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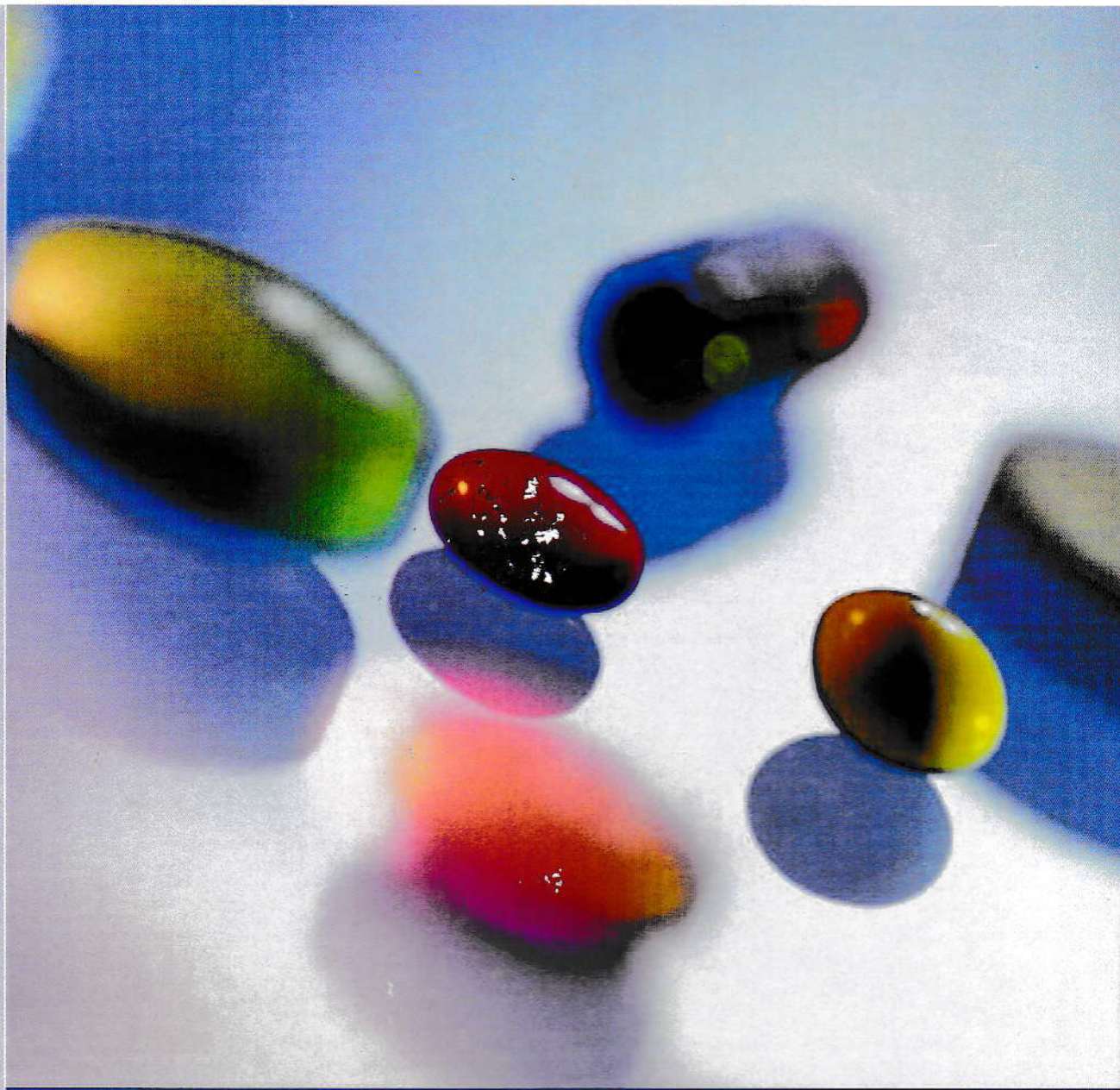

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Contents

Research Article

Antioxidant and Cytotoxic Activities and Phytochemical Screening of Beligo (*Benincasa hispida* Thunb. Cogn) Seeds Extract

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ABSTRACT

The aims of this research were to determine the antioxidant and cytotoxic activities and phytochemical screening of Beligo (*Benincasa hispida* Thunb. Cogn) seeds extract. The extraction of Beligo seeds by maceration using ethanol 96%. Antioxidant activity was determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The IC_{50} value for DPPH assay was 54,987 ppm. The cytotoxic activity was assessed by using brine shrimp lethality test (BSLT). The LC_{50} values of the tested samples was 4,666 ppm. Phytochemical screening showed positive results for flavonoids, terpenoids, and alkaloids.

Keywords:
Benincasa hispida Thunb. Cogn seeds, antioxidant, cytotoxic, phytochemical screening

INTRODUCTION

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants. (Cheeseman et al., 1993). Cell damage caused by free radicals appears to be a major contributor to cancer and degenerative diseases such as ischemic heart disease and cataract (Sies, H. et al., 1992)

Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. (Percival, 1998)

Benincasa hispida (Thunb.) Cogn., a member of the family Cucurbitaceae, is one of the famous crops that are grown primarily for its fruits and usually recognized with its nutritional and medicinal properties especially in Asian countries. Kundur (Synonym: Beligo) fruit has been valued as a nutritious vegetable as it provides a good source for natural sugars, amino acids, organic acids, mineral elements and vitamins. A number of medicinal properties such as anti-diarrheal, anti-obesity, anti-ulcer, and antioxidant and diuretic have been ascribed to this fruit of high economic value. As a rich source of functionally important bioactives and therapeutics such as triterpenes, phenolics, sterols, and glycosides, the fruit has been widely used for the treatment of epilepsy, ulcer, and other nervous disorders in the native medicine system of Asia (Zaini et al., 2011)

Antioxidant activity and phytochemical screening of *Benincasa hispida* fruit extract have been reported by badhani et al.(2013) and antioxidant activity of *Benincasa hispida* seeds extract investigated by using Conventional Soxhlet Extraction (CSE) was reported by Mandana et al.(2012). However, Antioxidant and cytotoxic activities and phytochemical screening of Beligo (*Benincasa hispida* Thunb. Cogn) seeds extract investigated by using maseration extraction have not yet been reported before. This research was performed to determine the antioxidant and cytotoxic activities and phytochemical screening of Beligo seeds extract.

