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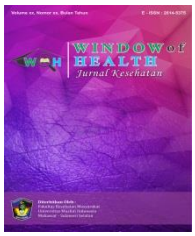

ARTICLE RESEARCH
URL artikel: http://jurnal.fkm.umi.ac.id/index.php/woh/article/view/woh7301

Toxicity of Standardized Ethanolic Extracts from *Duchesnea indica* (Jacks.) Focke Plants Using the BSLT Method

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ABSTRACT

Duchesnea indica (jacks.) focke., known as a medical plant that is used as raw ingredients for traditional medicines and herbs to increase the immune system because plants have the properties to cure and prevent various diseases. The objective of this study is to examine the quality of the duchesnea indica leaves extract by standardization method of specific and non specific parameters as well as the toxicity assay using using the Brine Shrimp Letality Test (BSLT). The results of specific parameter research include the identity of extracting duchesnea indica, Organoptic tests form extract samples that are used to extract thick, It's brown, Typical smell, astringent taste. The content of water soluble compounds is 18.67%, the content of ethanol soluble compounds is 0.03%, based in phytochemical screening test, the duchesnea indica leaves extract contain phenolic, flavonoid, tannin, Saponin and terpenoid. Non-specific parameters carried out include loss on drying of grounded Duchesne indica leaves at 0.029%, loss on drying of Duchesnea indica leaves extract at 0.026%, specific gravity determination at 0.83%, extract water content determination of 2.15%, total ash content is 3.70%, non-soluble acid of ash content is 1.32%. Thus, from these results, we can conclude that Duchesne indica leaf extract meets quality standards for extracting raw materials that can be used for traditional medicine. Research results: The standardized ethanol extract of duchesnea indica leaves is toxic to Artemia salina leach larvae.

Key words: Traditional medicine; Duchesnea indica (Jacks)Focke.; Toxicity; Standardization

PUBLISHED BY :
Faculty of Public Health
Universitas Muslim Indonesia
Address :
Jl. Urip Sumoharjo Km. 5 (Campus II UMI)
Makassar, Sulawesi Selatan.
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Article history :
Received 22 April 2024
Received in revised from 04 July 2024
Accepted 22 July 2024
Available online 24 July 2024

INTRODUCTION

Indonesia is one of the countries that has a wealth of traditional healing knowledge. Traditional medicine is believed to be medicine derived from plants which has been used by the community for generations to treat certain diseases. The development of research related to traditional medicine is currently growing rapidly, especially the use of medicinal plants as traditional medicine, therefore the use of medicinal plants is increasing every year through various research, one of which is the standardization of raw materials to obtain better and more effective quality of traditional medicine products. According to RI law no. 51 of 2009 concerning pharmaceutical work. In this law, pharmaceutical preparations are medicines, medicinal ingredients, traditional medicines and cosmetics. [(1)] Traditional medicine does not only come from plant materials but can also come from animal materials, mineral materials, galenic preparations, and even mixtures of these materials, which are traditionally used for treatment based on experience.

The use of medicinal plants as raw materials for traditional medicine and herbal medicine, when consumed, will increase the body's immune system because plants have medicinal properties that prevent various diseases. However, consuming herbal medicine does not necessarily mean healing; it is more about prevention by improving the body's immune system, so it is more suitable for health rather than healing. This is because medicinal plants are abundant, but their development is still minimal in herbal medicines, mostly for herbal medicine. [(2)]

Duchesnea indica is a wild plant that grows widely in several countries, including Afghanistan, Bhutan, India, Indonesia, Japan, Korea, Nepal, Africa, Europe, and North America. In Indonesia, especially in South Sulawesi, it is often found in highland areas such as the Bawakaraeng mountains and Malino Gowa. Local people use arbenan leaves as a traditional medicine to reduce fever & stimulants, reduce inflammation, and as a traditional medicine for burns. Findings made by Yang et al, 2019 that arbenan extract has antimetastatic activity in oral cancer [(3,4)]

