Nurul Izzati Osman et al /J. Pharm. Sci. & Res. Vol. 7(4), 2015, 185-188

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Pharmacological Activities of *Barringtonia Racemosa* L. (Putat), A Tropical Medicinal Plant Species

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Abstract
The interaction between plants and human has long been established since ancient times. Plants' medicinal properties have been acknowledged very well and considered as humans' living pharmacy for thousands of years. The knowledge of traditional medicine and ethno-botanical uses of plant species in each tribe may serve as a starting point for extensive pharmacological studies to be carried out in medicinal plant species. *Barringtonia racemosa* (L). which is also known as putat, fish poison tree or powder puff tree is a type of highly valuable plant species due to its medicinal values. Geographically found to be widely distributed from eastern Africa and Madagascar to Micronesian and Polynesian Island, this species is therefore has been associated very well in various tribes around the world with diverse ethno-botanical uses. The present article will discuss the ethno-botanical uses and pharmacological activities of *B. racemosa* which had been proven through various scientific researches.

Keywords Medicinal plants, *Barringtonia racemosa* L. (Putat), ethnobotany, phytochemistry, pharmacognosy, pharmacological activities

INTRODUCTION

Since ancient times, plants have been used as an important source of medicines due to their pharmaceutically significant contents of bioactive components. It has been recognised that medicinal plants may provide cure for certain ailments and disorders due to plant bioactive compounds isolated from plant secondary metabolites. Natural drug discovery which had been intensely developed in last few decades have proven the effectiveness of plant-derived remedies and verified the efficacy of folk medicinal preparations to be used for the treatment of certain ailments in human beings.

Barringtonia racemosa (L.) is a type of mangrove plants which is locally known as *putat* and fish-poison tree or powder-puff tree. In Singapore, the species is considered critically endangered. Meanwhile in Malaysia, according to a report presented by Malaysian Agricultural Research and Development Institute (MARDI) and Ministry of Agriculture of Malaysia in 2007 [www.fao.org/docrep/013/i1500e/malaysia.pdf], it has been stated that this species is classified as under-utilized crops since it gained less attention due to lack of effort in promoting their development and commercialization.

The species has long been used in traditional cuisines and ethno-medicinal practices. Through scientific studies, the medicinal uses of this species had been proven to be scientifically sound. Seen as an area which is not exhaustive and constantly expanding for further research to be carried out, the study of pharmacognosy in this species may provide another outlook for future development of plant-derived medicine from this species with possibly safer use and least side effects.

Botanical description

Taxonomically, this species is specified as follows: Kingdom: Plantae, Phylum: Tracheophyta, Class: Magnoliopsida, Family: Lecythidaceae, Genus: *Barringtonia*, Species: *Barringtonia racemosa* L.

Barringtonia racemosa L. is a type of mangrove plants species which grows well in wet and watery area such as along fresh water swamps, riverbanks and lakes with the height of approximately 4 to 8 m but can grow up to 15 m. Geographical distribution of the species includes the areas from eastern Africa and Madagascar to Sri Lanka, India and distributed in various regions of Southeast Asia and East Asia, Northern Australia as well as Micronesian and Polynesian Island. Known as a mangrove plant species, *B. racemosa* favours damp and watery areas. It can tolerate slightly saline conditions and on beaches near the high water level. However, the species cannot tolerate even a light frost. In addition, its distribution can also be found in primary and secondary forest, tropical rainforest areas, open lowlands and thickets.