MATERIALS AND METHODS

Preparation of Plant Samples

Sample of *Benincasa hispida* Thunb. Cogn seeds was collected from Tonra in Bone, South Sulawesi, Indonesian. Benincasa hispida Thunb. Cogn seeds were washed then cut to reduce the size and dried in an oven

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at a temperature of 45 ° C for 24 hours. The samples was extracted by maceration method using ethanol 96%.

DPPH scavenging method

The following procedure was modified from those described by Mensor (2001) and amudha et al.(2016). Antioxidant activity of *Benincasa hispida* Thunb. Cogn seeds extract was performed by pipetting 10 µL, 20 µL, 30 µL, 40 µL, and 50 µL of stoc solution 1000 ppm then poured into 5 mL volumetric flask and added with 1 ml 0.4 mM DPPH, finally it was added with methanol precisely until concentration 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm reached. The mixtures were homogenized by using vortex mixer, then covered with aluminiumfoil and incubated at room temperature in the for 30 min. The absorbance wasmeasured by Spectrophotometer UV Viseble at wavelength 513 nm

Brine Shrimp Lethality Test

The seawater was put in a small plastic container (hatching chamber) with a partition for dark (covered) and light areas. Shrimp eggs were added into the dark side of the chamber while the lamp above the other side (light) will attract the hatched shrimp. Two days were allowed for the shrimp to hatch and mature as nauplii (larva). After two days, when the shrimp larvae are ready. Ethanol extract of *B.hispida*, that will be tested each made in concentrations of 1, 10, 100, and 1000 ppm in sea water.

Pipette solution of ethanol extract from sample with concentration 1, 10, 100, and 1000 µg/mL then put into a test tube for each concentration and perform 3 repetitions. Control solution was performed without ethanol extract. Then added 3 mL of the seawater as well as 10 brine shrimps into each tube. Thus, there were 30 brine shrimps for each concentration. Then, volume of the test tubes was adjusted to 5 mL with seawater. The test tubes were left uncovered under lamp. The number of surviving shrimps was counted and recorded after 24 hours. By using probit analysis, lethality concentration (LC_{50}) was assessed at 95% of confidence interval.

Phytochemical screening

Phytochemical screening of the crude ethanol extract of *Benincasa hispida* Thunb. Cogn seeds was carried out according to standard methods (Mojab et al, 2003; Farnsworth, 1966; Ghasal and Mandal, 2012).

RESULTS AND DISCUSSIONS

Table 1. Avarage result of IC_{50} value of Beligo (*Benincasa hispida* Thunb. Cogn) seed extract

Repetitions	IC50 Value (ppm)	Avarage result of IC50 value (ppm)
1	44,539	54,987 ± 11,996
2	52,335	
3	68,088	

DPPH free radical scavenging assay is the simplest method for evaluating the antioxidant potential of an extract, which is based on the electron-transfer that produces a violet solution (kedare,2011; Huang,2005).

The results depicted in Table 1 reveal that the ethanol extract of seeds of *Benincasa hispida* Thunb. Cogn possess strong antioxidative activity against the DPPH radical with value of IC_{50} 54,987 ppm

Parameter of LC_{50} value of tested ethanol extract gained from linear regretion equation above is 4,666 ppm, so that it can be inferred that ethanol extract of *Benincasa hispida* Thunb. Cogn seeds has cytotoxic activity against *Artemia salina*, because its LC_{50} value is lower than 1000 ppm. So that extract of *Benincasa hispida* Thunb. Cogn seeds has considered potentially as cytotoxic (Meyer,1982),

Table 3. Results of phytochemical screening of crude ethanol extracts of (Benincasa hispida Thunb. Cogn) seeds

Phytochemicals	B. hispida Seeds
Alkaloids	(+)
Flavonoids	(+)
Saponins	(-)
Terpenoids	(+)
Tannins	(-)

The results of the phytochemical screening of Beligo (*Benincasa hispida* Thunb. Cogn) seeds extract showed the presence of alkaloids, flavonoids and terpenoids. Other secondary metabolites such as saponins and tannins were absent in the ethanol extract.

Table 2. Observation result and data analysis of cytotoxic activity of *Benincasa hispida* Thunb. Cogn seeds extract toward *Artemia salina* Leach Larvae after 24 hours treatment

Concentration (ppm)	Log of the concentrations	larvae mortality			% Mortality	Probits of Mortality
		1	2	3		
1000	3	10	10	10	90%	6,28
100	2	10	10	8	83,33 %	5,95
10	1	8	6	6	56,67%	5,18
1	0	4	5	4	33,33%	4,56
Control (-)		1	1	1		
Regression equation		y = 0,593 x + 4.603, R2 = 0,978				
LC50 Value		4,666 ppm				

CONCLUSIONS

Based on research results and discussion, hence we can conclude that of *Benincasa hispida* Thunb. Cogn seeds extract has antioxidant 54,987 ppm of IC_{50} value, cytotoxic activity 4,666 ppm of LC_{50} value and Phytochemical screening showed positive results for flavonoids, terpenoids, and alkaloids

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