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Saponin, Polyphenol, Flavonoid content and α -glucosidase Inhibitory Activity, Antioxidant Potential of *Launaea sarmentosa* Leaves grown in Ben Tre province, Vietnam

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Abstract. *Launaea sarmentosa* (Willd) Schultz-Bip.ex Kuntze, a creeping herb, belongs to family Asteraceae. It is distributed in sandy coasts of India, Sri Lanka, Malaysia, East Africa and Vietnam. In Vietnamese folk medicine, whole plant possess tonic, soporific, diuretic, laxative properties and the roots are used to benefit milk for mothers after childbirth, instead of *Lactuca indica*. Up to now, the phytochemicals and bioactivities of this plant are not yet been examined. The present study was designed to investigate the saponin, polyphenol and flavonoid contents as well as α -glucosidase inhibitory and antioxidant activities of the leaves of *L. sarmentosa*. Preliminary phytochemical screening of this plant were found to give positive reactions for flavonoid, saponin, alkaloid, tannin, and steroid compounds. The result of quantitative determination showed that the leaves contains high amount of polyphenol compounds (290.90 mg GAEs/g extract), lower amount of flavonoids (85.47 mg QEs/g extract) and the saponin content was found to be 10.8 % (per dry weight). As expected, the ethanol extract had a antioxidant potential with IC₅₀ value of 24.15 μ g/mL in DPPH experiment, compared to that of vitamin C. Meanwhile, the α -glucosidase inhibitory assay showed inhibited enzyme activity with IC₅₀ value of 67.09 μ g/ml.

1. Introduction

It is believed that medicinal plants play an important role in the health care system in Vietnam, based on their secondary metabolites, organic compounds synthesized by plants [1-4]. Due to these metabolites, the extracts or plants have been considered as a promising materials for the development new pharmaceuticals. Recently, polyphenol, flavonoid and saponin in plants are reported to possess many beneficial bioactivities, such as antioxidant, anti-inflammatory, anticancer, antihyperglycemic and antidiabetic activities [5]. *Launaea sarmentosa* (Willd) Schultz-Bip.ex Kuntze, a creeping herb,



belongs to family Asteraceae. It is distributed in sandy coasts of India, Sri Lanka, Malaysia, East Africa and Vietnam. According to Vietnamese folk medicine, the leaves is consumed as vegetable, whole plant possess tonic, soporific, diuretic, laxative properties and the roots are used to benefit milk for mothers after childbirth, instead of *Lactuca indica* [6]. Up to now, the phytochemical quantification and medicinal values of this plant are not yet been examined. As a part of our continuing studies on phytochemical and bioactivities of medicinal plants in Vietnam, this paper inform the phytochemical screening and to evaluate total polyphenol, flavonoid and saponin contents, as well as α -glucosidase inhibitory, free radical scavenger activities of the *L. sarmentosa* leaves [7-9].

2. Experimental

2.1. Chemicals and reagents

All reagents were of analytical grade and purchased from Merck (Darmstadt, Germany). Distilled and deionized water were obtained from Department of Pharmaceutical Biochemistry (Institute of Applied Materials Science, VAST, Vietnam). Others solvents and reagents were of analytical grade.

2.2. Plant Material

Leaves of *Launaea sarmentosa* were collected in the farm of Vietnam Sarmentosa Joint Stock Company, Ben Tre Province, Vietnam at August, 2018. Plant sample was authenticated by Southern Institute of Ecology, VAST, Vietnam, and voucher specimen (LSL1082018) was deposited at Department of Pharmaceutical Biochemistry, Institute of Material Science. The leaves sample were washed, afterthat cool dried and ground to powder.

2.3. Phytochemical screening

Phytochemical screening was carried out using standard methods to detect the plant secondary metabolites including of alkaloid, tannin, flavonoid, saponin, steroid, phenolic compounds etc. [10]

2.4. Total polyphenol content

Total polyphenol content was determined using Folin-Ciocalteu (FC) reagent [11]. The ethanol extract (10 mg) was dissolved in methanol (2 mL). Solution of 10% Folin-Ciocalteu reagent was prepared by adding Folin-Ciocalteu reagent (10 mL) in water (90 mL). Then, 5% Na_2CO_3 (3 g) was prepared by dissolving Na_2CO_3 (3 g) in water (50 mL). The plant extract (200 μL) was taken in a test tube and added 10% Folin-Ciocalteu reagent (1.5 mL). Then all the test tube was kept in a dark at room temperature for 5 min. Finally, 5% Na_2CO_3 (1.5 mL) was added to the solution and mixed well. The tube was kept again in the dark for 2 h. The absorbance was measured at 760 nm through UV-spectrophotometer, with gallic acid as a standard. The total polyphenol content were expressed as mg gallic acid equivalents (GAE)/ g extract.

