

Original Article

ANTIOXIDANT AND THE IMMUNOMODULATORY ACTIVITIES EXHIBITED BY THREE PLANTS FROM LAMIACEAE FAMILY

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ABSTRACT

Objective: The aim of this work was screening of the antioxidant activity and immunomodulatory effects of the crude extracts from the aerial parts of three Algerian endemic species from *Lamiaceae*.

Methods: DPPH radical scavenging assay was used to find the antioxidant activity of *Stachys circinata*, *Salvia verbenaca* and *Thymus guyonii* and the immunostimulant potential of these plants extracts on the phagocytic activity which was measured by the carbon clearance rate test.

Results: Our results obtained in this study shown that the IC50 of *Thymus guyonii*, *Salvia verbenaca* and *Stachys circinata* were 15,90;47,50 and 54,92 respectively. The phagocytic index was increased significantly in animals injected with *Stachys circinata* and *Salvia verbenaca* at doses of 200 mg/kg $P \leq 0,05$ but not significantly with *Thymus guyonii* when compared to the control group. The clearance rate of carbon was faster but not significantly $P \geq 0,05$ and the corrected phagocytic index α was increased at the group GIII with *Salvia verbenaca* but not significantly when compared with other groups $P \geq 0,05$.

Conclusion: The *Stachys circinata* and *Salvia verbenaca* extracts proved an immune-stimulatory effect on the reticuloendothelial system and *Thymus guyonii* extract revealed anti-oxidant activity.

Keywords: *Thymus guyonii*, *Salvia verbenaca*, *Stachys circinata*, Immunostimulatory activity, DPPH radical scavenging assay.

INTRODUCTION

The immune system comprises two functional divisions; the innate immune system consists of cellular components, soluble factors, physical barriers and the reticulo-endothelial system (RES) [1]. The adaptive immune system produces a specific reaction and immunologic memory to each pathogen and comprises cellular components and soluble factors [1].

The reticulo-endothelial system consists of the phagocytic cells such as monocytes and macrophages that kill the invading organism by Phagocytosis [2]. Immunomodulation is the regulation and modulation of immunity either by enhancing or by reducing the immune response. Modulation of immune response may involve induction, expression or amplification of immune response [3].

There are many plants having immuno stimulatory activity and some drugs are believed to enhance the natural resistance of the body to infection [3]. In recent times, there is a great focus on medicinal plants belonging to *Lamiaceae* family, which is a very large family represented by about 236 genera and 7172 species in the world [4] and well known with their therapeutic applications against many disorders such as common cold, throat infections, acaricidal, psoriasis, seborrheic eczema, hemorrhage, menstrual disorders, miscarriage, ulcer, spasm and stomach problems [5-7] related to their richness with polyphenols compounds from several classes [8].

Thymus guyonii de Noé, locally known for its antispasmodic, activities and antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* [9]. *Salvia verbenaca* locally named Essaffaya and particularly used in treating the healing of wounds [10], rich with flavonoids, tannins which can be used as a potential source of useful drugs [11] and *Stachys circinata* till now there is no photochemical studies about this species.

In numerous *in vitro* studies the antioxidant activity of *Lamiaceae* species has been already revealed [12-14]. It's known that natural's antioxidants from plants such as polyphenols play an important role

in the protection of cells from oxidative damage and consequently induce anticancer activities including pro-apoptotic, DNA damaging anti-angiogenic, and immunostimulatory effects [15]. These proprieties make this family a good source of dietary compared to much other foods. However, there is a very few studies concerning the immunomodulatory activity of herbs belonging to this family [8]. The objective of the present study was to evaluate the antioxidant and immunostimulatory effects of three crude extracts obtained from *Thymus guyonii*, *Stachys circinata* and *Salvia verbenaca* belonging to *Lamiaceae* family.

MATERIALS AND METHODS

Plant material

Collection

The aerial parts of *Thymus guyonii*, *Salvia verbenaca* and *Stachys circinata* were collected from zalfana (Ghardaia), Batna and Djebel El-Ouahch (Constantine, Algeria) respectively.

Preparation of the extract

Air-dried and powdered aerial parts (1 kg) of *Thymus guyonii*, *Salvia verbenaca* and *Stachys circinata*, were extracted with 70 % MeOH. The residue was suspended in water and extracted successively with petroleum ether, dichloromethane, Ethylacetate and n-BuOH. We used for both activities the n-BuOH extract.

Determination of antioxidant activity

The antioxidant activity was measured with bleaching of purple colored methanol solution of DPPH by using [16]. This spectrophotometric assay uses stable radical 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) as a reagent [16, 17].

30 μ l of various concentrations (10, 6, 5, 3, 2, 1 mg/ml) of each species extract in methanol was added to 3 ml of a 0.004 % methanol solution of DPPH. After 20 min of incubation in the dark

room, the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I %) was calculated in following way:

$$I \% = (\text{Ablank} - \text{Asample}) / \text{Ablank} \times 100$$

Where A blank is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound. Extract concentration providing 50 % inhibition (IC50) was calculated from the linear regression algorithm of the graph plotted inhibition percentage against extract concentration. For the calculation of these values, Microsoft Excel software was used.

Determination of immunostimulant activity

Animals

Adult male *Mus Musculus* mice (2.5-3 month old) from central pharmacy Algeria, weighing (22-36g) were used for determination of the phagocytic activity. The animals were kept under standard laboratory conditions of humidity, temperature (25±1 °C with 12:12 light: dark cycles), and allowed free access to food and water. The animal studies were conducted after obtaining clearance from Institutional Animal Ethics Committee and the experiments were conducted in strict compliance according to ethical principles and provided by the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA).

