Thakurta et al., 2007.

2.4. Preparation of extracts

Each sample (2.5 g) was extracted with 10 ml of aqueous EtOH (70%) at room temperature for two days and the obtained extracts filtered and evaporated under vacuum until total dryness. Then the residue was dissolved in methanol obtaining a concentration of 30 mg/ml. The solutions were stored at −5 °C until analysed (Toniolo et al., 2014).

2.5. HPTLC analysis

The HPTLC system (CAMAG, Muttenz, Switzerland) consisted of Linomat 5 sample applicator using 100 µl syringes and connected to a nitrogen tank; chamber ADC 2 containing twin trough chamber 20 × 10 cm; Immersion device III; TLC Plate Heater III; TLC visualizer –linked to winCATS software. Glass plates 20 cm × 10 cm (Merck, Darmstadt, Germany) with glass-backed layers silica gel 60 (2 µm thickness). Before use, plates were pre-washed with methanol and dried for 3 min at 100 °C. Filtered solutions of extract and standards were applied with nitrogen flow. The operating conditions were: syringe delivery speed, 100 nl s^{-1}; injection volume, 4 µl; band length, 8 mm; distance from bottom, 70 mm. The HPTLC plates were developed in ethyl acetate: dichloromethane: acetic acid: formic acid: water (100: 25: 10: 10: 11; *v*/*v*/*v*/*v*) using the automatic and reproducibly developing chamber ADC 2, saturated with the same mobile phase for 20 min at 25 °C. The developing solvents (i. e. type of solvents and ratios) were carefully optimised before the analyses. The length of the chromatogram run was 70 mm from the point of application (Nicoletti, 2011; Toniolo et al., 2014).

The developed layers were allowed to dry at 100 °C for 5 min and then derivatised with a selected solution, including Natural Product Reagent (NPR) (1 g diphenylborinic acid aminoethylester in 200 ml of ethyl acetate), the plate is heated at 100 °C for 2–3 min and then dipped into anisaldehyde-sulfuric acid (1 ml *p*-anisaldehyde, 10 ml H_2SO_4, 20 ml AcOH in 170 ml MeOH). Finally, the plates are dried for 5 min at 120 °C before inspection. All treated plates were then inspected under a UV light at 254 or 366 nm or under reflectance and transmission white light (WRT), respectively, at a Camag TLC visualizer, before and after derivatisation. WinCATS software 1.4.4 was used for the documentation of derivatised plates (Nicoletti, 2011; Toniolo et al., 2014).

2.6. Validation

Sample solutions of the extracts were found to be stable at 4 °C for at least 1 month and for at least 3 days on the HPTLC plates. Repeatability was determined by running a minimum of three analyses. RF values for main selected compounds varied ± 0.02%. The effects of small changes in the mobile phase composition, mobile phase volume, duration of saturation were minute and reduced by the direct comparison. On the contrary, the results were critically dependent on prewashing of HPTLC plates with methanol (Gallo et al., 2011; Nicoletti, 2011).

3. Results and discussion

3.1. Historical backgrounds

The first scientific description of neem was in the 4th volume of *Hortus Botanicus Malabaricus* by Rheede tot Drakenstein (1673) under the Malabar (*Malabarese*) name '*Aria Bepou*'. The nearest to that vernacular name is '*Ariyaveppu*' in Malayalam. In Rumphius' six volumes of *Herbarium Amboinense* (18th century), a wider description of Indonesian flora is carried out, but neem is not yet cited as a common species in Indonesian Archipelago, and he did not mention it among the many introduced species (Rumphius, 1741). Later, in Indonesia, neem is cited for Java, Bali, the Lesser Sunda Islands (particularly Lombok), and Sulawesi, even if its distribution is not wide (Miquel, 1869; Backer, 1925; Heyne, 1927; Backer and Bakhuizen van den Brink Jr., 1965).

