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No. 12, Thiruvannamalai Nagar, Saravanampatti - Kalapatti Road, Saravanampatti,
Coimbatore, Tamil Nadu 641035, India
Ph: +91 9385339863 | www.threatenedtaxa.org
Email: sanjay@threatenedtaxa.org

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Cover: ***Pipistrellus tenuis*** **recorded during the small mammalian fauna study, Manipur, India. © Uttam Saikia.**

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COMMUNICATION

Morphology characterization and phytochemical overview of the Moluccan Ironwood *Intsia bijuga* (Colebr.) Kuntze, a living collection of Purwodadi Botanic Garden, Indonesia

Melisnawati H. Angio [1], Elga Renjana [2] & Elok Rifqi Firdiana [3]

[1–3] Purwodadi Botanic Garden, Research Center for Plant Conservation, Botanic Gardens, and Forestry, National Research and Innovation Agency, Jl. Surabaya-Malang Km.65 Pasuruan, 67163, East Java, Indonesia.
[1] melisbio08@gmail.com (corresponding author), [2] elgarenjana@gmail.com, [3] elok.firdiana@gmail.com

Abstract: As one of the ex situ conservation sites, Purwodadi Botanic Garden (PBG) has *Intsia bijuga* as its collection with high economic value for its high quality wood. It is categorised as Near Threatened in the IUCN Red List. Its efficacy as herb is due to the presence of its various chemical compounds. The purpose of this study was to characterize the morphology of *I. bijuga* cultivated in PBG and to reveal its phytochemical compounds, as well as their health benefits. This research was conducted at the PBG in April–May 2020. The plant material was obtained from PBG collection. The plant morphology was characterized by direct observation in the field, while information regarding phytochemical compounds of *I. bijuga* along with their benefits was obtained by literature review. The data obtained was analyzed descriptively. The results showed that *I. bijuga* collected by PBG came from Maluku and Java. Both have morphological characteristics that are not very different, i.e., they are trees, they have compound leaves, pale stems with lenticels on their surface, panicle flowers, pod-shaped fruits, and buttress roots. *I. bijuga* contains polyphenol compounds that have medicinal benefits, such as anti-bacterial, anti-cancer, and anti-viral, hence it has enormous medicinal potential. Due to habitat shrinkage of the species, an effort to have it conserved ex situ is critical.

Keywords: Conservation site, Fabaceae, Merbau, plant morphology, phytochemical compounds.

Indonesian Abstrak: Sebagai salah satu kawasan konservasi ex situ, Kebun Raya Purwodadi (KRP) memiliki koleksi *Intsia bijuga* yang bernilai ekonomis tinggi karena kualitas kayunya. Spesies ini termasuk dalam kategori Near Threatened dalam IUCN Redlist. Efektivitasnya sebagai tanaman obat disebabkan oleh beragam senyawa kimia yang terkandung di dalamnya. Penelitian ini bertujuan untuk mengkarakterisasi secara morfologi *I. bijuga* yang berada di KRP dan mengungkap senyawa kimianya beserta manfaatnya untuk kesehatan. Penelitian ini dilakukan di KRP pada bulan April-Mei 2020. Material tumbuhan diperoleh dari koleksi KRP. Morfologi tumbuhan dikarakterisasi melalui pengamatan langsung di lapangan, sedangkan informasi mengenai senyawa fitokimia *I. bijuga* beserta manfaatnya diperoleh melalui tinjauan literatur. Data yang diperoleh dianalisis secara deskriptif. Hasil penelitian menunjukkan bahwa *I. bijuga* yang dikoleksi oleh KRP berasal dari Maluku dan Jawa. Keduanya memiliki karakteristik morfologi yang tidak jauh berbeda, yaitu keduanya memiliki habitus pohon, daunnya majemuk, batang pucat dengan lentisel pada permukaannya, bunga malai, buah polong, dan akar banir. *I. bijuga* mengandung senyawa polifenol yang bermanfaat bagi kesehatan, seperti anti-bakteri, anti-kanker, dan anti-virus sehingga memiliki potensial medis yang luar biasa. Karena adanya penyempitan habitat dari spesies ini, maka suatu upaya konservasinya secara ex situ menjadi hal yang sangat penting.