The leaves are arranged in alternate orientation and found crowded at the ends of the branches. The size of the leaves is large, obvate-oblong to lanceolate, with the measurement of approximately 8-35 x 4-13 cm. The flowers of *B. racemosa* are attractive with whitish pink colours which are attached to the staminal tube **(Figure 1)**. They are bloom at night and fall the following morning. After the flowers shed, ants will be attracted to the inflorescences due to the presence of nectar [http://www.worldagroforestry.org/treedb/AFTPDFS/Barringtonia_racemosa.pdf]. On the other hand, the fruits of *B. racemosa* are about the size of a small chicken egg. The shape of the fruits is conical to ovate with the size of approximately 1.5 x 2.5 inches **(Figure 2)**. The fruits are coated with fibrous tissues and cause them to be buoyant and can be carried by water current to a great distance [http://www.worldagroforestry.org/treedb /AFTPDFS/Barringtonia_racemosa.pdf].

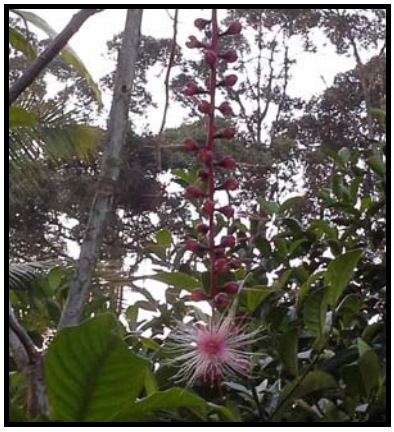

Figure 1: Whitish pink stamens of *B. racemosa* flower.

Figure 2: Fruit of *B. racemosa*.

Ethno-Botanical Uses

The fruits of *Barringtonia racemosa* are prescribed in the ayurvedic literature of Indian traditional medicines for the treatment of pain, inflammation and rheumatic conditions [1;2], ear ache and parturition[http://manoa.hawaii.edu/botany/plants_of_micronesia/index.php/full-database/288-barringtonia-racemosa-0]. The aromatic seeds are used to treat colics and ophtalmia [1]. In Karnataka, India, the species is used against dog bite wounds by different communities of Uttara Kannada district [3]. Apart from that, it has been reported that *B. racemosa* is medicinally used in the treatment of diarrhoea, asthma, coughs, jaundice, fever and functions as pain-killer as well. In Malaysia, the young shoots and leaves of *B. racemosa* are eaten raw as vegetable due to its medicinal values which is believed to be effective in high blood pressure treatment and management. The leaves are traditionally used to treat ulcer and cancer as well [4]. In addition, the pounded leaves, roots and barks are used to reduce itchiness and chicken pox [5]. Peter [6] reported that in South Africa, the Zulus use the fruit to treat malaria. Apart from medicinal uses, Barringtonia species is used as firewood [http://manoa.hawaii.edu/botany/plants_of_micronesia/index.php/full-database/288-barringtonia-racemosa-0] and for constructing planks in Sunderbans [http://mangrovesocietyofindia.in/mangrove_uses.php]. *B. racemosa* fibres are also used in producing hardboard, particle board and block board. Known as fish poison tree, the plant species is named after its properties which are used to stun fish due to its poisonous saponin in seeds, bark, wood and roots. High content of tannin in barks is frequently used in powdered form for poisoning purposes. According to Peter [6], extracts from the plant are known as effective insectides and used against *Citrus aphids*. Additionally, French [http://foodplantsinternational.com/articles/] had stated that the fruits are effectively used to poison wild pigs.

Pharmacological Activities

Antibacterial

In a study done by Khan *et al*., [7], the roots of *B. racemosa* had been reported to have antibacterial activity against several strains of both gram positive and gram negative bacteria. Later in 2013, Saha *et al.*, [8] were carried out another test on antibacterial activity from ethanolic bark extract. Six bacterial strains were used in disc diffusion assay which include *Staphylococcus aureus, Staphylococcus epidermidis, Eschericia coli, Shigella dysentriae, Vibrio cholerae and Proteus sp*. to assess the antibacterial potential of the species. The etahnolic bark extract of putat showed antibacterial activity against all bacterial strains tested with the zone of inhibition ranging from 6.96 to 14.12 mm.