It is believed that despite the long contact with the Indians (Ardika, 1999), population of neem trees in Indonesia is not abundant as in India and sparsely found (Heyne, 1927; Sukrasno, 2003). This phenomenon can be interpreted that neem might have been naturally dispersed by birds or if there has been human intervention it might have been planted by the indigenous people (i.e. the Austronesians) from India related to religious purposes; approximately similar to the cases of sacred fig or Bodhi tree (*Ficus religiosa* L.; Moraceae, related with the spread of Buddhism) and date palm (*Phoenix dactylifera* L.; Arecaceae, related with the spread of Islam) in Indonesia.

The possibility that neem was brought to the Indonesian Archipelago by the ancient Indonesian (i.e. Austronesian) sailors and traders from India in approximately the same time with the arrival of Hinduism to the Indonesian archipelago in at least in first century AD (see Schmutterer (1995), Koul and Wahab (2004), Supomo (2006)) seems to be fairly dubious. Miquel (1869) wrote that by the time he wrote his book neem has already well spread in Java, even cultivated in Sulawesi, but no mention on the origin of the species and how the species came into Indonesian Archipelago (then the Dutch East Indies).

Interestingly, neem is mentioned in the Balinese 16th century book of medicine (the *Usada*), which is believed to be written by a Javanese Hindu priest named Dang Hyang Dwijendra that went to Bali in the same century, approximately during the reign of King Waturenggong in Gelgel, Klungkung regency, Bali 1460–1550)

raises the possibility that neem has been cultivated in Java prior to the coming of the Europeans (Nala, 1996; Pringle, 2004); thus echoing the previous thought on the *anti*quity of the species introduction by the Austronesians (in which both the Javanese and Balinese are included) themselves (thus, not by Indians or other nations).

Then, based on the Balinese obvious adherent to Hinduism, it is believed that neem was in Bali Island, but not found in the Moluccas due to the lack of Hindu followers. In fact, the majority of Balinese are Hindu adherents, thus, it is reasonable that many plants, including neem, used in India for medicinal purposes (such as described in *Yajurveda*) are also mentioned and included in the ancient Balinese scripts on medicinal purposes, known as the *Lontar Usada* Bali (see Tengah et al. (1995) and Nala (1996)). In the *Lontar Usada* Bali, the usage of neem leaves for healing various kinds of ailments is described in great details, including the same practice known by other people, such as Javanese, Sasak (indigenous people in Lombok, the westernmost island in the Lesser Sunda Islands), and Balinese that live in Lombok (Tengah et al., 1995; Sujarwo et al., 2015).

In fact, in Indonesia, Bali is considered unique in the sense that it is a predominantly Hindu Island in the string of islands predominantly inhabited by Muslim societies. Thus, it is a kind like a relic of the pre-Islamic Indonesia, especially in the time when Indonesia was predominantly Hindu and full of Indianized kingdoms (Pringle, 2004; Supomo, 2006). Furthermore, due to their persistence on keeping the Hindu faith, it is assumed that the Balinese should have known 'neem' (as one of the important plant species described in the Hindu script *Ayurveda*) better than any other people in Indonesia and adjacent past Hindu influenced areas.

3.2. Ethnobotanical aspects

Among 50 informants interviewed, mainly constituted by male (90%) due to women tend to be less confident in providing information, only seven informants mentioned neem uses. Indeed, among the thirteen surveyed *aga* villages, information on neem uses came only from three villages, i.e. Sembiran, Tenganan, and Tigawasa, which are close to the coastline, where the plant grows, often as home gardens plant. In reality, most informants knew the plant and could give its popular names, i.e. intaran, mimba, mimbo, unfortunately they did not know the plant uses, also the plant does not exist in their villages.

The limited use of neem is also probably due to the fact that it is not an indigenous species; thus, not so familiar to most Balinese and Javanese (also presumably the Sasak people in Lombok Island). However, the informants declared that local people consumes herbal drink made neem leaves at least once/month. By the consequence, and according to our previous contribution, came from the same informants' set, neem 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 (Sujarwo and Caneva, 2016). The decoction of neem leaves is used overall for diabetes, and occasionally also as diuretic, headache, heartburn, and for stimulating the appetite (Table 1).

The modality of preparation is using decoction procedure by heating on a traditional stove the mixture of neem leaves and water until boiling. Leaves are often used fresh, after the collecting from the surrounding area. In case they need to be dried, our informants reported that drying the plant materials in direct sunlight might damage the "good ingredients" (the medicinal properties).