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INTRODUCTION

Intsia is a woody plant genus of the family Fabaceae (Leguminosae) which has a natural habitat in lowland tropical rain forests (Heyne 1987). They grow in forests up to 1,000 m and are often found in zones behind mangrove forests, brackish forests, and river banks (Samingan 1975). Their distribution comprises from Tanzania, West Indian Ocean, Taiwan to tropical Asia, and southwestern Pacific. Based on The Plant List (2013), *Intsia* has eight accepted species, i.e., *Intsia africana*, *I. attenuata*, *I. bijuga*, *I. bijuga* var. *retusa*, *I. bracteata*, *I. cuanzensis*, *I. palembanica*, *I. petersiana*, and *I. rhombodea.* On the other hand, Plants of the World Online (2022) states that *Intsia* consists only of *I. bijuga* and *I. palembanica*, while other species are included in genus *Afzelia*.

Intsia bijuga (Colebr.) Kuntze is a tree up to 40 m height and is known as Merbau or Moluccan Ironwood. It is native to southeastern Asia, Oceania, Madagascar, and eastern Africa (Image 1), while in Indonesia it can be found in almost all islands, especially Maluku, Kalimantan, and Papua (Baskorowati & Pudjiono 2015; GTA 2019). It lives in lowland tropical rain forests, capable of growing in the altitude of 1,000 m, and is often found in the riverside zone and behind mangrove forests (Yudohartono & Ismail 2013).

Intsia bijuga has high economic value for its high quality wooden structure. Until now, it was one of the favorites of Indonesian natural forest entrepreneurs (Tokede et al. 2013) and is very well known for export because it is widely used as furniture, plywood or woodworking (Pudjiono 2017). This has led to an increase in demand and massive logging of *I. bijuga* (Sirami et al. 2019). Forest destruction has exacerbated this situation, causing a reduction in the abundance of this species and declining wild populations (Margono et al. 2012; Vincent et al. 2015; Sirami et al. 2019). Therefore since 1998 it has been included in IUCN Red List (IUCN 1998). Concerns about its extinction have led to the inclusion of this species on the CITES list with Appendix III status (limited trading) (Tokede et al. 2013). Hence various rescue actions need to be taken to reduce the risk of extinction, one of which is ex situ conservation efforts.

In addition to considering the risk of extinction, awareness of plant conservation can also be raised by increasing the useful value of the plants. So far, *I. bijuga* has been used as traditional medicines in various countries including Philippines, Madagascar, Vanuatu, and Papua New Guinea to treat various diseases such as rheumatism, dysentery, urinary tract infections, asthma, diabetes, ulcers, and fractures (Norscia 2006; Koch et al. 2015), while in Indonesia, its bark is used as a medicine for flatulence and liver (Widodo et al. 2018). These various benefits are of course related to its various chemical compounds. Thus, the disclosure of information on phytochemical compounds in *I. bijuga* as well as its health benefits is also necessary in order to further increase its beneficial value.

As an ex situ conservation area, Purwodadi Botanical Garden (BG) has a collection of *I. bijuga* from Java and Maluku. Nevertheless, information on the morphology and content of phytochemical compounds about *I. bijuga* originating from Indonesia has not been widely published. This study aimed to characterize the morphology of *I. bijuga* from Indonesia cultivated in the Purwodadi BG. In addition, this study also revealed the phytochemical compounds contained in *I. bijuga* and their health benefits.

METHODS

This study was conducted at Purwodadi BG from April to May 2020. The leaves, stems, fruit and seeds as the study materials were obtained from Purwodadi BG (Image 2). Morphological characters observed included habit, root type, shape and size of leaves, fruit, and seeds. The morphological characterization was carried out based on Harris & Harris (2001). In addition, information regarding the name of the collector, the location of the original habitat, and location of the collection was obtained from the Plant Collection Catalog Information System (SIKATAN) of Purwodadi BG.

