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Screening of Three Species of Pasak Bumi (Bitter Charm) for Potential Toxicities as Active Drugs

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ABSTRACT
The utilization of traditional medicine or herbal medicine in a community is something inheritance, and it is inseparable from human lives. Support of scientific statements is necessary for comprehensive utilization. Pasak Bumi (Bitter Charm) is one of the raw material sources for traditional medicines that can be packaged inactive drugs that are benefits have been widely felt. This study is intended for preliminary screening of 3 types of Bitter Charm, including Yellow Bitter Charm (*Eurycoma longifolia*), Red (*Rennellia elliptica*) and Black (*Trivalvaria macrophylla*) as active drug ingredients through toxicity tests. Toxicity testing was carried out on all parts of Bitter Charm (roots, stems and leaves), which were first extracted (by ethanol, ethyl acetate and water solvent) using the BSLA (brine shrimp lethality assay) method through four test solutions with different concentrations, i.e. 10, 100, 500 and 1000 ppm. Results of toxicity testing of Bitter Charm extracts showed that based on the value of 50% Lethal Concentration (LC50), all Bitter Charm extracts showed that those extract had toxic potential (LC50 <1000 ppm). The lowest toxicity value on Yellow Bitter Charm extract was found in leaves with ethyl acetate solvent (132.08 ppm), the lowest toxicity value in Red Bitter Charm extract was in leaves with water solvent (287.59 ppm), and the lowest toxicity value in Black Bitter Charm extract was found in stems with water solvents (58.94 ppm). However, this recent study stated that all parts of the Bitter Charm of 3 different species contained high toxicity. As evidenced by the smaller LC50 value, the smaller LC50 value means the active compound has more toxic. Increasingly of toxicity inactive compound means potential highly as an anti-cancer, antibacterial, and antifungal.

Keywords: *Pasak Bumi, Potential Toxicities, Active Drug, Traditional Medicines*

1. INTRODUCTION

The utilization of traditional medicine or herbal medicine is essential in Indonesia. The traditional medicine trend towards increasing national and global levels was triggered by the global issue of "back to nature". The use of plant and plant products for the treatment of diseases is as old as humankind. Plant-based medicine's significant merits seem to be their perceived efficacy, low incidences of serious adverse effects, and low cost [1]. Medicinal plant species used to treat particular diseases are reported to have serious side effects on a large scale. Many drugs have originated from biologically active plant chemicals, and their medicinal uses are attributed to various active substances found in them [2]. Plant origin drugs are known to play a vital role in the management of various chronic diseases. The researcher has received a significant preference as an alternative source to allopathic pharmaceutical drugs in recent times [3].

Pasak Bumi (Bitter Charm) is one of the raw material sources for traditional medicines that can be packaged inactive drugs that are benefits have been widely felt. The utilization of Pasak Bumi as derived medicines has made considerable contributions to humans' health and well-being. Historically, Pasak Bumi has been used by local communities In Indonesia and Malaysia to treat various diseases. The plant's root is boiled in water, and the water is consumed as a health tonic for post-partum recovery, as an aphrodisiac, and the relief of fever, intestinal worms, dysentery,

Figure 1 Characteristic of (a) *Eurycoma longifolia* (Simaroubaceae) is yellow root and bitter taste; (b) *Rennellia elliptica* (Rubiaceae) is red root and not bitter taste; (c) *Trivalvaria macrophylla* (Annonaceae) is black root and not bitter taste (Doc. Supartini 2016).

diarrhoea, indigestion, and jaundice. In Vietnam, the flower and fruits are used to treat dysentery, and the root is used to treat malaria and fever. In Malaysia, a paste of the plant is applied topically to relieve headaches and stomach-aches [4,5]. This plant has been used as an aphrodisiac, including antimalarial, antidiabetic, antimicrobial, and antipyretic activities [6]. Its highly bitter root has been used as the basis for supplements and food and drink additives. As a supplement, it has been marketed for the supposed benefits of sexual health improvement, as an energy and st amina booster, for improving blood circulation, fat reduction, and as a testosterone booster. The drinks market is a common ingredient for coffee and functional beverages positioned as energy drinks [4,5,7].