The anti-diabetic activity is enhanced from the traditional method of serving the neem leaves as an herbal drink intended to treat high sugar level. This herbal drink is a kind of decoction or infusion, which is also a common method in the thousand years of Balinese (as well as in Javanese, Madurese, and Sundanese) traditional medicinal preparation called "*jamu*" or "*loloh*". This traditional use is common in the domestic level; for commercial level the preparations have been packaged in tea bags containing dried powdered leaves ready for use (Sujarwo et al., 2015).

3.3. The comparison of both datasets

Although there have been few populations of neem trees found in Bali, still neem is one of important plants for Balinese in medicinal purposes. It could be seen that *Balian usada*, an indigenous medical practitioner who is knowledgeable on plant uses, conserved the species by leaving some parts for future re-growth and sprouting.

Despite still following the practice written in *Yajurveda*, in Bali only leaves are harvested for their medicinal and insecticidal

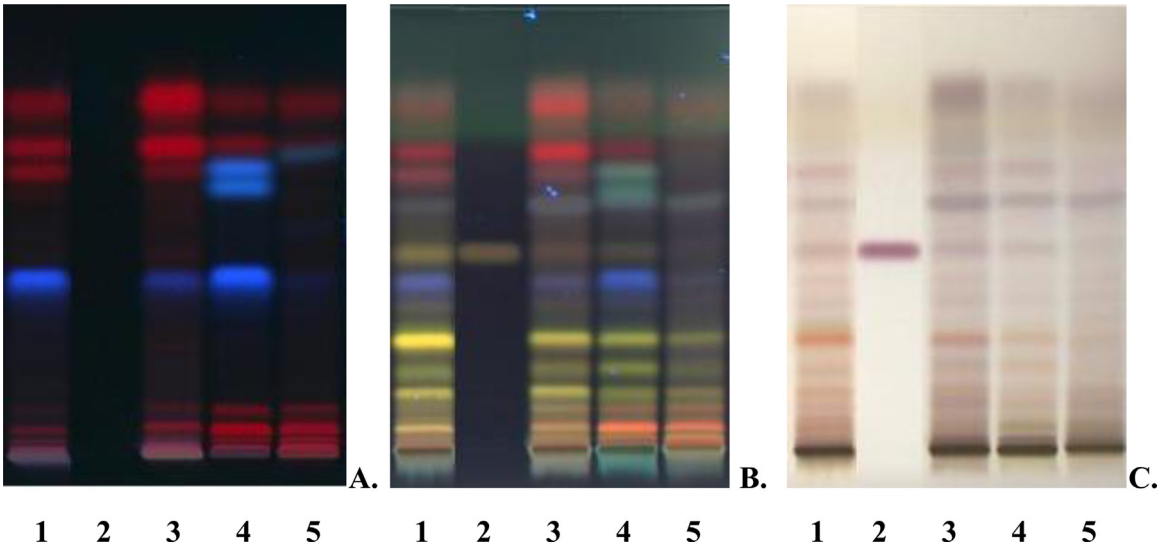

Fig. 3. HPTLC fingerprint of Neem Leaves extracts. A. Visualization under UV 366 nm before derivatization; B. Visualization under UV 366 nm after derivatization in anisaldehyde; C. Visualization under white light after derivatization in anisaldehyde. Tracks: 1. Neem fresh leaves from India; 2. Salannin (Standard); 3. Neem fresh leaves from Indonesia; 4. Neem dried leaves from Indonesia; 5. Neem powdered leaves from Indonesia.

