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Research Article

Total triterpene content, antioxidant activity and acute toxicity study of *Securidaca longepedunculata* Fresen

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Abstract

Plants are widely used in developing countries for traditional medicine. Knowledge of biological activities and phytochemistry is necessary for the safe use of these plants in traditional medicine. *Securidaca longepedunculata* is a medicinal plant whose roots are widely used in the treatment of several diseases. In this study the content of total triterpenes in leaf and root bark was assessed. The antioxidant activity by the ABTS method was evaluated as well as the effect of the extracts on lipid peroxidation of mice liver cell membranes. Acute toxicity was evaluated at the dose of 2000 mg / mL administered to the mouse. The results showed that the maximum values of the triterpenes contents of the leaves and of the root bark are 58 and 21 mg UAE / 100 mg respectively. Methanolic and chloroform extracts from leaves and root bark inhibited more than 50% of lipid peroxidation. The same extracts showed strong ABTS cation reduction activity. The leaf extract did not induce signs of acute toxicity in mice at a dose of 2000 mg / mL. The leaves of *S. longepedunculata* could be used in the management of pathologies associated with oxidative stress or which require biological properties relating to triterpene compounds.

Keywords: *Securidaca longepedunculata*, triterpenes, peroxidation, toxicity

INTRODUCTION

The involvement of oxidative stress has been reported in several age-related diseases such as neurodegenerative diseases, cardiovascular diseases, chronic kidney disease, chronic obstructive pulmonary disease and cancer ¹.

The high production of reactive oxygen species (ROS) causes malfunction and tissue damage during certain disease conditions. ROS oxidize lipids to generate peroxides and aldehydes which are more stable than the initial ROS. These stable derivatives diffuse from their site and cause damage to distant sites. Increased production of lipid peroxides and aldehydes has been observed in atherosclerosis, Alzheimer's disease, heart failure, cancer, rheumatic arthritis and other immunological disorders. Therefore, decreasing the formation of lipid peroxidation products or their chemical scavenging could be beneficial in limiting the deleterious effects of ROS in various pathological conditions.²

Studies have reported that the *Plasmodium*, in order to infect liver cells, causes lipid peroxidation of cell membranes ³. Lipid peroxidation is one of the steps in the infection of liver cells by *P. falciparum* ⁴. Induction of high lipid peroxidation by *Plasmodium* in children leads to the severe form of malaria which is fatal ⁵.

It has been established that free radicals (oxidative stress) are involved in some human infertility ⁶. ROS have been shown to

affect oocyte maturation to fertilisation, embryo development and pregnancy. Since most ovarian cancers appear in the surface epithelium, repetitive ovulation would be one of the causes of this disease. Oxidative stress has a negative effect on sperm quality and function. The imbalance between pro-oxidants and antioxidants can lead to certain diseases of the reproductive system such as endometriosis, polycystic ovary syndrome. Oxidative stress can be the cause of spontaneous abortions, recurrent pregnancy loss ⁷. Natural substances by their abundance could be an accessible alternative to the great mass of populations. In fact, these mainly vegetable substances contain bioactive compounds.

Medicinal plants owe their biological activities to the substances they contain. These substances are known to have shown various pharmacological activities. These substances include phenolic compounds, alkaloids, saponosides and terpene compounds. Each of these chemical groups have shown several biological properties ⁸. Terpene compounds have been reported to show anti-tumor, anti-inflammatory, hypoglycemic, antibacterial, antiviral, antimalarial activities. In addition, terpene compounds prevent and treat cardiovascular diseases. Studies have shown that terpene compounds have shown several uses such as against insect resistance, immunoregulation, antioxidant, slowing down old age and neuroprotection etc ⁹.

Oleanolic acid and ursolic acid which are triterpene have shown immunomodulatory activity. They are potential immunomodulators of macrophages during *Mycobacterium tuberculosis* infection¹⁰. Ginsenoside Re isolated from American ginseng has shown antioxidant activity capable of protecting cardiomyocytes from oxidative damage by internal and external oxidants¹¹. Ginsenoside Rg1 regulates the expression of regulatory factors in the cell cycle and thereby exerts its anti-aging effect. Ginsenoside Rd is commonly used for neuroprotection¹²⁻¹⁴. Ursolic acid can induce apoptosis in cancer cells and may protect hepatoma cells⁹. Total triterpenes have shown inhibitory activity on membrane lipid peroxidation of liver and neuronal cells in mice¹⁵.