2.5. Total flavonoid content

The total flavonoid content (mg/mL) was determined using aluminum chloride (AlCl_3) method [12]. The assay mixture consisting of 0.5 mL of the ethanol extract, 0.5 mL distilled water, and 0.3 mL of 5% NaNO_2 was stand at 25°C. This was followed by addition of 0.3 mL of 10% AlCl_3 immediately. Two milliliters of 1 M NaOH was then added to the reaction mixture, and the absorbance was measured at 510 nm. Quercetin was used as a standard. Total flavonoids content is expressed as quercetin equivalence (QE) mg/g extract.

2.6. Total saponin content

The determination of total saponin was done by slightly modified methods [13]. Briefly, 1 g of powdered leaf was extracted with 100 ml of 20% aqueous ethanol for half hour in an ultrasonic bath (Power sonic, Korea) equipped with digital sonication power, time and temperature controller with a useful volume of 10 L. The mixture was filtered by using Whatman filter paper no 1 and the residue again extracted with another 100 ml of 20% aqueous ethanol in same procedure. The total extract were concentrated under reduced pressure at 40°C to give 50 ml approximately. The residue was extracted

three times with 30 ml diethyl ether. Next, the aqueous layer was successfully extracted with 30 ml n-butanol. The n-butanol fraction was washed with 10 ml of 5% aqueous sodium chloride. Finally, the below layer was evaporated and dried until constant weight. Saponin content was calculated and express as a percentage against dried weight of leaves.

2.7. Antioxidant Assay

DPPH scavenging experiment was carried out as previously described [14]. The experiment was repeated three times. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. Vitamin C was used as standard compound. 1 ml of 0.1 mM DPPH prepared in ethanol was mixed with 1 ml of the leaf extracts ranging from 10 to 150 $\mu\text{g/mL}$. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance was measured on a spectrophotometer at 517 nm. The scavenging ability of the plant extracts was calculated using the equation:

$$\text{DPPH scavenging activity (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where $Abs_{control}$ is the absorbance of DPPH + ethanol; Abs_{sample} is the absorbance of DPPH radical + sample (i.e. extract or ascorbic acid)

2.8. α -Glucosidase inhibitory activity

The α -glucosidase inhibitory activity was determined according to the described method [15]. The principle of the method is that the enzyme α -glucosidase hydrolyzes the substrate *p*-nitrophenyl- α -D-glucopyranosid (pNPG) to give α -D-glucose and *p*-nitrophenol (pNP). The α -glucosidase inhibitory activity was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

$Abs_{control}$ is the absorbance without plant extract; Abs_{sample} is the absorbance with sample

3. Result and Discussion

Preliminary phytochemicals screening (Table 1) showed that *Launaea sarmentosa* leaves were found to be present alkaloid, flavonoid, triterpenoid, saponin, and only tannin was absent. As we known, the plant secondary metabolites are proved to possess interesting bioactivities such as anti-inflammation, antidiabetic, antibacterial, antioxidant activities, wound healing properties, protection of the skin, health promotion and disease prevention etc. Nowadays, phytochemicals are increasingly attracted to scientist for the isolation and structural determination, because they are not only used directly as drugs for treatment of disease but also as modeling compounds for development of new drugs with less toxic and side effects to humans. Previous authors already reported that flavonoid and polyphenol exhibited many bioactivities such as antioxidant, anti-inflammatory, antimicrobial, anticancer, α -glucosidase inhibition and anti-allergic activities [16,17]. Saponin are other type of phytoconstituents which are responsible for tonic and antimicrobial activities of plant [18]. Therefore, the appearance of different phytochemicals in *Launaea sarmentosa* may be responsible for its tonic effect, laxative, antibacterial activities which were used in Vietnam traditional medicine.