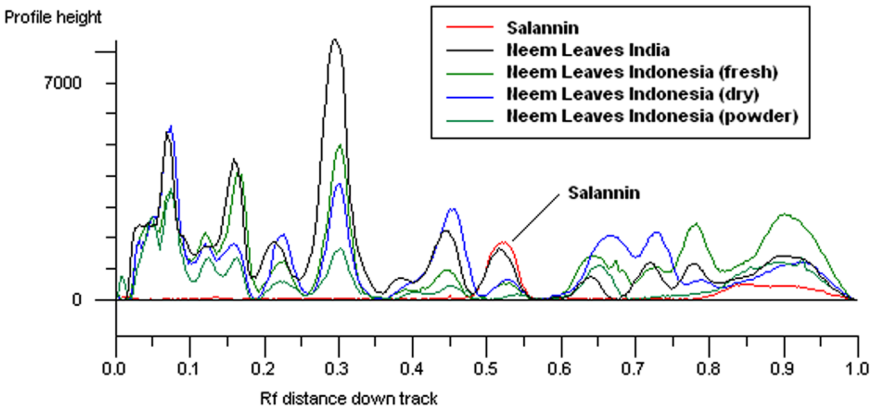

Fig. 4. HPTLC chromatogram of Neem Leaves extracts.

purposes (Förster and Moser, 2000; Paterson, 2009). Furthermore, neem is only fruiting once a year; thus the fruits and seeds are not always affordable. Therefore, harvesting fruits are regarded less efficient by the Balinese and Javanese and thus abandoned despite the fact that there is a great possibility that they may know that the seeds of neem have higher medicinal contents than the leaves through reading the *Ayurveda* (Tengah et al., 1995; Nala, 1996).

Leaves are proven to be a widely used plant parts (Martin, 1995; Cotton, 1996; see also Balangcod and Balangcod, 2011), and such result is confirmed for Bali (Sujarwo et al., 2015).

In the Indian subcontinent, neem leaves are used mainly to treat malaria fevers. Recent experiments have shown that one of neem's components, the pentacyclic triterpenoid gedunin, is as effective as quinine against malaria (Siddiqui et al., 2003; Siddiqui et al., 2004). It is also used by indigenous people in different parts of India for curing gastrointestinal disorders such as diarrhea and cholera are widely spread (Jain, 1991; Kala et al., 2004; Thakurta et al., 2007).

Traditionally, the Indians bathed in hot water soaked with neem leaves (Puri, 1999). This traditional method can prevent the various skin diseases or allergic reactions such as ringworm and scabies (see Bhowmik et al., 2010). Mixed dried neem leaves with stored grain or to place them among warm clothes is useful to repel insects and mosquito. The first scientific reports of the repellent action of neem against storage pests date back to the 1930s (Pruthi, 1937). Chewing neem leaves and seeds after a meal has been the traditional dental care practice in Indian rural areas. It is used in the treatment of gingivitis and periodontitis (Husain et al., 1992; Pai et al., 2004). The leaves can be also mixed with fodder in improving the quality of nutrients to livestock (Keher and Nagi, 1949). Ancient *Ayurveda* adherents believe that the bitterness tasted in the neem leaves can reduce the sugar level in human blood; thus, it is likely related with the property of the alcoholic extract of the leaves, which was found to possess a significant blood sugar effect and increased insulin release from the pancreas, potentially very useful against diabetes caused by high sugar level (Chattopadhyay, 1999; Khare, 2007; Bhowmik et al., 2010).

In some countries, like in Thailand, the leaves of neem are used as vegetable and medicaments, and crude extracts from leaves of Omani neem could be used as a vital source of natural antioxidant for food and pharmaceutical industry (Hossain et al., 2013; Nicoletti and Murugan, 2013).

3.4. Phytochemical analysis

Neem leaves possess excellent medicinal and insecticidal properties, and has been shown to contain crude fiber (11–24%), carbohydrates (48–51%), crude protein (14–18%), fat (2.3–6.9%), ash (7.7–8.5%), calcium (0.8–2.4%), phosphorus (0.134.24%), and a number of amino acids including the 10 essential ones (Keher and Nagi, 1949; Dakshinmurthi, 1954; Mitra and Misra, 1967; Koul et al., 1990).

We compared leaves from Bali with leaves from Coimbatore, Tamil Nadu using HPTLC. Fig. 3 shows the comparison between the different extracts of leaves. Track 1 reports the chromatographic fingerprint of the ethanolic extract of fresh leaves from India. It is followed by the spot of salannin, which is used as standard and marker of neem. Finally, three samples of Indonesian neem, i. e. fresh, dry and powdered leaves, reported in tracks 3, 4 and 5, respectively. In plate A, the red spots at high Rf values are corresponding to lipids and from degradation of chlorophylls, they are well present and evident in fresh raw materials.

