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Research Article

Antioxidant and Anti-Inflammatory Activities Development of Methanol Extract of *Lysimachia Cousiniana* Coss. et DR Growth in Jijel - Algeria

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ABSTRACT

Lysimachia Cousiniana Coss. et DR is Algerian endemic species growth in humid forest almost unnoticed on the scale of global biodiversity. This experiment tries to reflect using the antioxidant and anti-inflammatory activities. The radical scavenging capacity using 2,2- diphenyl-1-picryhydrazyl (DPPH) and the reducing power test were determined to evaluate the antioxidant activity and of the Human Red Blood Cell (HRBC) membrane stabilization method was used to evaluate the anti-inflammatory activity. The results concluded that the extract is a potential source of antioxidants.

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INTRODUCTION

Lysimachia Cousiniana Coss. et DR belongs to the tribe Lysimachieae Reich., and is commonly recognized as a primitive group of traditional Primulaceae [1, 2]. However, data from recent phylogenetic analyses suggested its re-location to the family Myrsinaceae [3], as members of subfamily Myrsinoideae [4]. The genus Lysimachia L. comprises about 200 species, wild and cultivated, that are native to temperate regions of Eurasia [5].

The chemistry of the genus Lysimachia has been studied by several research groups. It appears that the most typical chemical constituents in this genus are: flavonoids [6, 7], quinoids [8] and saponins, both triterpene and steroidal [9, 10].

The medicinal value of many Lysimachia species is well known, there are reports on their use as, for example, analgesic [11], anti-leishmanial [12], anti-helminthic [13] agents and to treat cholecystitis [14].

Lysimachia Cousiniana Coss. et DR is a Creeping prostrate plant. Isolated flowers in the armpit long stalked, Oval leaves 1-3 X 1-2 cm,Wet forests [15].

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In detail, the purpose was to valorise the biological activities of *Lysimachia Cousiniana* Coss. et DR to enrich the Algerian pharmacopeia and to preserve this endemic species from extinction.

MATERIALS AND METHODS

Plant Material

The random sampling were used during the harvesting, the areal parts of *Lysimachia Cousiniana Coss. et DR .,* were collected from Taza National Park Jijel – Algeria (36° 35' 16''-36° 48' 12'' north Latitude and between 5° 29' 07'' and 5° 40' 11'' Longitude East); Determined by Dr. Nouioua Wafa.

Preparation of Methanol Extracts

The aerial parts were powdered and macerated in 80 % methanol for 24, 48 and 72 hours, at the laboratory temperature (1:10 w/v, 10 g of dried herb). After maceration, the extracts were collected, filtered and evaporated to dryness under vacuum [16]. The dry extract was stored at a temperature of -18°C for later use.

Determination of Total Phenolic Content

For total polyphenol determination, the Folin-Ciocalteu method was used [17]. The samples (0.2 mL) were mixed with 1 mL of the Folin-Ciocalteu reagent previously diluted (10 % v/v). The solutions were allowed to stand for 4

minutes at 25°C before 0.2 mL of a saturated sodium carbonate solution (75 mg/mL) was added. The mixed solutions were allowed to stand for another 120 minutes before the absorbance was measured at 765 nm. Gallic acid was used as a standard for the calibration curve. The total phenolic compounds content were expressed as mg equivalent of Gallic acid per gram of extract (mg EAG/GE).

Determination of Total Flavonoids Contents

The flavonoids content in our extract was estimated by the Aluminium chloride solution according to the method described by Bahorun *et al*., (1996) [18]. Briefly, 1 mL of the methanol solution of the extract was added to 1 mL of 2 % $AlCl_3$ in methanol. After 10 minutes, the absorbance was determined at 430 nm. Quercetin was used as a standard. Results were expressed as mg equivalent Quercetin per gram of extract (mg EQ/GE).

DPPH Assay

The donation capacity of extract was measured by bleaching of the purple-coloured solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato *et al*., (1998) [19]. One millilitre of the extract at different concentrations was added to 0.5 mL of a DPPH-methanol solution. The mixtures were shaken vigorously and left standing at the laboratory temperature for 30 minutes in the dark. The absorbance of the resulting solutions was measured at 517 nm. The antiradical activity was expressed as IC_{50}. The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\ scavenging\ effect\ (\%) = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where:

A_0: the absorbance of the control at 30 minutes

A_1: is the absorbance of the sample at 30 minutes. BHT was used as standard [20].