Table 1. Phytochemical screening of ethanol extracts

| Phytochemical tests | <i>Launaea sarmentosa</i> extract |
|---------------------|-----------------------------------|
| Flavonoids | +the |
| Alkaloids | + |
| Triterpenoids | + |
| Tannin | - |

| | |
|---------|---|
| Saponin | + |
|---------|---|

Note: (+) indicates present; (-) indicates absent

The results of total flavonoid, polyphenol and saponin contents (Table 2) showed that *Launaea sarmentosa* leaves contained polyphenol (290.9 mg GAEs/g of extract), flavonoid (85.47 mg QEs/g) and saponin (10.8 %). The α -glucosidase inhibitory and antioxidant activities test (Table 3) showed the IC₅₀ values of 67.09 mg/ml and 24.15 μ g/mL, respectively. The α -glucosidase inhibition may be responsible for the antihyperglycemic effects in plasma blood. Enzyme α -glucosidase is one of the enzyme responsible for carbohydrates destruction to form smaller sugars, such as glucose and fructose. Thus, they will slowly digest glucide and delay glucose absorption in body, leading blood glucose levels following meals increase slowly. The high inhibition activity of *Launaea sarmentosa* leaves on α -glucosidase might be contributed by the high content of polyphenol and flavonoid compounds. Diabetes drugs with α -glucosidase inhibitors such as acarbose and voglibose, usually caused serious side effects. Therefore, it is necessary to discover and develop for alternatives with α -glucosidase inhibitory activity and without side effects to the body. In recent years, projects were deeply studied to discover potent α -glucosidase inhibitors from plant natural products have received great attention due on their highly abundant metabolites in nature and potential biological activities, among them *Launaea sarmentosa* was very promising, based on its alfa-glucosidase inhibitory activity.

Table 2. Selected phytochemical contents of *Launaea sarmentosa* leaves

| Items | Yield |
|--------------------------------------|--------|
| Total polyphenol content (mg GAEs/g) | 290.90 |
| Total flavonoid content (mg QEs/g) | 85.47 |
| Total saponin (% per dry weight) | 10.80 |

It was showed that the extract contains high amount of polyphenol (290.9 mg GAEs/g of extract), but low amount of flavonoid (85.47 mg QEs/g extract). This may be due to most of the polyphenol in the extract are non-flavonoid. The study also showed that the extract exhibited highly antioxidant activity through IC₅₀ value of 24.15 μ g/mL (in DPPH test) and high amount of polyphenol content may be the cause for their antioxidant ability. Because antioxidants (polyphenol and flavonoid) are able to stabilize or scavenging free radicals, so that they can not cause oxidative damage to the cellular structures. Free radicals and oxidants play a dual role as both toxic and beneficial compounds, since they can be either harmful or helpful to the body. When the balance between antioxidants and free radicals were shifted toward generating radicals, called oxidative stress and causing most of the degenerative diseases including atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia, etc. The human body has several methods to rebalance through generating endogenous or exogenous antioxidants such as vitamin C, vitamin E, coenzyme Q10, catalase superoxide dismutase and polyphenol or flavonoid supplements.

Our results were in good agreement with previous study that polyphenol compounds are potential antioxidants. The alfa-glucosidase inhibitory and antioxidant properties of *Launaea sarmentosa* leaves are meaningful to reduce oxidative damage associated with diabetic complications [19]. An antidiabetic herb with potent antioxidant activity may be prevented oxidative stress, which is considered supporting the relationship between chronic hyperglycemia and diabetes-associated complications.

Moreover, total saponin content of *Launaea sarmentosa* leaves were found to be 10.80 %. The bioactivities of saponin are normally assigned to their amphipathic properties, consisting of a hydrophobic triterpene/steroid skeleton and a hydrophilic carbohydrate molecules. It is known that potential biological activities of saponins are dependent on other aspects of their structure. Some

saponins are also important pharmaceuticals, and the underexplored biodiversity of plant saponins is likely to prove to be a vital resource for future drug discovery. Saponin compounds is believed to protect living body against hypercholesterolemia and antibiotic properties. Saponins were also responsible for central nervous system activities and being used to create tonics for treating depression, neurasthenia, etc. So, this result can be used to explain the ability of making people more strong when using *Launaea sarmentosa* in daily meals. Hence, *Launaea sarmentosa* may be a potential source of natural products for the development drug for treatment type 2 diabetes, stress oxidative and tonic property.

Table 3: *In vitro* α -glucosidase inhibitory and antioxidant activity of extract

| Sample | DPPH (IC ₅₀) | α -glucosidase inhibitory activity (IC ₅₀) |
|----------------------------------|--------------------------|---|
| <i>Launaea sarmentosa</i> leaves | 24.15 (μ g/mL) | 67.09 (μ g/mL) |
| Acarbose | | 138.2 (μ g/mL) |
| Vitamin C | 6.45 (μ g/mL) | |

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