There are three species of Pasak Bumi in Indonesia which are spread throughout in Kalimantan area (Borneo Island), i.e. Pasak Bumi Kuning/Yellow Bitter Charm (*Eurycoma longifolia*; Simaroubaceae family), Pasak Bumi Merah/Red Bitter Charm (*Rennellia elliptica*; Rubiaceae family), and Pasak Bumi Hitam/Black Bitter Charm (*Trivalvaria macrophylla*; Annonaceae family). Only *Eurycoma longifolia* has been widely researched and perceived as a commercial medicinal plant with some derivated products from those three species. However, most of the available information regarding the medicinal potential of *Rennellia elliptica* and *Trivalvaria macrophylla* is not provided with credible scientific data. For this reason, several types of research have been conducted to determine the toxicity of medicinal plants [8]. Toxicity is necessary to avoid some adverse effects obtained in local plants utilization as a source of medicine caused by overdosing and lack adequate knowledge of other harmful by-products in some plants [7]. A general bioassay that appears capable of detecting a broad spectrum of bioactivity present in crude plant extracts is the Brine Shrimp (*Artemia* sp.) Lethality assay (BSLA). BSLA is used as an indicator for general toxicity and a guide for the detection of antitumor and pesticidal compounds. The low cost and ease of performing the assay and the commercial availability of inexpensive brine shrimp eggs make BSLA a beneficial benchtop method. This assay has been noted as a useful tool for isolating bioactive compounds from plant extracts [9-

