

ANTI CANCER PRE-SCREENING FOR SEVERAL PLANT USING BRINE SHRIMP LETHALITY TEST

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Abstract

*Indonesia has high biodiversity especially its pharmacologically beneficial flora such as flora with anti cancer activity. The aim of this study is to screen several plant candidate for anti cancer therapy. Brine shrimp lethality test (BST) is the method that used in this study. By measuring the LC_{50} of the several plant extract to the *Artemia salina* larvae that analogue with the cancer cells. The result from the fifth plant were tested show various responses. With n-Hexane extract of *Physalis minima*, *Allium odoratum*, *Tabebuia chrysantha* has potential biological (anticancer) activity with LC_{50} 920,92 $\mu\text{g/ml}$, 805,62 $\mu\text{g/ml}$, and 652.868 $\mu\text{g/ml}$. And with methanol extract *Chentotecha longilamina*, *Impatiens balsamina*, *Tabebuia chrysantha*.shows the activity each with LC_{50} 908,58 $\mu\text{g/ml}$, 744,48 $\mu\text{g/ml}$ and 858 $\mu\text{g/ml}$.*

Key Word : anticancer activity, BST, plants

BACKGROUND

Cancer is still became most dangerous disease in the world. Based on World Health Organization in 2005; 7,6 million people death by cancer and 84 million people is going to be death 10 years onward (Diananda, 2007). At the advance country the cancer is the second disease that causing death after the cardiovascular diseases (Nafrialdi and Sulistia, 2007). As a development country the cancer diseases in indonesia being the sixth disease with high mortality number.

In the other word, Indonesia has many potential in its natural resources, especially its floral biodiversity that has many potential as natural drugs. Mangan (200), from 30.000. Floral species in Indonesia, about 1.260 species has pharmacological activities including anti cancer. Based on the potency of the floral species in Indonesia, the aim of this research are to get several potential plant for anticancer therapy.

The plant candidates were chosen by study its chemotaxonomy with plants that has close relationship in its chemical compound and its morphological taxonomy. *Physalis minima*, *Allium odoratum*, *Tabebuia chrysantha*, *Chentotecha longilamina*, *Impatiens balsamina* are plants that chosen to know its anticancer activity. Its chosen by literally study its morphological and chemical compound relation with the former plant that has know has anti cancer activity. Our effort to identify the bioactive compound of the plants we use bioassay test. Brine Shrimph Brine shrimp lethality test (BST) has been employed as an alternative bioassay technique to screen the plant extracts (Meyer et al., 1982; McLaughlin and Rogers, 1998).

METHODS

1. Plants Material

All plant materials were collected from several area Malang East Java Indonesia. *Physalis minima* (all part), *Allium odoratum* (all

part), *Tabebuia chrysantha* (caulis cortex), *Chentotecha longilamina* (all part), *Impatiens balsamina* (all part). Plant determination conducted in Balai Materia Medica Batu City and Purwodadi National Park.

2. Preparation of Plants Extract

Five hundred grams (500 g) of the plant powder were macerated with two solvent each 1,5 liters. First solvent n-hexane, the filtrate and residue from n-hexane maceration separated. The dry residues are macerate once again with methanol and separated to get the filtrate. Both filtrates evaporated with rotary evaporator with 40°C then dried with oven 40°C until gained dry extract.

3. Brine Shrimp Lethality Test

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of plant extracts. *Artemia salina* (Leach) eggs were added to a hatching chamber containing Ocean/Sea water. The hatching chamber was kept under an inflorescent bulb for 48h for the eggs to hatch into shrimp larvae. Different concentration of plant extract prepared by diluting extract with solvent 10, 50, 100, 500, and 100 µg/ml for both n-hexane extract and methanol extract. Four and a half millilitre (4,5ml) of Ocean/Sea water was added to each vial, and 10 larvae of *A. salina* Leach (taken 48 – 72h after the initiation of hatching) were added to each vial. The final volume of solution in each vial was adjusted to 5 ml with Ocean/Sea water immediately after adding the shrimps. One drop of dimethyl sulphoxide (DMSO) was added to the test and control vials before adding the shrimps to enhance the solubility of test materials. Each treatment is triplicate. LC₅₀ values were determined at 95% confidence intervals by analyzing the data with statistical analysis. The LC₅₀ values of the brine shrimps obtained for extracts of the plants studied were recorded.

4. Phytochemical Screening

The Phyto-chemist screening of bioactive compound of the plants including flavonoid, saponin Glycoside, Steroid Triterpenoid, Alkaloid, Tannin and Poliphenol screening.