Anti-tumour

B. racemosa also reported to be a potential candidate for the development of phyto-based anti-tumour agent. A study carried out by Thomas *et al.* [4] verified the efficacy of *B. racemosa* methanolic seed extract since it was found that the species exerted an even better anti-tumour effect than vincristine on mice challenged with 1 million Dalton's Lymphoma Ascitic (DLA) cells. The optimum dose for anti-tumour activity was found to be 6 mg/kg whereby such dose protected all the animals challenged with the tumour cells. Additionally, the extract was found to be devoid of conspicuous acute and short-term toxicity to mice through daily intraperitonial administration for 14 days up to a dose of 12 mg/kg.

Anti-nociceptive / Analgesic

Significant anti-nociceptive activity of *B. racemosa* had been reported from aqueous bark extract. The study was carried out at four different doses of 500, 750, 1000 and 1500 mg/kg body weight. The analgesic activity had been demonstrated upon hot plate and formalin test in experimental male mice without producing any unwanted side effects or toxicity. Interestingly, the extract did not alter fertility, gestational length, peri- and neonatal development and appears to be non-teratogenic. It was found that the antinociceptive effect was mediated mainly via opioid mechanisms [9].

In 2010, a study had been documented by Shikha *et al*., [10] to verify the analgesic potential of *B. racemosa* fruits through acetic acid-induced writhing response and its anti-lipid peroxidation properties. Upon administration of *B. racemosa* ethanolic extracts at three doses of 125, 250 and 500 mg/kg body weight, the writhing responses in mice were successfully inhibited at 68.02%, 79.5% and 91.8% respectively. The inhibition at 500 mg/kg dose was almost comparable to that of acetyl salicylic acid, the positive control (93.1%). Apart from that, *B. racemosa* at 150 µg/ml

showed the highest percentage of inhibition of Ferric chloride-acetic acid ($FeCl_2$-AA) stimulated rat liver lipid peroxidation *in vitro*. A reduction of malondialdehyde (MDA) production was observed following administration of *B. racemosa* hence signified its anti-lipid peroxidation effects.

Acetic acid-induced writhing test was also performed by Saha *et al*., [8]. The writhing test done had demonstrated significant dose-dependent analgesic activity in ethanolic bark extracts of *B. racemosa*. In such assay, peripherally acting analgesic activities of the samples were evaluated by inducing writhing through sensitization of pain receptors by prostaglandin releases. The results showed 36.3% and 63.8% inhibition of writhing at the doses of 250 mg/kg and 500 mg/kg body weight orally respectively.

Antioxidant

The study of antioxidant activities done on this species was found to be documented by Behbahani *et al.* [11], in which the samples were taken from the fully expanded leaf extracts. Three different assays were conducted which include the FTC (Ferric thiocyanate), DPPH (Diphenyl picryl hydrazyl) and (TBA) Thiobarbituric acid assays in which the radical scavenging activities were being assessed. From the results that they obtained, even though the *B. racemosa* extracts exhibited a bit lower antioxidant activity in DPPH assay (chloroform extract showed 54 µg/ml IC_{50} values as compared to 32 µg/ml in alpha-tocopherol), but the chloroform and hexane extracts exhibited higher activities than vitamin E (alpha-tocopherol) in the FTC and TBA assays.

Meanwhile, Nurul-Maryam *et al*. [12] had conducted the antioxidant activities study on this species as well. Different aerial parts of *putat* were assessed and the samples were taken from leaf, stick and bark. The results from the study showed a potent antioxidant activity found in *B. racemosa* in which the methanolic and ethanolic extracts in all aerial parts exhibited comparable activities to BHT (butylated hydroxytoluene), ascorbic acid and alpha-tocopherol.

