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## Identification of Anticancer and Antioxidant Potentials of Katang-Katang Flower Extract (*Ipomea pes-caprae* Linn) with Water Solvent

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**Abstract.** Cancer is a group of deadly diseases that attack human organs with an increasing number of cases and an increasing number of deaths from year to year. Free radicals as unstable molecules are one of the triggers for the emergence of cancer. Katang-katang or *Ipomea pes-caprae* Linn is a plant that could be found on almost all tropical coasts and reportedly contains bioactive compounds. So it has the potential to be developed as herbal medicine. This study used flowers of *I. pes-caprae* L. as the research sample. This study aimed to identify the anticancer and antioxidant potential of the flower extract of *I. pes-caprae* L. extracted with water as a solvent. This research included the phytochemical test of secondary metabolites, identification of anticancer potential based on the toxicity test using the Brine Shrimp Lethality Test (BSLT), and the antioxidant activity test using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The results showed that the flower extract of *I. pes-caprae* L. from water solvent contained active compounds including alkaloids, flavonoids, tannins, saponins, and terpenoids. The extract also has the potential as an anticancer with an LC<sub>50</sub> value of 170.8441 ppm and strong antioxidant activity with an IC<sub>50</sub> value of 71.7895 ppm.

## Introduction

Cancer is a disease that grows and develops into uncontrolled abnormal cells caused by external factors such as radiation, chemicals, tobacco, and infectious organisms as well as internal factors such as genetic mutations, hormonal influences, and immune conditions (ACS, 2015). In addition, the emergence of cancer can also be triggered by free radicals which are unstable molecules because they have unpaired electrons that come from environmental pollutants and unhealthy people's lifestyles. According to WHO (2018), cancer cases increased to 18.1 million new cases and 9.6 million deaths that occurred in 2018. In addition, the number of cancer sufferers worldwide was found to be 43.8 million and more than 50% of deaths in 2018 occurred in Asia, especially in

developing countries like Indonesia.

Cancer can be treated through several methods including surgery, radiation therapy, immunotherapy, chemotherapy, hormone therapy, stem cell transparency, and appropriate medicines (NCI, 2023). Some of these therapies certainly require high costs and not all cancer patients are able to undergo them, especially people in developing countries who live below the poverty line. Therefore, an alternative option is to use herbal medicines from plants that are cheap and easy to obtain.

*I. pes-caprae* L. or katang-katang is a plant that has the potential to be used in herbal medicine because it has a very abundant population, as well as it contains bioactive compounds and has clinical activities based on previous research. The abundance of these plant species can be found in almost all tropical coasts, including in Bantaeng Regency, South Sulawesi Province, Indonesia.

Several studies have reported the pharmacological activity of *I. pes-caprae* L. as an antioxidant, analgesic,

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anti-inflammatory, antispasmodic, antinociceptive, antihistamine, insulinogenic, hypoglycemic, immunostimulant, antimicrobial, antifungal, and antitumor (Ashish et al. 2015). However, most of these pharmacological activities were obtained from the leaves of *I. pes-caprae* L. including antitumor and antioxidant. In addition, the extraction process still uses organic solvents that have toxic properties such as methanol and chloroform. Therefore, researchers want to expand this research by conducting special studies on flowers from *I. pes-caprae* L. to explore their pharmacological potential in this study, namely the anticancer and antioxidant activity of flower extracts from *I. pes-caprae* L. with water as a solvent. The intention of the researchers to use water as a solvent in this study was to obtain non-toxic extracts and the public could apply the results of this study if the flowers of *I. pes-caprae* L. were identified as having potential as anticancer and antioxidant herbal medicines. One of the initial methods to identify potential anticancer compounds is to test the toxicity with the Brine Shrimp Lethality Test (BSLT) method. BSLT is one method that is widely used to search for new anticancer compounds derived from plants. The BSLT method has been shown to have a correlation with anticancer activity. In addition, this method is also easy to do, cheap, fast, and quite accurate (Aksono et al. 2022). While testing the antioxidant activity used the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method because it is the most effective method for determining the antioxidant activity of a sample with a correlation value ( $R > 0.98$ ) (Maesaroh et al. 2018).

## Experimental

### Material and Methods

The materials used in this study were flower leaves from *I. pes-caprae* L. obtained from the coast in Bantaeng district, water solvent (aquadest), Liebermann-Burchard, 1% HCl and concentrated, Mg powder, Wagner, 1% FeCl<sub>3</sub>, *A. salina* Leach, seawater, DPPH, methanol.