Several previous studies have been carried out regarding duchesnea indica extract, both its content and several activities have been carried out to explore the use of duchesnea indica extract for health. [(5)] Previous research data shows that arbenan extract contains phenolics, flavonoids and tannins. Its antioxidant activity is in the very strong category with an IC_{50} value of 30.20 µg/mL. [(6)] The results of previous research show that the ethanolic extract of duchesnea indica leaves has anti-inflammatory activity with an anti-inflammatory activity of 0.31%. [(2)]

This research aims to standardize duchesnea indica extract as a raw material for traditional medicine that meets quality standards through standardized testing of specific and non-specific standard parameters as well as testing the toxicity of extract compound components using the BSLT method.

METHOD

Duchesnea indica leaves were collected from Bawakaraeng mountain area of Gowa Makassar. A total of 500 g of powder samples were extracted using the maceration method with 70% ethanol until a thick extract was obtained.[7] Standardization testing of specific and non-specific parameters was carried out referring to the Indonesian Ministry of Health, 2020. [8,9]

This research is a type of experimental research using the BSLT method. The test begins by making a test solution of five variations of concentration 250, 300, 350, 400, and 450 ppm, 6 mL of each pipette is put into a test tube, and ten shrimp larvae are added. has been aerated for 48 hours. Each concentration was repeated three times and compared with the control. Observations were made for 24 hours regarding the death of Artemia salina larvae. Then, the number of dead and alive larvae from each vial was counted and calculated using probit analysis to determine LC_{50}. [10]

RESULT

The results of the extract yield research are to determine the quantity value of a natural compound extract. The yield value is related to the amount of bioactive compounds in the sample.

Table 1. Calculation results of % yield of ethanolic extract of duchesnea indica leaves

Extract type	Amount of Solvent (mL)	Initial weight (g)	Extract Weight(g)	Extract Yield (%)
Ethanol Extract of Duchesnea indica	2.700	300	32.49	10.83

Based on Table 1, it can be seen that the percent yield value of the extract is 10.83%. The higher the yield value, the higher the content of substances attracted to a plant.

Table 2. Results of checking the identity of Duchesnea indica extract

Extract Identity Parameters	Result
extract Name	Duchesnea indica extract
Parts Used	Leaf/Folium
Latin Name	(*Duchesnea indica* (Jacks.) Focke)
Name of Indonesian	Arbenan

Checking the identity of the extract was carried out to provide an objective identity of the specific name of the plant used,[8,11] the identification results in Table 2 show that the research sample used was duchesnea indica extract, the part of the plant used in this research was the leaves/folium. Plant

identification is carried out to minimize the occurrence of sampling errors as raw materials for traditional medicine.

Table 3. Results of organoleptic examination of ethanolic extract of Duchesnea indica leaves.

Parameters	Result
Form	Thick extract
Colors	Blackish brown
Smell	Distinctive
Flavor	Astringent

Then, an organoleptic examination is carried out as an initial introduction to an extract using the five senses.[5,9] The results of the organoleptic examination in Table 3 show that the form of arbenan extract used in this research is a thick extract, where the thick extract is obtained after undergoing an evaporation process and does not contain any more filter fluid, but the consistency remains liquid at room temperature. The color examination results are blackish brown, with a distinctive odor and astringent taste to the taste buds.

Table 4. Results of determining the water-soluble and ethanol content of Duchesnea indica leaf extract.

Parameters	Result (%)
Water Soluble Essence Content	18.67
Ethanol Soluble Essence Content	0.03

The results of determining the water-soluble essence content in Table 4 have a percentage value of 18.67%, and the results of determining the ethanol-soluble essence content are 0.03%.

The chemical content of Duchesnea indica leaf extract can be identified in Table 5, which shows that Duchesnea indica leaf extract contains phenolic chemical compounds and tannins. flavonoids, saponins, and terpenoids.