Reducing Power

The reducing power was determined according to the method of Oyaizu (1986) [21]. The extract (0.5 –10 mg/mL) in methanol (2.5 mL) was mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 10 mg/mL potassium ferricyanide. The mixtures were incubated at 50°C for 20 minutes; after cooling, 2.5 mL of 100 mg/mL trichloroacetic acid were added and the mixtures were centrifuged at 200g for 10 minutes. The upper layer (5 mL) was mixed with 5mL of deionized water and 1 mL of 1 mg/mL ferric chloride, and the absorbance was measured at 700 nm against a blank. A higher absorbance indicates a higher reducing power. EC_{50} value is the effective concentration at which the absorbance was 0.5 for reducing power. Ascorbic acid was used as standard.

The Human Red Blood Cell (HRBC) Membrane Stabilization Method

To prepare the HRBC suspension, fresh completely human blood (10 mL) was collected and transferred into the centrifuge tubes. These lasts were centrifuged at 3000 rpm for 15 minutes thrice and washed with equal volume of normal saline each time. The volume of blood was measured and reconstituted as 10 % v/v suspension with normal saline.

The principle involved here, was stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The mixtures contain 1mL phosphate buffer (pH 7.4, 0.15 M), 2 mL hypo saline (0.36 %), 0.5 mL HRBC suspension (10 % v/v) and 0.5 mL of plant extract or standard drug (diclofenac sodium) at various concentrations. The control was distilled water instead of hypo saline to produce 100 % haemolysis.

The mixtures were incubated at 37°C for 30 minutes and centrifuged at 2500 rpm for 5 minutes. The absorbance of haemoglobin content in the suspensions were estimated at 560 nm. The percentage of haemolysis of HRBC membrane can be calculated as follows:

$$Haemolysis\ (\%) = \frac{Optical\ density\ of\ Test\ sample}{Optical\ density\ of\ Control} \times 100$$

However, the percentage of HRBC membrane stabilization can be calculated as follows [22]:

$$Protection\ (\%) = 100 - \left[\frac{Optical\ density\ of\ Test\ sample}{Optical\ density\ of\ Control}\right] \times 100$$

Statistical Analysis

Results were expressed as mean ± standard deviation. Data were statistically analysed using one-way ANOVA, Newman-Keuls Multiple Comparison and to determine whether there were any significant with the criterion of P values < 0.05 between methanol extract and

standards, using Graphpad prism 5 Demo Software.

RESULTS AND DISCUSSION

The used extraction method gave a yield of 35% with 33.41±1.39mg EAG/GE of polyphenols and including 5.10±0.48 mg EQ/GE of flavonoids. These molecules play an important role to determine the power of methanol extract in biological activity.

An antioxidant can be broadly defined, as any substance that delays or inhibits oxidative damage to a target molecule [23]. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [24]. The extract show highest activity (IC_{50}=5.18±1.51 µg/mL; 94.56±0.28 % of radicals inhibition) against BHT (IC_{50} = 6.29±1.12 µg/mL; 94.64±0.31 % of radicals inhibition) Fig. 1:

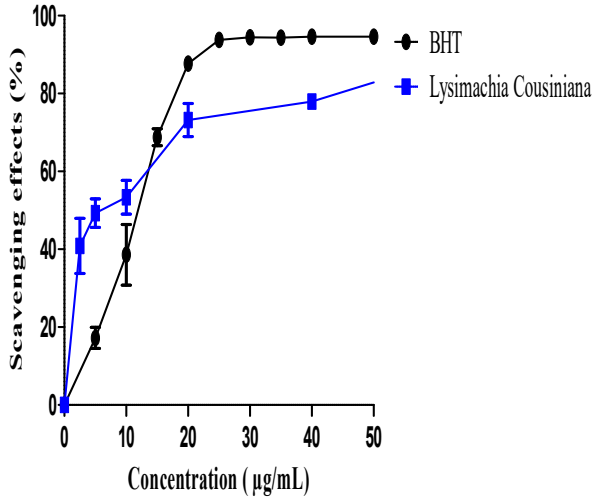

Figure 1: DPPH test of methanol extract of *Lysimachia Cousiniana* Coss. et DR

The free radical scavenging activity of antioxidant components is very much associated with their phenolic and flavonoid component [25].