The species identity confirmation was performed through morphological character approach of study material on herbarium specimen and morphology description from relevant articles and books. The references for herbarium were specimens from Herbarium Purwodadiense and Kew's Herbarium (The Herbarium Catalogue 2022), whilst the reference articles and books were "Studies in Malesian Caesalpinioideae (Leguminosae)", "The genera *Acrocarpus*, *Afzelia*, *Copaifera* and *Intsia*" (Hou 1994) and "Caesalpiniaceae (Leguminosae-Caesalpinioideae)" (Hou et al. 1996), a book chapter of Flora Malesiana.

Furthermore, the search for information on phytochemical compounds of *I. bijuga* and its benefits was carried out using the literature study method between 2010–2020 through Google Scholar, Mendeley, and Science Direct websites with the keywords "*Instia bijuga*", "phytochemical", and "natural compound". The

Image 1. World distribution of *Intsia bijuga* in wild population. [Source: GTA (2019)]

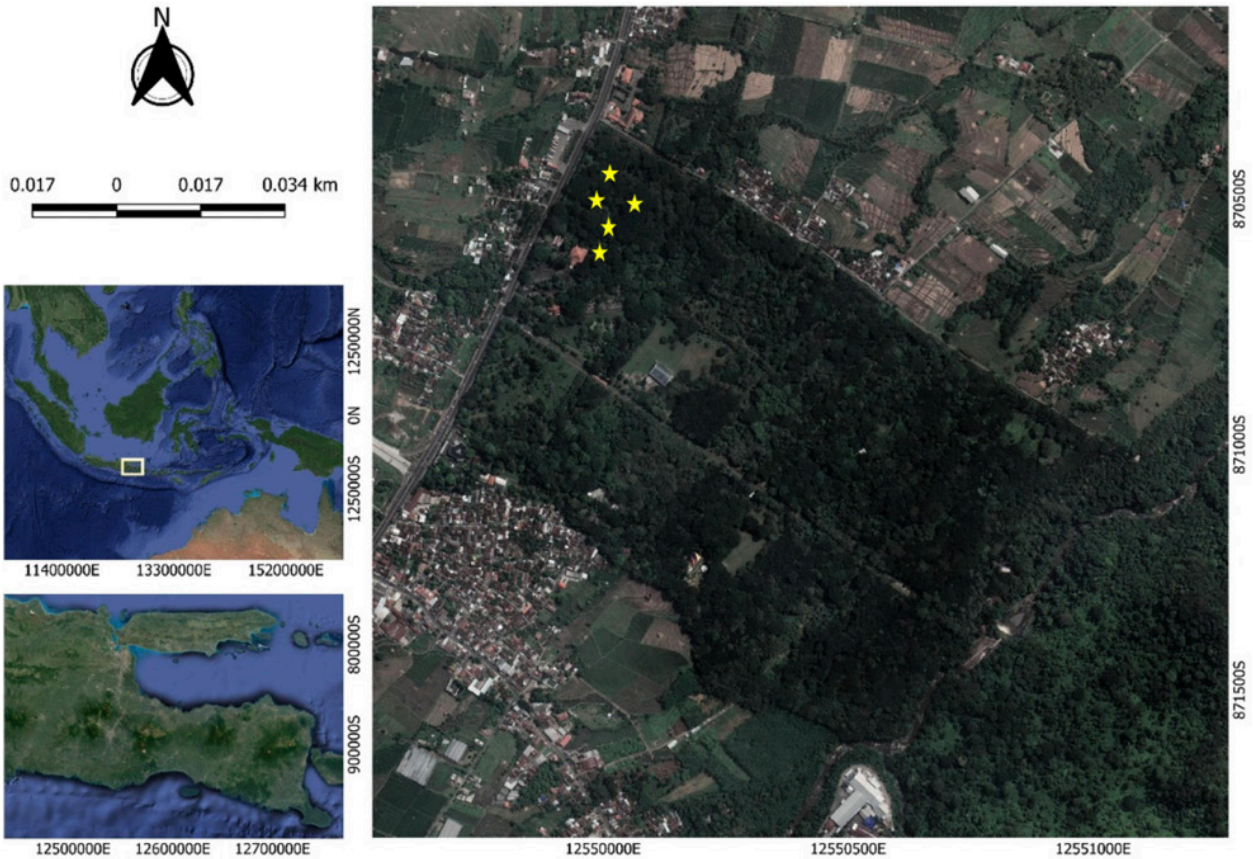

Image 2. Study site in Purwodadi Botanic Garden (the yellow asterisks indicate where *Intsia bijuga* collections are cultivated).

morphological character and phytochemical compounds data obtained were then analyzed descriptively to inform the potential of *I. bijuga* as a drug.