### Procedures

#### Sample Preparation and Extraction

Flowers of *I. pes-caprae* L. were cleaned and then dried at room temperature away from direct sunlight. The dried flowers of *I. pes-caprae* L. were then pollinated. As much as 500 g of *I. pes-caprae* L. flower powder was macerated with water for 3 x 24 hours, and then filtered to obtain a crude extract. Furthermore, each macerate is evaporated until a thick extract is obtained and then frozen in the dryer to form powder (Yusriadi et al. 2022).

### Phytochemical Test

Identification was carried out to determine the presence of alkaloids, flavonoids, steroids, terpenoids, saponins and tannins which are carried out as follows:

**Alkaloid.** A few drops of the *I. pes-caprae* L. extracts were placed on the drop plate and added with Wanger reagent. The formation of a brown precipitate indicated that the extract contained alkaloids.

**Flavonoid.** A few drops of *I. pes-caprae* L. extracts were placed on the drip plate and a few drops of concentrated HCl and Mg powder. A positive test marked red solution.

**Terpenoid and steroid.** A few drops of *I. pes-caprae* L. extracts are placed on the drip plate and added with a few drops of Liebermann-Burchard reagent. The color changes that occurred were observed, the presence of terpenoids was indicated by the occurrence of red to purple colors while the presence of steroids was indicated by the formation of green to blue colors.

**Saponin.** As much as 0.5 g of *I. pes-caprae* L. extracts were added to 0.5 mL of hot water and shaken for 1 minute. Observe the solution if it creates foam, then add 1% HCl and wait for 10 minutes, if the foam persists the positive extract contains saponins.

**Tannin.** As much as 0.5 g of sample extract from the extraction results was added with 2 mL of water and 2 drops of 1% FeCl<sub>3</sub>. The extract solution was observed if it produced a bluish-green color, and then the positive extract contained tannins.

### Toxicity Test of Flower Extract of *I. pes-caprae* L. from Water Solvent with BSLT Method

The eggs of *A. salina* Leach were put into a container filled with seawater. The eggs are then aerated and left under the lighting for 48 hours so that the hatching is perfect. *A. salina* Leach eggs that have hatched into larvae are used for toxicity tests. Ten larvae of *A. salina* Leach were put into a test tube filled with seawater. Furthermore, *I. pes-caprae* L. flower extract was added with various concentrations of 1, 10, and 100 ppm, while for the control no extract solution was added. The treatment consisted of three replications for each concentration of the extract and control, then observations were made after 24 hours by counting the number of dead larvae from the total larvae included in order to obtain the percent mortality. The LC<sub>50</sub> value was obtained using LC<sub>50</sub> probit analysis at a 95% confidence interval using the SPSS program (Nerdy et al. 2021).

### Antioxidant Test of Flower Extract of *I. pes-caprae* L. from Water Solvent with DPPH Method

As much as 4 mg of *I. pes-caprae* L. flower extract, put into

two 25 mL volumetric flasks. The first flask was then filled with absolute ethanol up to the mark, then the second flask was filled with water up to the mark. Then, from the mother liquor, concentration series of 10 ppm, 20 ppm, 40 ppm, 60 ppm, and 80 ppm were made. Then each concentration of the extract solution was pipetted as much as 4.5 mL, put into a test tube and then 0.5 mL of 1 mM DPPH solution. The solution was then homogenized and incubated at 37°C for 30 minutes, and then the absorbance of the solution was measured with a UV-VIS spectrophotometer at a wavelength of 517 nm. All work was carried out in a room protected from direct sunlight. The absorbance of the blank solution was also measured to perform the calculation of the percent inhibition. A blank solution was prepared by reacting 4.5 mL of methanol with 0.5 mL of 1 mM DPPH solution in a test tube. Percent inhibition is calculated by the following equation:

$$\% \text{ Inhibition} = \frac{(AA - AB)}{AA} \times 100\% \quad (1)$$

Explanation:

AA: Blank Absorption

AB: Sample absorbance

## Result and Discussion

### Sample Preparation

Flower extract of *I. pes-caprae* L. was obtained by

maceration technique using water as a solvent. At first the flowers of *I. pes-caprae* L. collected were dried by means of aerated aiming to remove the water content and prevent damage to the compound in the sample, then cut into small pieces to increase the surface area of the sample and macerated with a water solvent of approximately 3x24 hours so that the sample maceration process can be maximized. The macerate obtained was made into powder using a freeze dryer so that the flower extract of *I. pes-caprae* L. was obtained in the form of powder (Baliyan et al. 2022).