As expected, the quantities of fatty acids and triglycerides are totally different and lower in comparison with the seeds oil (Del Serrone et al., 2015). In plate B, a large quantity of constituents is evident, including the marker salannin, and in general, the fingerprints are very similar, with few differences, mainly in quantities. As evident in the comparison of the spots concentration (Fig. 4), the amount of salannin is higher in leaves from India than leaves from Indonesia. Therefore, on the basis of the reported analysis, differences in utilisation can not be related to chemical differences and other constituents besides limonoids must be investigated and related to the multipurpose activity of neem. It will be interesting to follow in the future the adaptation of neem trees in very different habitats of other continents.

4. Conclusions

The differences of traditional use of neem between Bali and the Indian subcontinent is believed to the fact that neem is an introduced plant to Bali and the Balinese have their own medicinal system rooted in the already established Austronesian civilisation long before the contact with the Aryan Sanskrit speaking civilisation; thus, the use of the exported neem into the Balinese system of medicine would be regarded as “implementing thing of an uncertain result”. Consequently, the Balinese –as other people in the world when faced with something new– would naturally implement the most widely used plant parts that regarded most safe, i.e. leaves.

Indian and Balinese people use neem differently, and Balinese community recognised neem leaves are used as a diuretic and for headache, heartburn, and stimulating the appetite, while the local people in the Indian subcontinent supported neem leaves are used for dental care, gastrointestinal disorders, insect repellent, malaria, and various skin diseases. On the contrary, neem leaves are believed to treat diabetes in both cultural communities.

On the basis of the analyses, limonoids can not be considered

the only responsible of digestive properties, and other constituents must be investigated. The amount of collected ethnobotanical data let us consider reliable the beneficial properties of neem leaves in gastroenteric disorders and further research would be the validation of this study by enzymatic tests and the identification of active constituents.