RESULTS AND DISCUSSION

Collection Database

Based on the database of PBG or Sistem Informasi Katalog Koleksi Tanaman Kebun Raya Purwodadi (2020), there are 12 collection numbers of the *I. bijuga* cultivated in five different locations/vak. The plants were the results of exploration in Indonesia (Maluku Islands and Java Island) and spontaneously collected. The age of the collection plants also varied, 24–65 years (Table 1).

Morphology of *Intsia bijuga*

So far, studies on morphology of *I. bijuga* in Indonesia have not been widely carried out and the information provided is still general and not specific (Rimbawanto & Widyatmoko 2006; Yudohartono & Ismail 2013; Pudjiono 2017). Based on direct observations, *I. bijuga* is a perennial tree with a height of about 15–23 m (Image 3A). This is slightly different from *I. bijuga* growing in Papua New Guinea, where at the age of eight it has reached a height of 12 meters with a trunk diameter of 15 cm (Gunn et al. 2004). The growing conditions and altitude factors were thought to have an indirect effect on plant growth, thus affecting plant height (Finkeldey & Hattemer 2007). The growth direction is erectus, hardwood and strong stems, elongated round shape like cylindrical, rough surface, showing lenticels on the surface of the stem and the length of the free stem before branching is about 7–10 m, releasing a little lymph and pale stems slightly greenish due to the lichen covering the stem surface (Image 3B).

Intsia bijuga has folium compositum consisting of 2–4 leaflets with a length of 7–12 cm per leaflet (Image 4A). The layout of the leaves is opposite and the shape is elipticus. The arrangement of penninervis with the costa is sloping so that the two parts of the leaf on the right and left of the leaf bone are asymmetric (Image 4B). The nerves lateralis are clearly visible and stop before reaching the leaf margins, the veins are smaller, forming a mesh and are not too prominent. Flat leaf edges are integer, thin but quite stiff with thickness of 0.05–0.1 mm, green leaf color with smooth abaxial and adaxial surface, hairless and not wrinkled. When compared with the leaves of *I. bijuga* in the Pacific Islands (Thaman et al. 2006), the leaf color of *I. bijuga* in Purwodadi BG is slightly darker. The shape of the tip of the leaf is acutus with the two edges of the leaf on the right and left of the costa gradually going upward and meeting at the tip of the leaf to form an acute angle. The base of the leaf is rotundatus and attached to the petiolus. Petiolus is cylindrical with slightly flattened upper side, green in color, thickened at the base with a diameter of 0.3-0.5 cm and does not show any wrinkles, hair, scales, lenticels or supporting leaves (Image 4C).

Direct observation of *I. bijuga* in Purwodadi BG showed that the cultivated plants had not yet entered the flowering season which usually occurs in January and lasts for 30–45 days. Slightly different from *I. bijuga* in the Northern territory where the cultivated plants flower in June and December, in its natural habitat, *I. bijuga* flowers in March (Cowie & Westaway 2012). Based on the observations conducted by Baskorowati & Pudjiono (2015), *I. bijuga* has unlimited bisexual inflorescentia centripetala and grows terminally and monopodially at the end of a branch. The peduncle grows steadily with branches that can branch again and the flowers bloom with acropetal type.

Although at the time of observation, *I. bijuga* had not yet entered the flowering season, part of the fruit was left over from the previous fertilization season. Fruit of *I. bijuga* is single true fruit dry pods with a length of 8–24 cm, smaller than the fruit of *I. bijuga* from Vanuatu which has a size of 10–30 cm (Thaman et al. 2006). The fruit is formed from one fruit leaf, it has a room with pseudo barriers where each fruit has a number of seeds, about 2–10 (Image 5). When young, the fruit is green and it is brown when ripe. Flat seeds, with 3–4 cm length

Table 1. *Intsia bijuga* cultivated in Purwodadi Botanic Garden.