### Phytochemical Test

Phytochemical tests were carried out to determine the class of active compounds contained in the flower extract of *I. pes-caprae* L. from water as a solvent. Based on the results of the phytochemical test (Table 1) it is known that the flower extract of *I. pes-caprae* L. from water solvent contains alkaloids, flavonoids, tannins, saponins, and terpenoids. These results are supported by the exploration data carried out by Akinniyi et al. (2022) that in general *I. pes-caprae* L. contains secondary metabolite compounds including alkaloids such as tetrahydroxy congeners, flavonoids, tannins such as quercetin, terpenoids such as  $\alpha$ -amyryn acetate, saponin, and steroids. The content of these compounds will correlate with the bioactivity ability of the extract.

**Table 1.** Phytochemical test of *I. pes-caprae* L. flower extract from water solvent

Compound Groups	Observations	Results
Alkaloid	Brown precipitate	+
Flavonoid	Pink	+
Tannin	Blue	+
Saponin	Foam	+
Terpenoid	Red	+
Steroid	Red	-

### Toxicity Test of Flower Extract of *I. pes-caprae* L. from Water Solvent with BSLT Method

One way to find out the potential of a compound as an alternative to a new drug is to do a biotoxicity test. The principle of toxicity tests is that bioactive components are always toxic when given in high doses and are medicinal when given in low doses. The toxicity test with the BSLT method can be used as a preliminary test in research that leads to cytotoxic tests (Yusriadi et al. 2019). The test animal used was *A. salina*. The shrimp larvae of *A. salina* have similarities with mammals such as the type of DNA-dependent RNA polymerase (DNA directs the process of

RNA transcription). This causes compounds or extracts that have activity in the system to be detected through this method. The correlation between this acute toxicity test and the cytotoxic test is if the mortality against *A. salina* caused has an LC<sub>50</sub> value of <1000  $\mu$ g/mL. This method has several advantages, namely faster, cheaper, easier, does not require aseptic conditions and a 95% confidence level. The mechanism of larval death is related to the function of the compounds contained in the cells which can inhibit the eating power of the larvae (Aksono et al. 2022). The relationship curve between the probit value and log[sample] able to be shown in Figure 1.

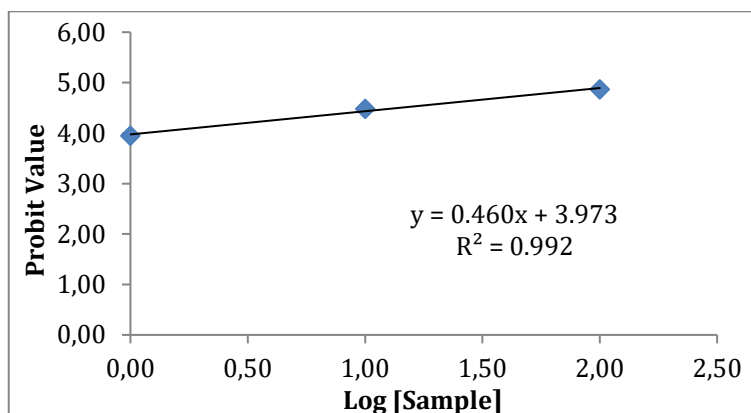


Figure 1. Relationship curve between probit value and log[sample]

Table 2. Test results of *I. pes-caprae* L. flower extract from water solvent using the BSLT method

Sample	[sample] (ppm)	x-axis ([sample] logs)	% death larvae - control	y-axis (Probit value)	LC <sub>50</sub> (ppm)
<i>I. pes-caprae</i> L. flower extract	1	0	15	3.95	170.8441
	10	1	30	4.48	
	100	2	45	4.87	

From the results of testing the flower extract of *I. pes-caprae* L. from water solvent using the BSLT method in Table 2, the LC<sub>50</sub> value of 170.8441 ppm was obtained. These results prove that the flower extract of *I. pes-caprae* L. from water has the potential as an anticancer because the LC<sub>50</sub> value is <1000 µg/mL, which means that the sample is toxic (Aksono et al. 2022). The toxicity properties produced from *I. pes-caprae* L. flower extract from water solvents against *A. salina* are due to the active compounds contained therein which include alkaloids, flavonoids, tannins, saponins and terpenoids.