Antioxidants can prevent basic oxidative damage induced by ovulation and DNA damage in the ovarian epithelium¹⁶. The use of antioxidants or products containing antioxidant molecules could restore fertility^{17,18}.

Therefore, any compound capable of inhibiting lipid peroxidation of liver cells could prevent *Plasmodium* infection of the liver¹⁹.

Studies have shown that plants contain compounds which are capable of preventing or neutralising agents that induce lipid peroxidation in human cells²⁰. Results of some work have reported that antioxidants such as melatonin and vitamin C have the ability to enhance the antiplasmodial properties of certain compounds and also protect liver cells from lipid peroxidation that leads to their apoptosis during infection¹⁹.

Identified biomarkers of oxidative stress can provide important information on the antioxidant capacity of a plant extract²¹.

The use of medicinal plants is growing exponentially around the world. It is necessary that these plants used do not exhibit toxicity.

Changes in organ weights are a sensitive indicator of biochemical modifications induced in organs. Comparison of organ weights between control and treated groups is used to predict the toxic effect of a substance. Organ weight is an index of swelling, atrophy or hypertrophy. Relative organ weights are essential for diagnosing organ exposure to injury. The heart, liver, kidneys, spleen and lungs are the main organs affected by metabolic reactions caused by toxins²².

The objective of this work is to assess the total triterpene content and the antioxidant properties of *S longepedunculata* as well as the identification of acute toxicity signs induced by the plant.

METHODS

Total triterpene content assesment

Total triterpenoid content was determined by using the following procedure²³. Briefly, 100 μ L (10 mg/ mL, in methanol) of each sample was mixed with vanillin-glacial acetic acid solution (150 μ L, 5% w/v) and perchloric acid solution (500 μ L). The sample solutions were heated for 45 min at 60°C and then cooled in an ice-water bath to the ambient temperature. After the addition of glacial acetic acid (2.25mL), the absorbance of each sample solution was measured at 548nm. Ursolic acid (in methanol) was used as a standard. Results were expressed as milligram ursolic acid equivalents /g dry extract (mg UAE/g).

Reduction of radical ABTS assay

The method described by Re et al.²⁴ with some modifications was used to evaluate the sample ABTS^{•+} scavenging ability. To 50 μ L of extract, was added 200 μ L of diluted ABTS^{•+} solution for 5 min dark incubation. The absorbance was read at 734 nm

with microplate reader. (Ascorbic acid) was used for generating standard curve and the result were expressed in mg ascorbic acid equivalents /g dry extract (mg AAE/g).

Inhibition of lipids peroxidation in rat liver homogenate

The inhibition activity of extracts on lipid peroxidation (LPO) was determined according to the thiobarbituric acid method. FeCl₂ and H₂O₂ was used to induce the liver homogenate peroxidation with slightly modification^{25,26}. In this method, 0.2 mL of extract was mixed with 1.0 mL of 1% liver homogenate, then 50 μ L of FeCl₂ (0.5 mM) and 50 μ L of H₂O₂ (0.5 mM) was added. Final concentration of extract was 100 μ g/mL. The mixture was incubated at 37°C for 60 min, then 1.0 mL of trichloroacetic acid (15%) and 1.0 mL of 2-thiobarbituric acid (0.67%) was added and the mixture was heated up in boiled water for 15 min. The absorbance was recorded at 532 nm. The control without extract was use. The result is expressed as a percentage of inhibition.