Vak	Collection number	Access number	Collector	Date of cultivation	Origin	Note
XIII.E.I.	01-01abcd	P1986080306	LHP 08	1986-12-11	-	Spontaneous collection
XIII.F.	25	P1986070183	IS 221	1986-12-11	South west Maluku	Obtained from exploration
XIII.H.	48	P1955020044	-	1955-03-02	West Java	Obtained from exploration
XIII.I.	11-11a-11bc	P1995070068	IS 68	1996-11-11	Maluku: Halmahera	Obtained from exploration
XIII.K.	01-01a	P196002126	-	1962-01-11	Java	Obtained from exploration

Image 3. The habit (A) and stem (B) of *Intsia bijuga* cultivated in Purwodadi BG. © E. Renjana.

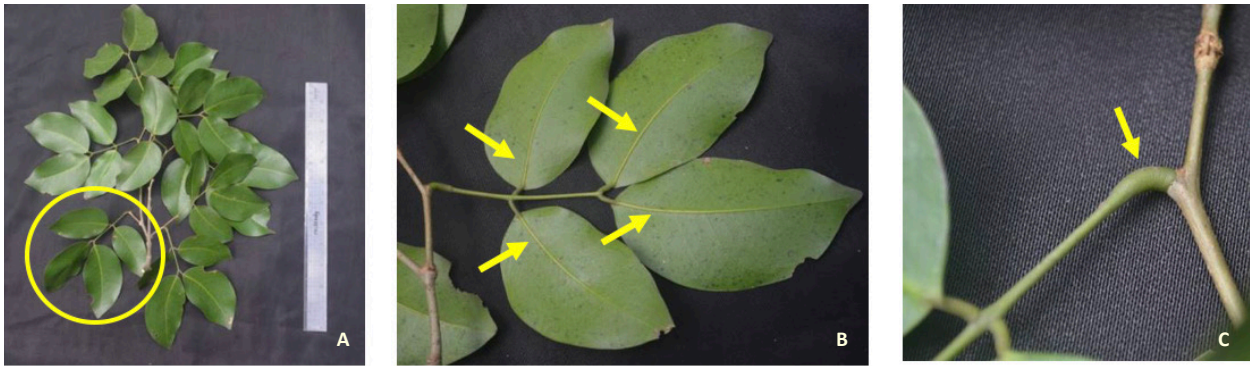

Image 4. Leaf morphology of *Intsia bijuga* cultivated in Purwodadi BG: A—folium compositum | B—penninervis | C—petiolus. © E. Renjana.

Image 5. Fruit and seed morphology of *Intsia bijuga* cultivated in Purwodadi BG. © E. Renjana.

with a black surface. The spermodermis is quite hard, where the tesla has a stiff texture and is like wood or stone. The hilus is clear, rough and brownish in color, slightly different from the surface color of the seed coat. The roots of *I. bijuga* are buttress type, where the roots grow high above the ground, and are flat like boards with a thickness of about 5–8 cm (Image 6). With the age of more than 20 years, the root of *I. bijuga* cultivated in Purwodadi BG is quite small compared to that of the root of *I. bijuga* growing in forests which can reach 4 m (Thaman et al. 2006). The root bark is pale, slightly greenish in color because it is covered by lichen and the surface is rough with scattered lenticels, and there are no spines near the roots.

In general, the plant morphology of *I. bijuga* does

Table 2. Phytochemical compunds in *I. bijuga* and their health benefits.