RESULT AND DISCUSSION

The result from the fifth plant were tested show various responses. With n-Hexane extract of *Physalis minima*, *Allium odoratum*, *Tabebuia chrysantha* has potential biological (anticancer) activity with LC₅₀ 920,92 µg/ml, 805,62 µg/ml, and 652,868 µg/ml. And with methanol extract *Chentotecha longilamina*, *Impatiens balsamina*, *Tabebuia chrysantha* shows the activity each with LC₅₀ 908,58 µg/ml, 744,48 µg/ml and 858 µg/ml (Table I).

According to Meyer et al. (1982) several naturally extracted products which had LC₅₀ < 1000 µg/mL using brine shrimp bioassay were known to contain physiologically active principles. Although toxicity test using BST does not give a clear depiction on cytotoxicity against cancer cell, however this method has been reported useful for screening anticancer from plant/natural sources.

The LC₅₀ result from *Physalis minima*, *Allium odoratum*, with n-hexane extract shows that the more potential bioactive compound from this plants are soluble in non polar solvent than polar solvent. But from *Chentotecha longilamina*, *Impatiens balsamina* are vice versa. One plant under this research that has potential both in polar and non polar solvent are *Tabebuia chrysantha* 858 µg/ml

Table I. Brine Shrimp Test of Cytotoxicity of plant extract under study

Plant	Part Used	n-Hexane LC50	Methanol LC50
<i>Physalis minima</i>	all part	920,92	1281,39
<i>Allium odoratum</i>	all part	805,62	1005,75
<i>Tabebuia chrysantha</i>	cortex of stem	652,87	858,63
<i>Chentotheca longilamina</i>	all part	2193,26	908,58
<i>Impatiens balsamina</i>	all part	1167,75	744,49

Table II. Result from phytochemical screening from plant bioactive substances

Plant	Screening Result							
	Flavonoid		Glycoside saponin		Steroid Triterpenoid		Tannin & Poliphenol	
	n Hexan	Methanol	n Hexan	Methanol	n Hexan	Methanol	n Hexan	Methanol
<i>Physalis minima</i>	+	X	+	X	+	X	-	X
<i>Allium odoratum</i>	+	X	+	X	+	X	-	X
<i>Tabebuia chrysantha</i>	+	X	-	X	+	X	+	X
<i>Chentotheca longilamina</i>	X	+	X	-	X	+	X	-
<i>Impatiens balsamina</i>	X	+	X	-	X	+	X	+

*X: not tested

According to Table II, there are shows that for *Physalis minima* and *Allium odoratum* n-Hexane extract. Its bioactive compound which has cytotoxicity effect has flavonoid, saponin glycoside, and steroid triterpen content. *Chentotheca longilamina* and *Impatiens balsamina* both has Saponin Glycoside and Steroid triterpen content but plus Tannin & polyphenol in *I. balsamina*. Also in *Tabebuia chrysantha* look has flavonoid, steroid triterpenoid and tannin & polyphenol.

All plant above has steroid/triterpenoid substances (Table II). This maybe the main factor for that plants has cytotoxic activity and potential as anti cancer agents. Thoppil and Bhisayee (2011) mention that terpenoid has potential as chemopreventive and therapeutic for cancer. Former identified terpenoid such as Geraniol and Limonene are stated has anti cancer activity. Geraniol, an acyclic dietary monoterpene, represents the only monoterpene that has been studied *in vitro* against liver cancer cells. Geraniol was shown to inhibit the growth of HepG2 human hepatic carcinoma cells by decreasing 3-hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the major rate-limiting enzyme in cholesterol biosynthesis in mammals (Thoppil and Bhisayee, 2011).

From Table II. there are also shows that all plants extract has flavonoid content. Ren et al. (2003) mentioned that many mechanisms of action have been identified from flavonoid, including carcinogen inactivation, anti-proliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation and reversal of multidrug resistance or a combination of these mechanisms. Based on these results, flavonoids may be promising anticancer agents.

CONCLUSION

Based on this reseach, it can be conclude that all the plants (*Physalis minima*, *Allium odoratum*, *Tabebuia chrysantha*, *Chentotecha longilamina*, *Impatiens balsamina*) has cytotoxic activity and potential to become anti

cancer agent. From these plant also know triterpenoid and flavonoid can be the main substance which has the cytotoxic activity and potential as anticancer agent. Based on this research there are good chance for researchers to continue this topic deeper. Analyzing and purifying the bioactive compound and testing it to human cell line *in vitro* and propably testing it *in vivo* and step forward with clinical trial if necessary.

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