The results of this research showed that the reducing power of crude extract of ***Lysimachia Cousiniana*** was weaker than standard with EC_{50} of 47.54±0.037 µg/mL*** against the BHA 9.09±0.21 µg/mL (Fig. 2).

The reductive ability was measured in terms of Fe^{3+} to Fe^{2+} transformation in the presence of different concentrations of the extract. The presence of reductants in extract causes the reduction of potassium hexacyanoferrate K3 FeCN6 to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the absorbance, where it is directly proportional to the reducing power of test substance. The reducing ability of a compound generally depends on the presence of reluctant [26]. In the case of ***Lysimachia Cousiniana ,*** reductive ability was moderate to strong but still lower than BHA.

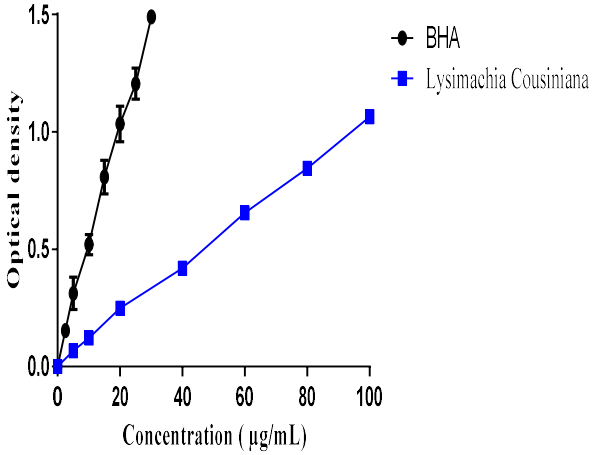

Figure 2: Reducing power activity of methanol extract of *Lysimachia Cousiniana* Coss. et DR.

The results of the anti-inflammatory activity of methanol extract of *Lysimachia Cousiniana* are shown in Fig. 3.

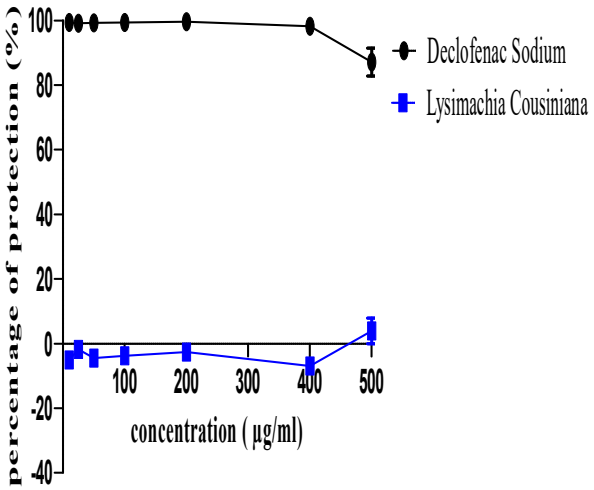

Figure 3: The percentage inhibition of hypotonicity induced haemolysis of HRBCs (%) of methanol extract of *Lysimachia Cousiniana* Coss. et DR.

The results show an haemolytic effect of extract reach the level of – 6.96±2.04 % at concentration of 400 µg/mL of against 98.17±0.49 % of haemolysis inhibition of the standard at the same

concentration. However, the maximum of protection was reached at a concentration of 200 µg/mL by 99.54±0.28 %.

It has clearly admitted that the crude extract of *Lysimachia Cousiniana* is not an anti-inflammatory agent. The level of protection of erythrocytes membranes is null or even show more release of haemoglobin in solutions expressed by negative percentages. These results can be explained by the presences of molecules, which causes the destruction of erythrocytes membranes.

CONCLUSION

The study demonstrated a powerful antioxidant activity of crude extract of *Lysimachia Cousiniana*, especially in free radical scavenging against DPPH and a moderate to strong reducing power. However, the effect of crude extract in anti-inflammatory activity was very weak or caused the destruction of erythrocytes membranes. More molecular studies are needed to explain the above phenomena and reflect others benefits sides of this endemic species.

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