#### Antioxidant Test of Flower Extract of *I. pes-caprae* L. from Water Solvent with DPPH Method

Flower extract antioxidant activity *I. pes-caprae* L. of water solvent was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The DPPH method is considered very sensitive for testing antioxidant activity

compared to other methods with a high reproducibility rate with a very significant correlation found in this method ( $R > 0.98$ ) (Maesaroh et al. 2018). In addition, the procedure is also simple, fast, and accurate. The compound 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a free radical that is commonly used as a standard test model in measuring the scavenging power of free radicals or to test the antioxidant activity of a test sample (Mishra and Chaudhury, 2012). The principle of the DPPH testing method is based on the reduction of DPPH free radical solutions by antioxidant compounds contained in certain plant extracts. When the DPPH solution interacts with an electron donor solution which in this case is an antioxidant, the single electrons in the free radicals of the DPPH solution become paired (Munteanu and Apetrei, 2021). The reaction of solutions containing antioxidant compounds with DPPH either by transferring electrons or hydrogen radicals on DPPH will then form reduced DPPH (Figure 2).

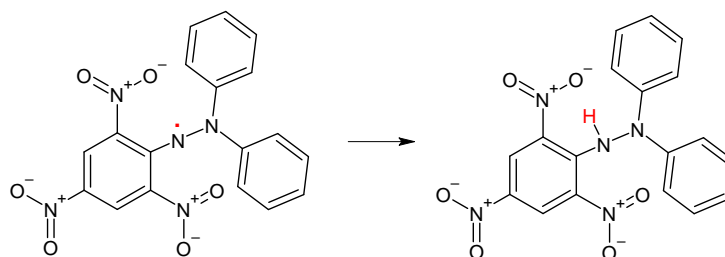


Figure 2. Relationship curve between probit value and log [sample]

This reaction causes the color of the solution to change from dark purple to pale yellow, along with the amount of DPPH reduced. The results of the decolorization of the DPPH solution by these antioxidant compounds are

equivalent to the number of electrons captured or the amount of hydrogen absorbed (Moon and Shibamoto, 2009).

**Table 3.** Results of antioxidant testing of *I. pes-caprae* L. flower extract from water solvent using the DPPH method

Sample	IC <sub>50</sub> value (ppm)			Average value of IC <sub>50</sub> (ppm)
	Simplo	Duplo	Triplo	
<i>I. pes-caprae</i> L. flower extract	72.9187	72.5913	69.8586	71.7895

Based on the antioxidant test using the DPPH method in table 3, the results of flower extract *I. pes-caprae* L. from water solvent has an IC<sub>50</sub> value of 71.7895 ppm. According to Molyneux (2004) that IC<sub>50</sub> values between 50-100 ppm are in a strong category, so flower extract *I. pes-caprae* L. from water solvent has a strong antioxidant activity. The strong antioxidant activity of *I. pes-caprae* L. flower extract from the water solvent is due to the presence of active compounds in the extract such as alkaloids, flavonoids, tannins, saponins, and terpenoids that are shown in the phytochemical test.

Alkaloids function as antioxidants because they contain nitrogen atoms which have lone electron pairs in their structure to reduce free radical activity. Tannin is a polyphenol compound which is capable of acting as an electron donor to stabilize free radical compounds. Saponin compounds are able to reduce superoxide through the formation of hyperoxide intermediates, thereby preventing biomolecular damage by free radicals (Hasan et al. 2022). Meanwhile, the hydroxyl groups of Flavonoids help to eliminate the free radicals (Yang et al. 2020).. Terpenoids are able to reduce the formation of new free radicals by breaking the chain reaction and converting them into more stable products (Zhao et al. 2020).

## Conclusion

According to the results of the research conducted, it could be concluded that the flower extract of *I. pes-caprae* L. from water solvent contains active compounds including alkaloids, flavonoids, tannins, saponins and terpenoids. In addition, the extract also has anticancer potential with an LC<sub>50</sub> value of 170.8441 ppm and strong antioxidant activity with an IC<sub>50</sub> value of 71.7895 ppm.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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