Acute toxicity study

The acute toxicity study of *Securidaca longepedunculata* leaves was carried out with the methanolic extract according to the guidelines 423 of OECD²⁷. Female mice (strain NMRI) 6 weeks old were used. The animals were randomly selected and grouped into two group. After a 12-hour fast, the mice were weighed. A control group of 3 animals received each 0.2 mL of sodium chloride solution (NaCl, 0.9%) by gavage using a feeding probe. The test group (two) animals consisting of 6 females received each a limit dose of 2000 mg / kg of body weight of extract (in NaCl 0.9%). The animals were fed one hour after administration of the extract. Animals were observed continuously during the first 30 minutes after administration of the extract, and regularly during the first 24 hours, then daily for 14 days for behavioural signs of toxicity (changes in coat and muzzle, contortions, convulsions, salivation, tremors, restlessness, grooming, stool appearance, mobility, and death of mice). The mice were sacrificed at the end of the observation by cervical dislocation. The liver, kidneys, lungs and heart were removed and weighed.

Statistical analysis

One way analysis of variance (ANOVA) followed by Tukey test of Graph Pad Prism software was used to determined statistical significance; p value \leq 0.05 was considered significant (n=3).

RESULTS

Triterpene content

The assessment of total triterpenes showed (**Figure 1**) that their contents in chloroformic extracts from leaves and root bark harvested in the warm period are 56, 27 \pm 0.83 and 14.50 \pm 0.19 mg UAE /100 mg and 57.54 \pm 0.69 and 21.18 \pm 0.23 for the samples from the cold period respectively.

Leaves and root bark methanolic extracts contents showed 58, 65 \pm 1.78 and 15.82 \pm 0.63 mg UAE/100 mg for warm periods samples and 15.0 \pm 0.22 and 18.11 \pm 0.05 mgAUE /100 mg for sample from cold periods respectively.

Statistical analysis of leaves chloroformic extracts contents showed that there was no significant difference (P > 0.05) between the samples from warm and cold periods. However, using the root bark chloroformic extracts, the sample from the cold period showed a higher content than the sample from the warm period. The results also showed that leaves chloroformic extracts contents are three times higher than root bark chlorofomic extract. With regard to leaves methanolic extracts, samples from warm period showed three times higher content than that of cold period. Root barks

harvested in the warm period showed a lower content than the same organ from the cold period using methanolic extracts. The results showed that the leaves generally contained higher contents than the root bark. In addition, both methanol and chloroform make it possible to extract the same quantities of terpene compounds from leaves harvested in warm or cold periods.

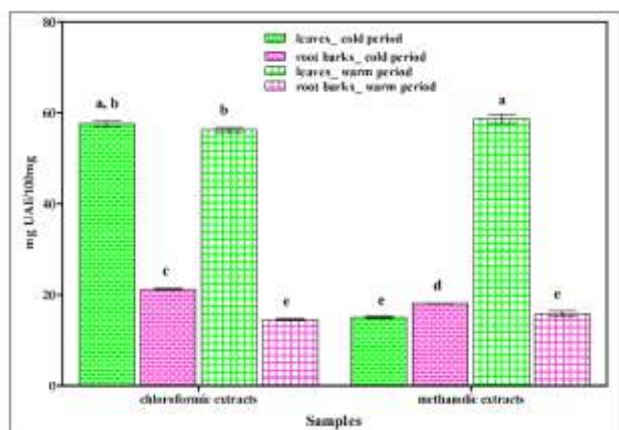


Figure 1: Total triterpene content of leaf and root bark. Different letters indicate statistically different values ($P < 0.05$)

Lipid peroxidation inhibitory activity

The lipid peroxidation inhibitory activity of *S. longepedunculata* leaves and root bark extracts is shown in **Figure 2**. The activities of chloroformic extracts from leaves harvested during cold and warm periods are statistically identical ($57.00 \pm 0.48\%$ and $58.29 \pm 0.74\%$). The same result is also observed with methanolic leaves extracts ($64.09 \pm 1.22\%$ and $62.80 \pm 0.48\%$). With regard to leaves (cold and warm periods sample), methanolic extracts activity is higher than that of chloroformic extracts. However, root bark extracts (chloroformic and methanolic) from sample harvested in warm period are more active than those of samples from the cold period. The methanolic extracts from leaves and root bark harvested during the warm period showed similar activities ($p > 0.05$). Also, chloroformic extracts from leaves and root bark harvested during the cold period showed identical activities ($p > 0.05$). The variation of the values shows that leaves activity would be related to extraction solvent and that of the root bark would be related to the harvesting period. Methanol, which is a polar solvent, therefore extracts more bioactive compounds from the leaves than chloroform. These results show that *S. longepedunculata* extracts inhibited more than 50% lipid peroxidation of hepatic cell membranes from rat induced by FeCl_2 - and H_2O_2 .