	Phytochemical compunds	Compound Group	Part of Plant used	Health Benefits	References
1	Dihydromyricetin (Ampelopsin)	Flavonoid	Stem	Provides neuroprotective effect on the brain injured by an ischemic stroke	Ye at al. 2017
				Induces cell growth inhibition and apoptosis in breast cancer cells	Zhou et al. 2014
				Acts as anti-bacterial, anti-inflammatory, anti-tumor, hepatoprotective, anti-hypertensive anti-oxidant, plasma lipid and blood sugar regulator; provides neuroprotection	Liu et al. 2019
2	Naringenin	Flavonoid	Stem	Acts as anti-hepatitis C, anti-aging, anti-Alzheimer's, anti-asthma, anti-chikungunya virus, anti-seizure epilepsy, anti-dengue virus, anti-diabetic, anti-edwardsiellosis, anti-hyperlipidemic, anti-inflammatory, anti-microbes, anti-oxidants, anti-platelets in cardiovascular disease, anti-damage due to stroke, cardioprotective, chronic kidney disease, expectorants, eye protective, hepatoprotective, radioprotective; provides aids against infertility, immunodepression, and constipation	Salehi et al. 2019
3	Myricetin	Flavonoid	Stem	Acts as anti-oxidant, anti-cancer, anti-inflammatory, anti-diabetic; provides protective effects against Parkisone and Alzheimer's	Semwal et al. 2016
4	Robinetin	Flavonoid	Stem	Acts as anti *Proteus vulgaris*, anti-tumor, anti-HIV-1	Kumar & Pandey 2013
5	3,5,3',4'-tetratrihydroxystilbene (Piceatannol)	Stilbene	Stem	Acts as anti-oxidant, anti-cancer, anti-parasitic, anti-bacterial; plays a role in cell signaling	Piotrowska et al. 2012
6	3,5,4'-trihydroxystilbene (Resveratrol)	Stilbene	Stem	Acts as anti-oxidant, cardioprotective, chemopreventive agent against cancer, anti-inflammatory, neuroprotective; has anti-viral properties	Liu et al. 2019

not vary too much in habit, stem growth direction, and root type between vaks/locations (Table 2). Although differences in morphological characters can be influenced by environmental factors, *I. bijuga* cultivated in Purwodadi BG do not significantly differ in morphology because they are in relatively the same environmental conditions. In addition, their vak/locations are quite close each other in Purwodadi BG area.

Quite different morphological variations can be seen in leaf size, where *I. bijuga* collected from Maluku, Halmahera planted in Vak XIII.I seems to have larger leaves than other *I. bijuga* planted in other vaks, with the ratio of length to width is 2: 1. Meanwhile, other leaf morphological characters such as leaf type, leaf layout, leaf shape, and leaf venation do not show clear differences (Image 7).

According to morphological characterization conducted in this study, the state character of *Intsia bijuga* lies in its compound leaves, which is 2-jugate leaf. This is in consonant with morphological descriptions of *I. bijuga* stated by Arifiani (2018), Hou et al. (1996), dan Hou (1994). Additionally, they also mentioned that another state character is the glabrous petiole.

Phytochemical compounds of *I. bijuga* and their benefits

From the identification of chemical compounds by Hillis & Yazaki (1973), it can be inferred that the wood of *I. bijuga* has six polyphenol compounds, consisting of four flavonoids and two stilbenes. The main polyphenol in this plant is flavonoid, namely robinetin, while the other three flavonoids are dihydromyricetin (ampelopsin), myricetin, naringenin. The two stilbenes in *I. bijuga* include 3,5,4'-trihydroxystilbene (resveratrol) and 3,5,3 ', 4'-tetratrihydroxystilbene (piceatannol). Based on literature studies, each of these chemical compounds has many health benefits (Table 2).

The variety of health benefits of *I. bijuga* is related to the role of each compound it contains at the organ and cellular levels. It's main potential is the prevention and treatment of tumors and cancer is due to its anti-oxidant, anti-tumor, and anti-cancer properties. According to Zhou et al. (2014) ampelopsin as one of the compounds contained in *I. bijuga* inhibited the growth of breast cancer cells as well as induced apoptosis. Furthermore, *I. bijuga* also plays a role in the maintenance of body organs such as the heart (cardioprotective), liver (hepatoprotective), eyes, and the nervous system (neuroprotective). Neuroprotective properties are very important for stroke, Alzheimer's, and Parkinson's patients in order to reduce the symptoms they suffer from.

The compounds of *I. bijuga* also have anti-inflammatory benefits. Inflammation is an immune system response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation.

Image 6. Root type of *I. bijuga* cultivated in Purwodadi BG. © E. Renjana.