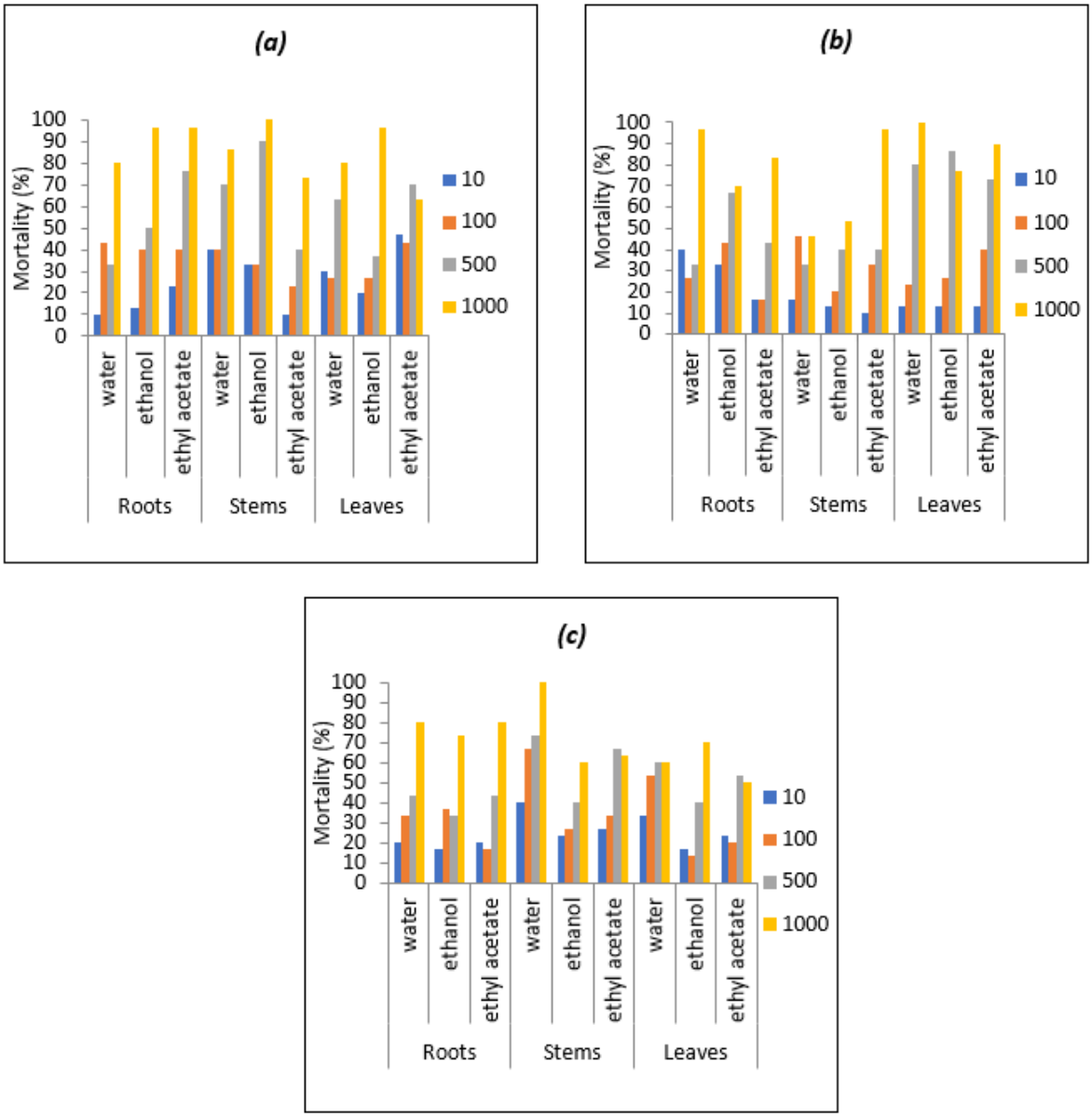

Figure 2 Shrimp larva mortality by (a) *E.longifolia* extracted with ethanol, ethyl acetate and water solvents; (b) *R.elliptica* extracted with ethanol, ethyl acetate and water solvents; (c) T. macrophylla extracted with ethanol, ethyl acetate and water solvents.

11]. The recent study is intended for the initial screening of three species of Pasak Bumi (*E. longifolia*, *R. elliptica*, and *T. macrophylla*) as a preliminary study cytotoxic and antitumor agents. Thus, this present work's findings would give baseline information on the most promising these three species that could be used as a basis for the development of new ingredient for active drugs.

2. MATERIALS AND METHODS

Pasak Bumi were collected from Kotawaringin Timur District, Central Kalimantan, Indonesia in 2017. Toxicity testing was conducted at Biopharmaca Research Center, IPB, Bogor on all parts of Pasak Bumi (roots, stems and leaves), which were first extracted by ethanol, ethyl acetate and water solvent. Toxicity testing was carried out using the BSLA (brine shrimp lethality assay) method (Artemia salina Leach shrimp eggs). A total of 10 mg was hatched in 250 ml of seawater with TL lamps and aerators' help, then the shrimp larvae that had hatched and were approximately 48 hours. Each part of Pasak Bumi extracted (20 mg) was put in 10 ml of seawater, then homogeneous was carried out and was stocked in sample solution with a concentration of 2000 ppm obtained. Test solutions were made in 4 (four) different concentrations, i.e. 10, 100, 500 and 1000 ppm. Into a 2000 µL vial, 1000 µL of seawater was added containing ten shrimp larvae and an extract solution with concentrations, i.e. 10, 100, 500 and 1000 ppm. Vials are covered with aluminium paper and incubated for 24 hours. Each extract concentration was calculated on the average per cent of mortality of shrimp larvae.

The lethal concentration value of 50% Pasak Bumi extract (Lethal concentration/ LC50) was determined from the relationship curve between extract concentration (x-axis) and the average per cent mortality of shrimp larvae (y-axis). Furthermore, the toxicity of extracts/ingredients can be associated with anti-cancer properties if the LC50 value is below 1000 ppm [12, 9-11].