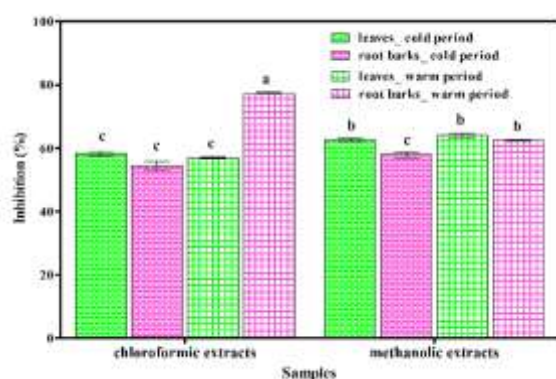


Figure 2: Inhibitory activities of leaf and root bark extracts on lipid peroxidation. Different letters indicate statistically different values ($P < 0.05$)

Reduction of cation radical ABTS^{•+}

The result of ABTS^{•+} cation reduction by *S. longepedunculata* extracts is shown in **Figure 3**. Analyses have shown that using methanol as extraction solvent, the leaves harvested during the warm and cold periods show identical activities ($p > 0.05$). The methanolic extract of root bark from the warm period is more active than that from the same organ harvested during the cold period. In addition, the results showed that leaves and root bark extracted with methanol had similar activities in contrast to the samples from the cold period.

With regard to the chloroformic extracts of *S. longepedunculata*, the leaves harvested during the cold period were more active than those harvested during the warm period. Chloroformic extracts of root bark from cold and warm periods showed similar activities. These root bark extracts showed higher activity than those of the leaves.

Leaves methanolic extracts showed a better activity compared to their chloroformic extracts.

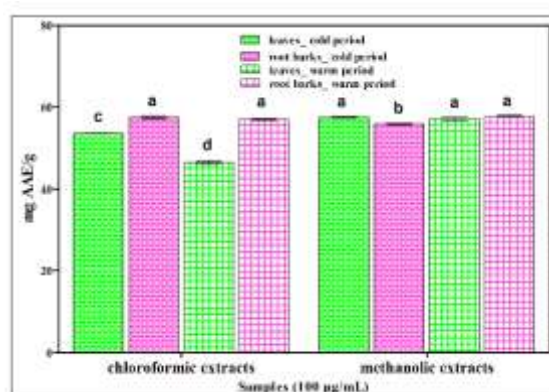


Figure 3: Antioxidant activity of reduction of ABTS cation of one gram of extracts in equivalence of a mass of ascorbic acid expressed in milligrams. Different letters indicate statistically different values ($P < 0.05$).

Acute toxicity

The acute toxicity of the methanolic extract of the defatted powder of *S. longepedunculata* was assessed according to the OECD procedural guide and the result was showed in **table 1**.

Oral administration of the extract at a single dose of 2000 mg/Kg body weight resulted in no signs of acute toxicity or death, throughout the observation of mice from the test group compared to the control group.

Estimation of organ weights and assessment of relative organ weights (Table 1) of mice from the test group showed values that were statistically similar ($p > 0.05$) to those of mice from the control group.

Table 1 : Comparison of the relative organ weights of the test and control groups.

	Relative organ weights :			
	mouse weight (m_w) on day 14 / organ weight (o_w)			
	liver	kidney	heart	lung
control group	21.02 ^a	87.58 ^b	212.91 ^c	141.80 ^d
test group 1	18.83 ^a	81.18 ^b	197.84 ^c	136.62 ^d
test group 2	19.71 ^a	80.83 ^b	219.68 ^c	143.90 ^d

Values with the same superscript letters are statistically identical ($p > 0.05$).

DISCUSSION

The determination of terpene quantities showed that the leaves contain the highest concentrations. This contents results show that in order to assess biological activities or to carry out phytotherapy related to the terpene compounds of *S. longepedunculata*, it is advisable to choose its leaves rather than its roots.