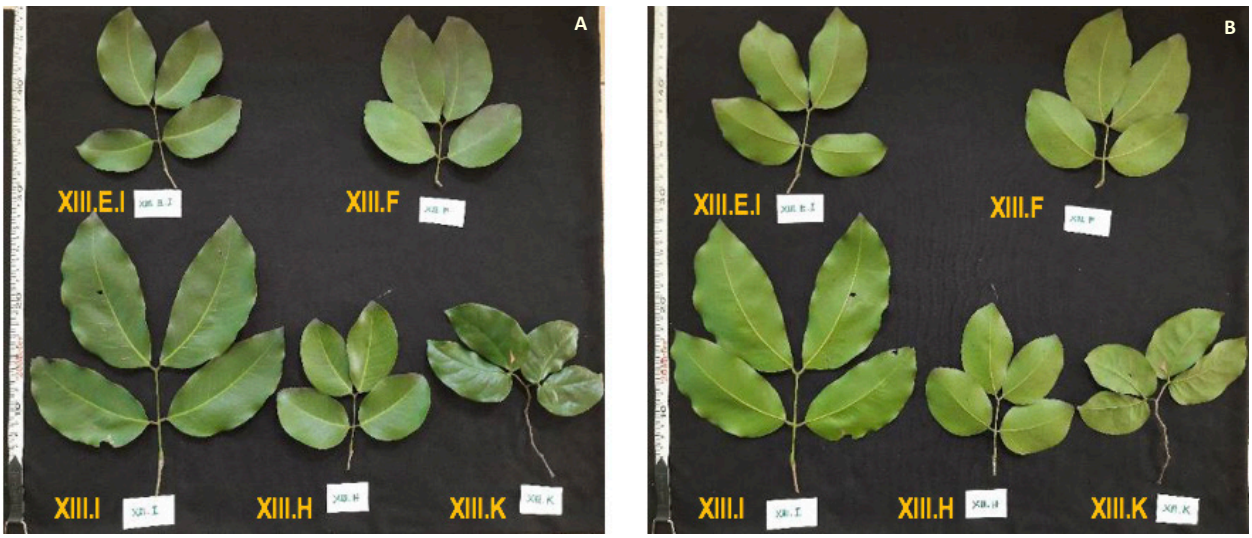

Image 7. Leaf shape and size of *I. bijuga* in various vak/locations of Purwodadi BG: A—abaxial | B—adaxial. © E. Renjana.

Inflammation causes discomfort since it causes swelling, redness, burning sensation, pain and reduced tissue function (Takeuchi & Akira 2010) so often anti-inflammatory drugs are used to reduce these symptoms. However, these drugs have many side effects, including gastric ulcers and cardiovascular complications, damage to kidney and the respiratory system (Henry 1988; Sostres et al. 2010). Various herbal plants have been developed to overcome the side effects of using synthetic anti-inflammatory drugs and *I. bijuga* can be a potential natural ingredient for this purpose with its anti-inflammatory components.

I. bijuga also has the potential to play a role in helping the body's immune system fight pathogens with its anti-bacterial, anti-parasitic, and anti-viral properties. One of the flavonoids of *I. bijuga* with anti-viral activity is robinetin. According to Kumar & Pandey (2013), robinetin is able to inhibit HIV-1 proteinase activity. In addition, *I. bijuga* might play a role in the maintenance of body homeostasis with its anti-hypertensive properties and its action in regulating blood sugar in diabetics and also its capacity as plasma lipids regulator. With all the active compounds that are useful and work synergistically for the health of the body, *I. bijuga* has the potential to be one of the leading medicinal plants.

Recent Conservation Status of *Intsia bijuga* and Prospective Research for its Conservation

Intsia bijuga is distributed across several regions of Indonesia, including Sumatra, Kalimantan, Sulawesi, Maluku, East Nusa Tenggara, and Papua. However, its distribution in Indonesia has begun to decrease due to illegal logging activities, so that it only remains in the Papua and Maluku regions (Rimbawanto & Widyatmoko

2006). With its superior quality of wood, this species has become a major production target for timber entrepreneurs in Papua, which raises concerns that it will face extinction in nature (Tokede et al. 2013). In 2020, it has been assessed into the category of Near Threatened (NT) with a decreasing population trend on the IUCN Red List v2.3 (Barstow 2020). Thus it can be inferred that it faces a high risk of extinction in the wild in the near future.