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References

Ahmed, S., Bamofleh, S., Munshi, M., 1989. Cultivation of neem (*Azadirachta indica*; Meliaceae) in Saudi Arabia. Econ. Bot. 43 (1), 35–38.
Alexiades, M.N., Sheldon, J.W., 1996. Selected Guidelines for Ethnobotanical Research: A Field Manual. The New York Botanical Garden Press, New York.
Ardika, I.W., 1999. Ancient trade relation between India & Indonesia. In: Behera, K.S. (Ed.), Maritime Heritage of India. Aryan Books International, New Delhi, pp. 80–89.
Badan Pusat Statistik, 2014. Bali Dalam Angka. Badan Pusat Statistik Press, Denpasar, Indonesia, p. 2014.
Backer, C.A., 1925. Handboek voor de Flora van Java: Vol. 1. Drukkerijen Ruygrok, Batavia.
Backer, C.A., Bakhuizen van Den Brink Jr., R.C., 1965. Flora of Java Vol II. Wolters Noordhoff N.V, Groningen, The Netherlands.
Balangcod, T.D., Balangcod, A.K.D., 2011. Ethnomedicinal knowledge of plants and healthcare practices among the Kalanguya tribe in Tinoc, Ifugao, Luzon, the Philippines. Indian J. Tradit. Knowl. 10 (2), 227–238.
Benelli, G., Conti, B., Garreffa, R., Nicoletti, M., 2014. Shedding light on bioactivity of botanical by-products: neem cake compounds deter oviposition of the arbovirus vector *Aedes albopictus* (Diptera: Culicidae) in the field. Parasitol. Res. 113, 933–940.
Bernard, H.R., 2002. Research Methods in Anthropology: Qualitative and Quantitative Approaches. Altamira Press, Walnut Creek California.
Bhowmik, D., Yadav, C.J., Tripathi, K.K., Sampath, Kumar, K.P., Pradesh, U., 2010. Herbal Remedies of *Azadirachta indica* and its Medicinal Application. J. Chem. Pharm. Res. 2 (1), 62–72.
Brahmachari, G., 2004. Neem–an omnipotent plant: a retrospection. Chembiochem 5 (4), 408–421.
Chattopadhyay, R.R., 1999. A comparative evaluation of some blood sugar lowering agents of plant origin. J. Ethnopharmacol. 67 (3), 367–372.
Cotton, C.M., 1996. Ethnobotany: Principles and Applications. Wiley, London.
Dakshinmurthi, K., 1954. The amino acids in the leaf of *Azadirachta indica* (*Melia*). Curr. Sci. 23, 125–126.
D'Arey, W.G., 1967. Annotated checklist of the Dicotyledons of Tortola, Virgin Islands. Rhodora 69 (780), 385–450.
Del Serrone, P., Toniolo, C., Nicoletti, M., 2015. Neem (*Azadirachta indica* A. Juss) seed oil a natural preservative to control meat spoilage. Foods 4 (1), 3–14.
EPA US Environmental Protection Agency, 2012. Biopesticide Registration Action Document: Cold Pressed Neem Oil PC Code 025006.⟨http://www3.epa.gov/pesticides/chem_search/reg_actions/registration/decision_PC-025006_07-May-12.pdf⟩. (accessed 13.01.16.).
Förster, P., Moser, G., 2000. Status Report on Global Neem Usage Division 45: Rural Development. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), Eschborn, Germany.
Gallo, F.R., Multari, G., Federici, E., Palazzino, G., Giambenedetti, M., Petitto, V., Poli, F., Nicoletti, M., 2011. Chemical fingerprinting of *Equisetum arvense* L. using HPTLC densitometry and HPLC. Nat. Prod. Res. 25 (13), 1261–1270.
Gallo, F.R., Multari, G., Federici, E., Palazzino, G., Nicoletti, M., Petitto, V., 2012. The modern analytical determination of botanicals and similar novel natural products by the HPTLC fingerprint approach. In: Ur Rahman, A. (Ed.), Studies in Natural Products Chemistry. Elsevier, Oxford, pp. 217–258.
Girmansyah, D., Y., Santika, A., Retnowati, W., Wardani, I., Haerida, E.A., Widjaja, M. M.J., van Balgooy, 2013. Flora of Bali: an annotated checklist. Yayasan Pustaka Obor Indonesia, Jakarta.
Hatti, K.S., Muralitharan, L., Hegde, R., Kush, A., 2014. NeeMDB: convenient database for neem secondary metabolites. Bioinformation 10 (5), 314–315.
Heyne, K. 1927. De Nuttige Planten van Nederlandsch Indië ed. 2. Department van Landbouw, Nijverheid en Handel in Nederlandsch Indië, Batavia.
Hossain, M.A., Al-Toubi, W.A.S., Weli, A.M., Al-Riyami, Q.A., Al-Sabahi, J.N., 2013. Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. J. Taibah Univ. Sci. 7, 181–188.
Husain, A., O.P., Virmani, S.P., Popli, L.N., Misra, M.M., Gupta, G.N., Srivastava, Z., Abraham, A.K., Singh, 1992. Dictionary of Indian Medicinal Plants. Vap Enterprises, New Delhi.
Hwee-ling, K., Tung-kian, C., Chay-hoon, T., 2009. A Guide to Medicinal Plants: An Illustrated, Scientific and Medicinal Approach. World Scientific Publishing Company, Singapore.
Jain, S.K., 1991. Dictionary of Indian Folk Medicine and Ethnobotany. Deep Publications, New Delhi.
Jones, P.S., Ley, S., Morgan, E.D., Santafianos, D., 1989. Focus on Phytochemical Pesticides, The Neem Tree. CRC Press, Florida.
Kala, C.P., Farooquee, N.A., Dhar, U., 2004. Prioritization of medicinal plants on the basis of available knowledge, existing practices and use value status in Uttaranchal, India. Biodivers. Conserv. 13, 453–469.