Terpene compounds are known to have antioxidant activity against the radical cation ABTS^{•+} 28. These chemical groups have shown neuroprotective and hepatoprotective 29,30, cardioprotective 31 properties et antiplasmodiale 32. These different properties of the triterpenes would explain the use of *S. longepedunculata* against pathologies such as heartache, hepatitis, epilepsy and malaria 33. The anti-plasmodial activity of terpene compounds has been reported by several authors, such as artemisinin³⁴. This richness of the leaves of *S. longepedunculata* in terpene compounds would explain the use of this plant against malaria.

The leaves showed significant lipid peroxidation inhibitory activities in this study. In traditional medicine, the generally used extraction solvent is water which is more polar than methanol, so the leaves would be more appropriate for treatments aimed at inhibiting the lipid peroxidation induced by *Plasmodium falciparum* during hepatocyte infection.

Some authors have reported that during infection of hepatocytes by *P. falciparum*, peroxidation of the cell membranes occurs, leading to apoptosis 19,35. The antioxidant effect of *S. longepedunculata* leaves observed in this work could contribute to inhibit this lipid peroxidation and thus the apoptosis of hepatocytes during *P. falciparum* infection. The lipid peroxidation inhibitory activity observed with the leaf extracts indicates that this organ of *S. longepedunculata* could be associated in the management of atherosclerosis, ischemia-reperfusion, heart failure, Alzheimer's disease, rheumatic arthritis, cancer, and other immunological disorders 2.

Methanol is therefore indicated for extracting bioactive compounds from leaves than chloroform, unlike root bark, for which both solvents are suitable.

These results therefore show that polar compounds in the leaves reduce the radical cation ABTS^{•+} better than non-polar compounds. The activity of the root bark showed that the apolar compounds are as active as the apolar compounds. The antioxidant capacity of the extracts observed shows that the leaves of *S. longepedunculata* could contribute to mitigate the effects of oxidative stress especially on cortical cells. Indeed, it has been reported that neuronal cells are very vulnerable to oxidative stress due to their high oxygen consumption and lack of adequate antioxidant defence mechanisms 36.

Neurons consume a lot of oxygen throughout life making the brain more vulnerable to oxidative damage. In addition, the brain contains high concentrations of polyunsaturated fatty acids and transition metals, as well as weak antioxidant defense mechanisms 37.

Body weight and internal organs such as liver, kidney, heart, spleen, thymus, etc. are sensitive indicators of toxicity following exposure to a toxic substance. Toxicity data are needed to predict the associated safety before the use of medical products 22.

This lack of statistical difference shows that the organs of the mice would not have undergone atrophy or hypertrophy following the taking of the extract. Furthermore, the lethal dose of this extract would therefore be higher than 2000 mg/kg. The extract is therefore classified in the risk category (LD50 >2000 mg/kg) according to the OECD 423 toxicity scale

27. In the literature, it is reported that plants with an LD50 greater than 1000 mg/kg orally are considered nontoxic 38.

According to the results of our research no studies on the acute toxicity of *S. longepedunculata* leaves have been performed. However, studies on *S. longepedunculata* roots have reported toxicity without mortality 33. Studies have reported that extracts from the leaves and roots of *S. longepedunculata* neutralized the toxicity of the snake venom *Naja nigricollis* 39.

In poor countries, people do not have the means to buy food supplements and they are not part of their dietary habits. Taking plant organs like coffee could help prevent the occurrence of certain diseases (cancers, neuropathology, premature ageing, certain factors of sterility).

Plants that are rich in terpene compounds could show immunomodulatory and neuroprotective properties 40,41. The use of *S. longepedunculata* in the treatment of epilepsy would be linked to these properties due to its richness in terpene compounds.

CONCLUSION

The leaves of *S. longepedunculata* are very rich in triterpene compounds than the roots regardless of the harvesting period. Leaves and root bark significantly inhibit lipid peroxidation in liver cells and strongly reduce the ABTS radical. The methanolic extract of the leaves did not induce any signs of acute toxicity in mice. The biological investigation of the leaves should be continued with studies on neuroprotection, genoprotection, immunomodulation and chronic toxicity of this widely used plant.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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