



Cytotoxicity Activities of Ethanol Extract of Hooks *Uncaria tomentosa* West Kalimantan

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ABSTRACT

Uncaria tomentosa is a member of the plant family Rubiaceae. It has been used as medicinal plants in West Kalimantan. The cytotoxic of ethanol extract from the hooks of *U. tomentosa* was determined. This study used Brine Shrimp Lethality Test (BSLT) method with solution concentration 1,000; 5,000 and 10,000 ppm. The extract has LC₅₀ values of 21,754 ppm. It is indicated the extract not toxic. This extract is potent to be used as drugs.

INTRODUCTION

Plants of the genus *Uncaria* (Rubiaceae) contains approximately 34 species. The genus is distributed mainly in tropical regions, such as Southeast Asia, Africa, and Southeast America. *Uncaria* is liana or scandent shrub that climb by hooks (Turner, 2018). *Uncaria tomentosa* is one of the species in West Kalimantan (Iskandar, 2020). It is known as the cat's claw because of its claw-shaped thorns (Honório et al., 2016).

U. tomentosa has been known as medicinal plants. Medicinal plants have been used for various therapeutic purposes. *U. tomentosa* has been traditionally used to treat asthma, abscesses, fever, urinary tract infections, viral infections, and wounds (Batiha et al., 2020). It also has been used to treating diabetes, cancer, intestinal affections inflammation, cancer, menstrual disorders (de Paula et al., 2015; Zhang et al., 2015). The plants also have a potent for treating most parasites (Santos et al., 2016).

The chemical constituents of *U. tomentosa* have been reported. Fifty compounds have been identified and isolated from *U. tomentosa*, including hydroxybenzoic acids, hydroxycinnamic acids, flavan-3-ols monomers, procyanidin dimers, and trimers, flavonolignans, propelargonidin dimers tetracyclic and pentacyclic alkaloids (Hoyos et al., 2015). Alkaloids, flavonoids, polyphenols, triterpenoids, steroids, saponin and tannin were identified as secondary metabolites from ethanol extracts of leaves *U. tomentosa* (Iskandar, 2020). Pentacyclic oxindole alkaloids, ursane type pentacyclic triterpenes, and quinovic acid glycosides, carboxyl alkyl esters, catechin monomers, and proanthocyanidins were isolated from *U. tomentosa* leaves (Kośmider et al., 2017).

U. tomentosa has several pharmacological activities such as antitumor, anticancer, antioxidant, anti-neoplastic, antiinflammation, antimicrobial, antiprotozoal, and antiviral activities (Batiha et al., 2020). Cytotoxic activity of ethanol extract from hooks *U. tomentosa* from West

Kalimantan is not known. The extract was tested for the cytotoxic against *Artemia salina* Leach using Brine Shrimp Lethality Test (BSLT) methods. The methods were used to see the toxicity of plant extracts.

METHODS

Collection and Identification of Plants

Hooks of *U. tomentosa* were collected from Kapuas Hulu, West Kalimantan. The plants were identified in the Biology Laboratory, Department of Biology, FMIPA, Tanjungpura University. The cytotoxic of the plant extracts have been carried out in Chemistry Laboratory, Department of Chemistry, FMIPA, Tanjungpura University.

Preparation of Plants and Plant Extract

The hooks of *U. tomentosa* was prepared by air-dried at room temperature. Dried samples powdered and macerated with ethanol. Whatman no.1 filter paper was used to filter the mixture. The filtrate was concentrated by rotary evaporation to evaporate the residue of the solvent. Then, ethanol extract of hooks *U. tomentosa* stored in the refrigerator for further use.

Hatching of Brine Shrimp Nauplii

Brine shrimp eggs were hatched in sterilized seawater. It soaked for 24 hours with aeration in a transparent container. After 48 hours, the nauplii (larvae) were collected by pipette as many five larvae for each replication. The larvae were used for the brine shrimp lethality test (Pisutthanan et al., 2004).