The non-specific standard parameters of *Duchesnea indica* extract in Table 6 contain several test parameters where the drying loss of the extract is 0.26%, the drying loss of the powder is 0.029, the specific gravity determination is 0.83%, the water content of the extract is 2.15% which is required to be ≤ 10 (A), total ash content 3.7% and acid insoluble ash content 1.32%.

Test data shows that the concentration load of the extract in the media can kill *A. salina* Leach larvae, respectively, with concentrations of 250, 300, 350, 400, and 450 ppm. The number of deaths of *A. salina* Leach larvae in each test glass in various concentrations of *Duchesnea indica* leaf extract treatment is shown in Table 7.

Table 5. The results of the examination identified the chemical content of Duchesnea indica leaf extract.

Phytochemical Analysis	Reagent	Result
Fenoliks	$FECl_3$	Positive (+) occurs when the color changes to dark black
Tanins	$FECl_3$	Positive (+) changes the color to blackish green
Flavonoids	Concentrated HCl + Magnesium Powder	Positive (+) changes the color to black and yellow
Saponins	+ Hot Aquadest	Positive (+) there is foam or foam on the surface of the sample
Alkaloids	Dragendorf + H_2SO_4	Negative (-) there is no red or orange precipitate
Terpenoids	Liebermann Burchard	Positive (+) changes the color to orange-red

Table 6. Calculation results of non-specific parameters of Duchesnea indica extract.

Test Parameters	Result %	Condition
Drying loss of extract	0.26	-
Powder drying shrinkage	0.029	-
Determination of specific gravity	0.83	-
Determination of extract water content	2.15	≤ 10 (A)
Total ash content	3.7	-
acid insoluble ash content	1.32	-

Table 7. Observation data on the death of Artemia salina Leach shrimp larvae after 24 hours in the ethanolic extract of Duchesnea indica leaves using the *Brine Shrimp Lethality Test* (BSLT) method.

Test sample	Replications	The number of dead shrimp larvae per series of test sample solution concentrations					
		250	300	350	400	450	Control
Ethanol Extract of Duchesnea indica	1	1	3	6	5	9	0
	2	3	4	6	9	8	0
	3	2	3	6	9	8	0
Average		2	3.3333	6	7.6667	8.3333	0
Percent of deaths (%)		20	33.333	60	76.666	83.333	0
Probit value		4.16	4.56	5.25	5.71	5.95	-

From this table, it can be seen that variations in the concentration of soyogik leaf extract in this experiment showed different effects on the death of *A. salina* Leach larvae. Total mortality was obtained by adding up the larvae that died at each concentration. The death response occurred more quickly at a concentration of 450 ppm, with a percentage of larval death of 83.333%.

DISCUSSIONS

Testing the toxicity of the *Duchesnea indica* extract began with standardization testing of specific and non-specific parameters of the ethanol extract, followed by a toxicity test using the BSLT method using *Artemia salina* Leach shrimp larvae.

The toxicity test of *duchesnea indica* extract was carried out as a basis for determining the bioactivity of a sample. This test is to determine various biological activities of a plant, such as cytotoxic, phototoxic, pesticide, enzyme inhibition, and regulatory activities. (12.13) According to Janakiraman and Johnson (2016), the BSLT test can be used as a basis for testing cell line toxicity, anti-tumor, and anti-cancer activity. (14) The advantages of this test are that it is fast and easy, the results can be repeated, and it does not require a large cost. (15,16.)

Duchesnea indica ethanolic extract is used for standardization and toxicity testing. Examination of an extract was carried out to provide an objective identity of the specific name of the plant used (8,17) identification showed that the research sample used was duchesnea indica extract, the part of the plant used was leaves/folium. Then, an organoleptic examination is carried out as an initial introduction to an extract using the five senses. (8) Parameters for levels of compounds that are soluble in water and soluble in ethanol are carried out by dissolving the extract with water or ethanol as a solvent to determine the level of compounds based on their polarity, where polar compounds can be soluble in water and semi-polar to non-polar compounds can dissolve in ethanol. (3) The results showed that the extract dissolved more in water compared to ethanol. So it can be said that in *duchesnea indica* leaf extract there are more compounds that are polar in nature compared to compounds that are semi-polar to non-polar. Identification of chemical ingredients is carried out to determine the quality standards of extracts containing compound components for the use of medicinal plants as disease prevention.(10)