In addition to that, in a study done by Kong *et al.* [13], water, ethanol, ethyl acetate and hexane were used as solvents for the extraction of antioxidant from leaves and stems of the shoots of *B. racemosa*. It was found that the leaf water extracts had the highest ferric reducing activities and scavenging activities against ABTS (2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), DPPH (diphenyl picryl hydrazyl) and superoxide anion radicals. It was found that the leaf extracts had the highest polyphenol and ascorbic acid contents as well. The superiority of anti-oxidative activities shown by the species was clearly manifested due to its higher and comparable activities to that of BHT, ascorbic acid, rutin and Gallic acid. Upon UHPLC analyses, Gallic acid, protocatechuic acid, ellagic acid, quercetin and kaempferol were detected and these compounds were identified to be responsible for its anti-oxidative actions.

Additionally, Kong *et al.* [14] performed another series of *in vitro* antioxidant analyses by using biological samples. In such assay, the inhibition of oxidation in serum, LDL (low density lipoprotein) and haemoglobin were assessed by using leaf and stem extracts of *B. racemosa*. In the LDL oxidation assay, *B. racemosa* leaf extract (IC_{50} = 73.0 µg/ml) was better than stem extract (IC_{50} = 226 µg/ml) at inhibiting the formation of TBARS (Thiobarbituric acid reactive substances) and lipid hydroperoxides. Similar trends were observed for serum and haemoglobin oxidation. In serum oxidation assay, *B. racemosa* leaf extract at its highest concentration (1000 µg/ml) inhibited 46% of TBARS as compared to its stem extract at similar concentration which only exerted 19% inhibition. *B. racemosa* leaf extract was better than its stem extract in delaying the time required to oxidise haemoglobin to MetHb (methaemoglobin) and showed a concentration-dependent increase in the inhibition of MetHb (methaemoglobin) with the highest inhibition recorded at 500 µg/ml (79.51%).

Anti-inflammatory

Griess assay for nitric oxide (NO) inhibitory activity had been carried out to investigate the anti-inflammatory activity from the leaves of *B. racemosa*. The cell viability study was also conducted to assess the cytotoxicity effect of the samples. Moderate activity of 73.85% of NO inhibition capacity had been observed from 200 µg/ml chloroform extract of *B. racemosa* leaves with no cytotoxic effect. The results suggested that chloroform extract of *B. racemosa* leaves would be potentially used as anti-inflammatory agents with the absence of cytotoxicity effects [11].

The ethanolic fruit extract of *B. racemosa* had been reported to have anti-inflammatory activities through carrageenan-induced paw oedema and formalin-induced paw oedema in experimental albino rats. *B. racemosa* fruit extract at 500 mg/kg body weight significantly inhibited the carrageenan-induced paw oedema at 75.00% inhibition, slightly less than the positive control (indomethacin) which produced 78.33% inhibition. On the other hand, another comparable anti-inflammatory activity to indomethacin had been manifested by formalin-induced paw oedema. *B. racemosa* fruit extract at 500 mg/kg body weight produced 81.66% inhibition of oedema, comparable to that of indomethacin with 83.3% inhibition [10].

Another anti-inflammatory activity had been further identified following a research done by Patil *et al.* [2] in which the anti-arthritic potential of the species was being evaluated. Through the preliminary study, the results for carrageenan-induced acute inflammation in rats had been significantly reduced by ethyl acetate fraction of fruit extracts of *B. racemosa*. Furthermore, this species may have the potential to be an immunosuppressive agent in addition to its anti-inflammatory activity due to suppression of secondary lesions in rats which is shown as a manifestation of cell-mediated immunity. This effect was reported to be more potent than the conventional drug, diclofenac.

Alpha-glucosidase inhibitor

B. racemosa was reported to have alpha-glucosidase inhibitory activities. A study was conducted by Gowri *et al.* [15] to assess the potency of alpha-glucosidase inhibitory activity in the seed extract of *B. racemosa*. Hexane, ethanol and methanol were used for the extraction and it was found

that those extracts displayed potent yeast and intestinal alpha-glucosidase inhibitory activities. Among them, methanol was identified to be most superior with the lowest IC_{50} value of 26.96 g/ml, followed by hexane (131.68 g/ml) and ethanol (163.67 g/ml). Such findings verified the potential of *B. racemosa* to be used as a potential remedy for alpha-glucosidase-related diseases such as diabetes mellitus and hepatitis C.