Plant Extracts Solution Preparation

Small amounts of ethanol extract of hooks *U. tomentosa* were evaporated. Then, the extract was dissolved in DMSO to prepare a stock solution. A serial dilution was prepared by diluting stock extract in several concentrations 1,000; 5,000; and 10,000 ppm.

BSLT Test

The procedure for BSLT was modified from the assay described previously (Meyer et al., 1982). The stock solution pipetted and put in vials with varied concentrations. Each concentration has five replications. Then, the vials were added up to 10 mL of seawater containing ten nauplii. The control had no extract in it. The

test vials were incubated at room temperature for 24 hours. The numbers of dead nauplii in each vial were counted, and LC₅₀ values were estimated.

RESULTS AND DISCUSSION

Maceration is an extraction method by soaking samples with solvent. The technique does not damage the chemical entities of the plant. Hooks of *U. tomentosa* were macerated using ethanol. It is a semipolar solvent that can dissolve polar and non-polar compounds. The solvent is not associated with toxic effects (Marzuki et al., 2019). The solvent penetrated the cell wall of plants and disrupting the cell membrane. It caused secondary metabolites to come out of the cell and dissolved into the solvent.

Cytotoxic activities were conducted using the BSLT method. This study was a preliminary test before the plants extract applied in vivo. The result of larvae mortality percentage for each concentration of ethanol extract of *U. tomentosa* showed in Table 1.

Table 1. Percentage of larvae mortality for each the concentration of ethanol extract of hooks *U. tomentosa*

Concentration (ppm)	Mortality (individual)					Mortality (%)
	1	2	3	4	5	
1,000	3	1	1	0	0	0.1
5,000	2	1	2	1	1	0.14
10,000	4	3	3	2	2	0.28
Negative control	0	0	0	0	0	0
DMSO	1	1	0	0	0	0.04

Based on the results study, each concentration of extract showed different mortality of *Artemia salina* Leach larva (nauplii). The highest percentage of mortality showed plant extract in 10,000 ppm. Higher concentrations of extract increased the percentage of mortality. The cytotoxic tests of the ethanol extract of hooks *U. tomentosa* using *Artemia salina* Leach showed the LC₅₀ value of 21,754 ppm. The value of LC₅₀ > 1,000. It means that ethanol extract of hooks *U. tomentosa* was not toxic. It is indicated the extract not toxic to humans and safely used as a drug candidate. In bioactivity evaluation of plant extracts by BSLT, LC₅₀ value greater than 1,000 µg/mL is considered non-toxic. The cytotoxic activity was considered toxic when the LC₅₀ 31 mg/L ≤ LC₅₀ ≤ 1000 mg/L, as strong when LC₅₀ ≤ 30 mg/L (Gaikwad et al., 2017). Cytotoxic of other species of *Uncaria* was reported. The Uc7, terpenoid compound from ethyl acetate extract

of *U. cordata* (Lour.) Merr has a powerful cytotoxic activity with LC₅₀ 2,75 µg/mL (Rahmawati et al., 2016). The methanol extract of bark and wood of the roots *U. nervosa* Elmer was very toxic with LC₅₀ values of 1.76 dan 2.66 ppm, respectively (Maulina and Pratiwi, 2019).

Artemia salina Leach has highly sensitive to the changes in environmental conditions and chemical agents in the environment. Brine shrimp eggs hatched after 48hr incubation. The whole body of nauplii (larvae) was formed completely (Muaja et al., 2013). The nauplii death caused by the presence of toxic secondary metabolites. The compounds of the extracts entered through the mouth of nauplii and absorbed into the alimentary tract. The absorption process occurs through the cell membrane. After the absorption process, the toxic compounds distributed into the body of nauplii. Finally, the metabolism of nauplii was damaged.

CONCLUSIONS

The ethanol extract of hooks *U. tomentosa* from West Kalimantan was determined the cytotoxic activities. The LC₅₀ values of the extract are 21,754 ppm. It showed the extract not toxic to the human.

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