The drying loss parameter is basically one of the requirements that must be fulfilled when standardizing medicinal plants. measurement of the remaining substance after drying was carried out at a temperature of 105^{o}C for 30 minutes. At this temperature, the water will evaporate, and compounds that have a lower boiling point than water will also evaporate. The aim of this test is to provide a maximum limit on the range of compounds lost in the drying process.[8] The parameter for determining specific gravity aims to provide a limit on the amount of mass per unit volume, which is a specific parameter for liquid extracts to concentrated extracts that can still be poured. (8) According to the Ministry of Health, 2000, the water content in the extract should not be more than 10%. (8) Water content that is too high will cause the growth of microbes, which will reduce the stability of the extract during storage. (18) The water content results obtained were 2.15%, which means it meets the requirements set

out in the literature. Then, the total ash content is used as a basis for determining the mineral content of an extract from internal and external (non-physiological ash) sources originating from the initial processing process to the end of extract production. The total ash content value was obtained at 3.7%, this parameter does not have a permissible value or range. The determination of acid-insoluble ash content in the high category indicates the presence of silicate content originating from soil and metal elements such as silver, lead, and mercury, which will be toxic to the human body if consumed because they cannot be decomposed in the body. [18] The acid-insoluble ash content parameter reflects the presence of acid-insoluble mineral or metal contamination in a sample. [19]

The toxicity test of *duchesnea indica* extract showed that all series of concentrations caused death in larvae except the control group which contained sea water. at a concentration of 450 ppm caused the highest average larval death, while at a concentration of 250 ppm it caused the lowest larval death. The number of larval deaths shows that the higher the concentration of an extract, the higher the influence of the extract on larval death. This shows that sea water does not affect larval death. The research results and data obtained are based on calculation results using the probit analysis method. The LC_{50} value of *duchesnea indica* extract is 326.661µg/mL, so it can be concluded that the ethanol extract of *duchesnea indica* leaves has a toxic effect. According to Mayer et al 1982, a compound is categorized as very toxic if it has an LC_{50} value of less than 30 ppm, is categorized as toxic if it has an LC_{50} value of 30-1000 ppm, and is categorized as non-toxic if it has an LC_{50} value above 1000 ppm. [17]

Previous research by Mus S. 2020 regarding toxicity testing of kopasanda ethanolic extract containing flavonoid, saponin, triterpenoid, alkaloid and tannin compounds had toxicity test results with an LC50 value of 101.62 ppm. [12,20] *Duchesnea indica* extract also contains phenolic compounds, flavonoids, saponins, terpenes and tannins, so it can kill *artemia* larvae significantly starting from a concentration of 250 ppm so it has a toxic effect. The larval mortality rate is not only caused by the concentration of the extract given *to Artemia salina* larvae. However, this is also caused by chemical interactions of other secondary metabolites, such as triterpenes and polyphenols, which work synergistically with each other. Polyphenolic compounds such as flavonoids influence the death of artemia larvae. According to M I Rasyd, 2020, phenol can act as a toxin for plasma at high concentrations by damaging the cell wall system and collecting proteins in cells, while it can act to inhibit the multiplication of enzymes in vitro at low concentrations. [21]

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of the research that has been carried out, it can be concluded that the standardized ethanolic extract of Duchesnea indica leaves that has been carried out provides results that meet the quality standard requirements for extracting raw materials that can be used for traditional medicine. Then, testing the toxicity of the ethanolic extract of Duchesnea indica leaves was toxic to Artemia salina Leach larvae.

ACKNOWLEDGEMENTS

Thank you to the Chancellor of the Universitas Muslim Indonesia (UMI) and the UMI LP2S Resource Research and Development Institute through the 2023 internal lecturer research grant.

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