In addition to illegal logging activities, the decline in the population of *I. bijuga* is also caused by its natural growth factors. Naturally, this plant only bears fruit once a year from September to December (Baskorowati & Pudjiono 2015). In its natural habitat, seeds are very easy to obtain, but sometimes seedlings under the mother tree, especially on sandy and loamy soils, are difficult to find (Sirami et al. 2019). Thus, research on phenology and seed viability in ex situ conservation areas is expected to initiate a solution to this problem. Intensive maintenance and fertilization carried out in this area may have a positive impact on flowering and seed viability of *I. bijuga*.

Study on the propagation of *I. bijuga* can be done to increase the quantity of individuals. When deemed sufficient, the population of *I. bijuga* can be reintroduced in their natural habitat. Population reintroduction is henceforth a common practice in conservation to alleviate the loss of plant species. Generally, the aim of population reintroduction is to establish genetically variable populations, to increase gene flow and to minimize the probability of population extinction (Kaulfuß & Reisch 2017).

Based on the description of the medicinal potential of *I. bijuga*, the six active compounds of *I. bijuga* are mainly found in wood. However, if only the wood is used, then the sustainability of this species will be threatened. Therefore further research is needed on the content of its active compounds in other parts, such as leaves and seeds which are more abundant. In addition, the use of technology such as callus culture can be considered to produce the active compound in *I. bijuga* without having to extract it directly from nature. Callus cultures have gained commercial potential for the manufacture of secondary metabolites of therapeutic significance. Callus culture has been reported to be more reliable than collecting plant materials from the wild for extracting the therapeutic metabolites. They can be used for the generation of multiple clones of plants using micropropagation, and can also be used to develop single-cell suspension cultures employing either batch or continuous fermentation to produce the preferred secondary metabolites. Previous studies reported that callus cultures have been used for the production of tropane alkaloids, ajmaline, serpentine, reserpine, flavonoids, scopolamine, paclitaxel, stilbene, resveratrol and anthocyanins (Chandran et al. 2020).

CONCLUSION

Purwodadi Botanic Garden has a collection of *I. bijuga* originating from the Maluku Islands (Maluku: Halmahera) and Java. The results showed that all collections of *I. bijuga* observed had morphological characters that were not much different, namely tree habitus, compound leaves, pale stems with lenticels on the surface, panicle flowers, pod-shaped fruit and buttress roots. Based on literature studies, the wood of this species contains polyphenol compounds with medicinal benefits, such as anti-bacterial, anti-cancer, anti-viral, and so on. It shows that it has enormous medicinal potential. Therefore, ex situ conservation of these plants is very important considering that their numbers have decreased in their natural habitat

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Author details: Melisnawati H. Angio achieved her master in Plant Biology from Bogor Agricultural University and she is presently working as a researcher in Research Center for Plants Conservation, Botanic Gardens, and Forestry, BRIN. She is interested in botany and plants conservation, especially local fruit plants. Recently, she is studying rare and endemic plants in Sulawesi and also seeds conservation of National Park. Elga Renjana is a researcher at the Research Center for Plants Conservation, Botanic Gardens and Forestry, National Research and Innovation Agency (BRIN). He earned his M.Sc. degree from Biology Department, Airlangga University in 2017. His research interests are conservation of plants (Pteridophyte in particular), seeds, and modelling. He is also interested in predicting the potential of medicinal plants using docking analysis. To complete his task as plant conservation scientist, he also actively explores Indonesian forest. In addition of research activities, he is also active as an editor of Jurnal Penelitian Kehutanan Wallacea and the member of the Indonesian Researcher Union (PPI). Elok Rifqi Firdiana completed her Biology master degree from Brawijaya University. She is currently a researcher in National Research and Innovation Agency. She is interested in the conservation of threatened species in Indonesia hence she joined the Research Center for Plants Conservation, Botanic Gardens and Forestry. Beside working on in vitro propagation of certain species of Dipterocarpaceae, she is also involved in seed conservation of wild bananas project.

Author contributions: MHA built the concept, did the field work, and initiated the manuscript writing. ER helped the field work, did the data analysis, and developed the manuscript. ERF compiled the data, developed the manuscript, and translated it. All authors were involved in the finalization of the manuscript.

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