Keher, N.D., Nagi, S.S., 1949. Neem leaves as a feed for livestock. Curr. Sci. 18, 325.
Khare, C.P., 2007. Indian Medicinal Plants: An Illustrated Dictionary. Springer, New York.
Koul, O., Isman, M.B., Ketkar, C.M., 1990. Properties and uses of neem, *Azadirachta indica*. Can. J. Bot. 68, 1–11.
Koul, O., Wahab, S. (Eds.), 2004. Neem: Today and in the New Millennium. Kluwer Academic Publishers, Dordrecht, The Netherlands.
Lewis, W.H., 1983. Neem (*Azadirachta indica*) cultivated in Haiti. Econ. Bot. 37 (1), 69–70.
Mabberley, D.J., Pannell, C.M., Sing, A.M., 1995. Meliaceae. In: Kalkman, C., Kirkup, D.W., Nooteboom, H.P., Stevans, P.F., de Wilde, D.J.J.O. (Eds.), Flora Malesiana I. 12(1). National Herbarium of Nederland, Leiden, pp. 1–407.
Martin, G.J., 1995. Ethnobotany: A Methods Manual. Springer, Falmouth.
Miquel, F.A.G. 1869. Annales Musei Botanici Lugduno-Batavi: Vol. 4. Apud C.G. van der Post, Amsterdam.
Mitra, C.R., Misra, P.S., 1967. Amino acids of processed seed meal proteins. J. Agric. Food Chem. 15, 697–708.
N., Nala, 1996. Usada Bali. Upada Sastra, Denpasar, Indonesia.
Natarajan, B., Paulsen, B.S., Pushpangadan, P., 1999. An ethnopharmacological study from the Coimbatore District, Tamil Nadu, India: traditional knowledge compared with modern biological science. Pharm. Biol. 37, 378–390.
National Research Council, 1992. Neem: A Tree for Solving Global Problems. National Academy Press, Washington, USA.
Nicoletti, M., 2011. HPTLC fingerprint: a modern approach for the analytical determination of botanicals. Braz. J. Pharm. 21 (5), 818–823.
Nicoletti, M., Serafini, M., Mariani, S., Ciocchetti, T., Murugan, K., 2012. Neem cake: chemical composition and larvicidal activity on Asian tiger mosquito. Parassitol. Res. 111 (1), 205–213.
Nicoletti, M., Murugan, K., 2013. Neem the tree of 21st century. Pharmacologyonline 3, 115–121.
C., Orwa, A., Mutua, R., Kindt, R., Jamnadass, S., Anthony, 2009. Agroforestree Database: A Tree Reference and Selection Guide Version 4.0. World Agroforestry Centre, Kenya.
Pai, M.R., Acharya, L.D., Udupa, N., 2004. Evaluation of antiplaque activity of *Azadirachta indica* leaf extract gel—a 6-week clinical study. J. Ethnopharmacol. 90 (1), 99–103.
Pankaj, S., Lokeshwar, T., Mukesh, B., Vishnu, B., 2011. Review on neem (*Azadirachta indica*): thousand problems one solution. Int. Res. J. Pharm. 2 (12), 97–102.
P., Paterson, 2009. Neem the Wonder Tree: Its Pesticide and Medicinal Applications. Scholarly Paper Masters of Chemical and Life Sciences University of Maryland, Maryland.
Pringle, R., 2004. A Short History of Bali: Indonesia's Hindu Realm. Allen And Unwin, Crows Nest.
Pruthi, H.S., 1937. Report of the Imperial Entomologist. Scientific Report from the Agriculture Research Institute, New Delhi.
Puri, H.S., 1999. Neem: The Divine Tree (*Azadirachta indica*). Hardwood Academic Publishers, Amsterdam.
Rheede tot Drakenstein, H.Avan, 1673. *Hortus Indicus Malabaricus*. Johannes van Someren, Johannes van Dyck. Hendrijk & Theodore Boom Publication, Amsterdam.
Rosenthal, J.P., 2006. Politics, culture, and governance in the development of prior informed consent in indigenous communities. Curr. Anthropol. 47 (1), 119–142.
Ross, I.A., 2005. Medicinal Plants of the World: Chemical Constituents, Traditional & Modern Medicinal Uses. 2. Humana Press, New Jersey.
Rumphius, G.E., 1741. *Herbarium Amboinense*. Franciscus Changuion, Amsterdam.
Ruskin, F.R., 1992. Neem: A Tree for Solving Global Problems. The National Academies Press., Washington DC.
Saikia, A.P., Ryakala, V.K., Sharma, P., Goswami, P., Bora, U., 2006. Ethnobotany of medicinal plants used by Assamese people for various skin ailments and cosmetics. J. Ethnopharmacol. 106, 149–157.
Sara, S.B., Folorunso, O.A., 2002. Potentials of utilizing neem tree for desertification control in Nigeria. In: Ukwe, C.N., Folorunso, A.O., Ibe, A.C., Lale, N.E.S., Sieghart, L. (Eds.), Sustainable Industrial Utilization of Neem Tree (*Azadirachta indica*) in Nigeria. UNIDO Regional Development Centre, Lagos, pp. 45–51.
Schmutterer, H., 1995. The tree and its characteristics. In: Schmutterer, H. (Eds.), The Neem Tree – Source of Unique Natural Products for Integrated Pest Management, Medicine and Other Purposes. Weinheim, New York, pp. 1–34.
Siddiqui, B.S., Afshan, F., Gulzar, T., Sultana, R., Nagui, S.N., Tariq, R.M., 2003. Tetracyclic triterpenoids from the leaves of *Azadirachta indica* and their insecticidal activity. Chem. Pharm. Bull. 51 (4), 415–417.
Siddiqui, B.S., Afshan, F., Gulzar, T., Hanif, M., 2004. Tetracyclic triterpenoids from the leaves of *Azadirachta indica*. Phytochemistry 65 (16), 2363–2367.
Sujarwo, W., Arinasa, I.B.K., Salomone, F., Caneva, G., Fattorini, S., 2014. Cultural erosion of Balinese indigenous knowledge of food and nutraceutical plants.