Anti-fungal

The antifungal activity of methanolic extracts of *Barringtonia racemosa* leaves, sticks and barks had been verified to have anti-fungal activity against *Fusarium* sp., *Tricoderma koningii*, *Penicillium* sp., *Ganoderma tropicum*, *Ganoderma lucidum*, *Aspergillus* sp. and *Rhizopus* sp. at concentration of 50 mg/ml. The strongest inhibitory activity effect was observed in the methanolic extract of leaf against *Fusarium* sp. (53.45%), *G. lucidum* (34.57%), *Aspergillus* sp. (32.27%) and *T. koningii* (20.99%). Apart from that, remarkable effects of anti-fungal activity ware also identified in the boiling water extract of leaf against *Fusarium* sp. (51.72%) and the ethanolic extract of bark against *Rhizopus* sp. (37.50%). Among different fungi tested, *Fusarium* sp. was found to be more sensitive to *B. racemosa* extracts when compared to others [16].

Anti-mycobacterial / Anti-Tuberculosis

The activity of *B. racemosa* against mycobacteria which is the causative factor of tuberculosis (TB) was evaluated by Mmushi *et al*. [17]. Different solvents of various polarities were used to extract the leaves of *B. racemosa. Mycobacterium smegmatis* was used in the study and the MIC (minimum inhibitory concentration) of leaf extracts towards the strain was evaluated. Acetone extract was found to show promising antimycobaterial activities against *M. smegmatis* with MIC value of 0.107 mg/ml which is having greater activity as compared to Rifampicin standard with 0.125 mg/ml MIC.

Anti-arthritic

The anti-arthritic properties of *B. racemosa* had been validated in an *in vivo* study done on Complete Freund's Adjuvant (CFA)-induced arthritis rats. Chromatographic isolation and characterization confirmed the presence of pharmacological substance in *B. racemosa*, identified as bartogenic acid. The compound was further tested for its anti-arthritic properties in CFA-induced arthritis rats. Bartogenic acid in *B. racemosa* fruit extracts exerted potent protective effect against adjuvant-induced arthritis in rats. Upon radiological analysis by x-ray radiograph it was found that the soft tissue swelling around the joints, periarticular bone resorption, periarticular bony erosions and joint space narrowing have been protected from the CFA-induced arthritis-related joint changes in bartogenic acid-treated rats [2].

Anti-diarrhoeal

A study to assess anti-diarrhoeal activity in *B. racemosa* was documented in 2013. The test was performed *in vivo* which involved the induction of diarrhoea by castor oil in experimental mice. Ethanolic bark extract had increased the latent period of diarrhoea and declined the defecation frequency in dose-dependent manner. The mice treated with the ethanolic bark extract of *B. racemosa* at 250 mg/kg and 500 mg/kg doses demonstrated 45.94% and 68.46% inhibition of defecation as compared to the control group (84.68%) which were treated with a conventional anti-diarrhoeal drug, loperamide at 3 mg/kg [8]. The *B. racemosa* extracts exhibited significant reduction in the total number of faeces and prolongation of onset of diarrhoea.

CONCLUSION

Ethno-medicinal uses and promising phytomedicinal values of this species which had been proven through scientific studies have indeed verified its worth exploring properties and may serve as a potential candidate for a future drug development.

ACKNOWLEDGEMENT

The authors would like to thank the Ministry of Education of Malaysia under Higher Education Department for the funds allocated under Fundamental Research Grant Scheme (FRGS) with the grant number of FRGS/1/2014/STWN03/UITM/02/01 facilitated by Research Management Institute, Universiti Teknologi MARA.

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