Table 1. Toxicity potential of three species of pasak bumi by BSLA method (LC50)

Extracts	Part of plant	Solvent	LC_{50} (ppm)
E.longifolia	Roots	Ethanol	370.68
		Ethyl acetate	239.01
		Water	550.90
	Stems	Ethanol	167.32
		Ethyl acetate	634.47
		Water	208.86
	Leaves	Ethanol	445.13
		Ethyl acetate	132.08
		Water	391.82
E.elliptica	Roots	Ethanol	310.12
		Ethyl acetate	549.83
		Water	390.65
	Stems	Ethanol	858.20
		Ethyl acetate	443.79
		Water	>1000
	Leaves	Ethanol	384.46
		Ethyl acetate	321.90
		Water	287.59
T. macrophylla	Roots	Ethanol	616.78
		Ethyl acetate	562.88
		Water	505.93
	Stems	Ethanol	750.02
		Ethyl acetate	469.73
		Water	58.94
	Leaves	Ethanol	675.56
		Ethyl acetate	814.79
		Water	324.23

3. RESULTS AND DISCUSSION

All extracts were subjected to BSLA for possible cytotoxic action. Generally, all parts of Pasak Bumi extract with different solvents (water, ethanol and ethyl acetate) differ in brine shrimp larvicidal activity. The mortality of shrimp larvae in *E. longifolia* extracts are highest compared to *R. elliptica* and *T. macrophylla* extracts, respectively. While separately, 100% brine shrimps larva mortality were occured in the highest concentration in stems of *E.longifolia* extract by ethanol solvent; in leaves of *R. elliptica* extract by water solvent; in stems of *T. macrophylla* extract by water solvent (Figure 2). Variations of mortality value shown are very dependent on the type of solvent during the extraction process. Therefore, choosing the type of solvent for the extraction process is vital for toxicity testing because different solvents show different extracting potential [12]. If possible, extraction should be carried out under mild conditions utilizing low reactivity solvents [13].

Furthermore, the results of toxicity testing of Pasak Bumi extract are also presented in Table 1. All extracts showed that those extract had toxic potential (LC50 <1000 ppm). The lowest toxicity value on *E. longifolia* extract was found in leaves with ethyl acetate solvent (132.08 ppm). The lowest toxicity value in *R. elliptica* extract was in leaves with water solvent (287.59 ppm). Then, the lowest toxicity value in *T. macrophylla* extract was found in stems with water solvents (58.94 ppm).

The observed lethality of three species of Pasak Bumi extracts to brine shrimps indicated the presence of potent cytotoxic and probably some anti- components of these species. In this study, 50% of Lethal Concentration (LC50) in all Pasak Bumi extracts showed that the extract had toxic potentially. Plant extracts have very toxic properties if the toxicity value about LC50 <30 ppm, harmful if the toxicity value is 31 ppm <LC50 <1000 ppm, and not toxic if LC50> 1000 ppm [14]. LC50 value was inversely proportional to the high and low percentage of mortality of shrimp larvae. The greater LC50 value will indicate a lower mortality rate of shrimp larvae [15]. Afterwards, LC50 with values <1000 ppm could potentially be anti-cancer, antibacterial, and antifungal [11].

The toxic effects of extracting a compound may be caused by polar or semipolar compounds such as alkaloids and glucosides. The toxicity of bioactive compounds to organism cells are included alkaloids, flavonoids, and steroids [16]. Bioactive compounds that have bioactivity containing anti-cancer, i.e. flavonoids, triterpenoids and saponins [17]. Preliminary phytochemical screening revealed the presence of alkaloid, steroid, and extractives of plant extracts necessary to be observed since these compounds are also known to show cytotoxicity in some biological toxicity assay tests [17,18].

4. CONCLUSIONS

The recent study was provided information that different solvent of the extraction process in all parts of Pasak Bumi can be influenced by the individual mortality rate of brine shrimps larvae as a biological assay. The selection of the right solvent type and the right extraction conditions should be considered in toxicity testing using bioassays. This study stated that all parts of three species of Pasak Bumi were contained high potent cytotoxic and probably some anti-components of these species. The high toxicity potential of three species of pasak bumi by the BSLA method indicated a smaller LC50 value. Smaller in LC50 value means active compound contains more toxic. Increasingly of toxicity inactive compound means potential highly as an anti-cancer, antibacterial, and antifungal. Thus, some active drugs derived from these three species can be developed based on this research work.

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