Econ. Bot. 68 (4), 426–437.
Sujarwo, W., Keim, A.P., Savo, V., Guarrera, P.M., Caneva, G., 2015. Ethnobotanical study of Loloh: traditional herbal drinks from Bali (Indonesia). J. Ethnopharmacol. 169, 34–48.
Sujarwo, W., Caneva, G., 2016. Using quantitative indices to evaluate the cultural importance of food and nutraceutical plants: comparative data from the Island of Bali (Indonesia). J. Cult. Herit. 18, 342–348.
Sukrasno, 2003. Mengenal Lebih Dekat Mimba: Tanaman Obat Multifungsi. Agromedia Pustaka, Tangerang, Indonesia.
Supomo, S., 2006. Indic transformation: the sanskritization of Jawa & the Javanization of the Bharata. In: Bellwood, P., Fox, J.J., Tryon, D. (Eds.), The Austronesians: Historical and Comparative Perspectives.. Australian National University Press, Canberra, pp. 309–332.
Tengah, I.G.P., Arka, I.W., Sritamin, N.M., Gotama, I.B.K., Sihombing, H., 1995. Studi Tentang: Inventarisasi, Determinasi dan Cara Penggunaan Tanaman Obat Pada "*Lontar Usada*" di Bali. Puslitbang Farmasi Balitbang Kesehatan Departe(men Kese)hatan Republik Indonesia, Jakarta.
Thakurta, P., Bhowmik, P., Mukherjee, S., Hajra, T.K., Patra, A., Bag, P.K., 2007. Antibacterial, antisecretory and antihemorrhagic activity of *Azadirachta indica* used to treat cholera and diarrhea in India. J. Ethnopharmacol. 111, 607–612.
The Plantlist, 2015. The Plantlist Database. ⟨www.theplantlist.org⟩. (accessed 18.12.15.).
Toniolo, C., Nicoletti, M., Maggi, F., Venditti, A., 2014. HPTLC determination of chemical composition variability in raw materials used in botanicals. Nat. Prod. Res. 28 (2), 119–126.
van der Nat, J.M., van der Sluis, W.G., de Silva, K.T.D., Labadie, R.P., 1991. Ethnopharmacognostical survey of *Azadirachta indica* A. Juss. (Meliaceae). J. Ethnopharmacol. 35, 1–24.