Phagocytic index

The phagocytic index was determined by the method of [18]. For each species extract we used the same concentrations, Animals were divided into four groups, consisting of six mice in GI, GII, GIII and GIV. Group I (control) was given 0, 9 % NaCl (0, 5 ml/mouse I. p.), Groups II-III-and IV administered by I. P injection with different concentrations of each plant extracts (50, 150 and 200 mg/kg/) respectively.

After 48h of I. P injection, mice were administered with carbon ink suspension at a dose of (0.1 ml/10g through the tail vein; the mixture consisted of black carbon ink 3 ml, saline 4 ml and 3 % gelatin solution 4 ml. Blood samples were taken from the retro orbital vein by using glass capillaries, at 5 and 15 min. Blood sample drops (14) were mixed with 0.1 % sodium carbonate solution (4 ml) for the lysis of erythrocytes and the absorbance measured at 675 nm using a spectrophotometer. The animals were sacrificed and the liver and spleen dissected and weighed immediately in the wet state.

The phagocytic activity is expressed by the phagocytic index K which measures all the reticuloendothelial system function in the contact with the circulating blood and by corrected phagocytic index α which expresses this activity by unit of active weight organs: liver and spleen.

The clearance rate is expressed as the half-life period of the carbon in the blood ($t_{1/2}$, min). These are calculated by means of the following equations [18, 19].

$$K = \frac{\ln OD1 - \ln OD2}{t2 - t1}$$

$$t_{1/2} = 0,693 / K$$

$$\alpha = \sqrt[3]{K} \times \frac{\text{Body weight of animal}}{\text{Liver wt} + \text{spleen wt}}$$

Where OD1 and OD2 are the optical densities at times t1 and t2 respectively.

Statistical analysis

Results were analyzed for differences between the groups across dietary treatments by one-way ANOVA test and Tukey's multiple comparison tests (SPSS version 9), P-values* < 0.05 was considered as statistically significant.

RESULTS

The scavenging activity against DPPH radical

The present data showed that the three extracts presented a good antioxidant activity which was increased by increasing the concentration of the samples extracts. The highest antioxidant

activity of *Stachys circinata* was 90 % at concentration 0,1 mg/ml (fig. 3) and 95 % at the same concentration for *Salvia verbenaca* (fig. 2) however the highest antioxidant activity of *Thymus guyonii* at concentration 0,03 mg/ml was 0, 99 % (fig. 1).

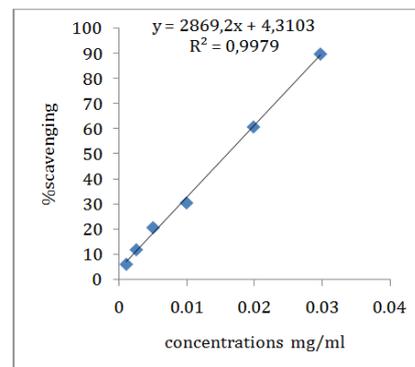


Fig. 1: It shows the scavenging activity of *Thymus guyonii*

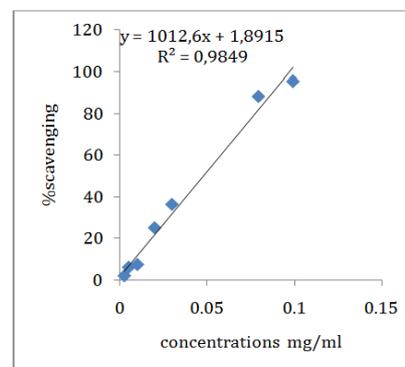


Fig. 2: It shows the scavenging activity of *Salvia verbenaca*

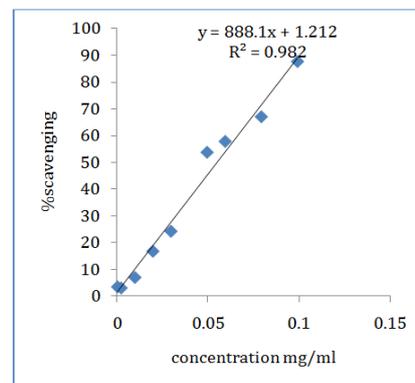


Fig. 3: It shows the scavenging activity of *Stachys circinata*

The measurement results of the half-inhibition concentrations (IC50), which is inversely proportional to the antioxidant potential value, were calculated from the linear regression of the % antioxidant activity versus extracts concentrations.

The Results are shown in table 1.

Table 1: It shows IC50 of the tested extracts

Tested extracts	IC50 (µg/ml)	R ²
<i>Thymus guyonii</i>	15,90	0,997
<i>Salvia verbenaca</i>	47,50	0,982
<i>Stachys circinata</i>	54,92	0,984

The results shown in table 1 indicated that the *Thymus guyonii* extract has the lowest IC50 and exhibited the highest DPPH free radical scavenging ability, three fold more potent compared to *Salvia verbenaca* and *Stachyscircinata* extracts.

Immuno stimulant activity

The present data showed that there is a difference in the means for the phagocytic index (K) between groups (GI, GII, GIII, and GIV) and the phagocytic index for groups GIV of *Stachys circinata* (0,105±0,018) and *Salvia verbenaca* (0,095±0,012) have significant difference when compared to the control GI P≤0,05.

However, the *Stachys circinata* extract showed a higher effect than *Salvia verbenaca*, the remaining extract of *Thymus guyonii* showed no significant increase at all doses.

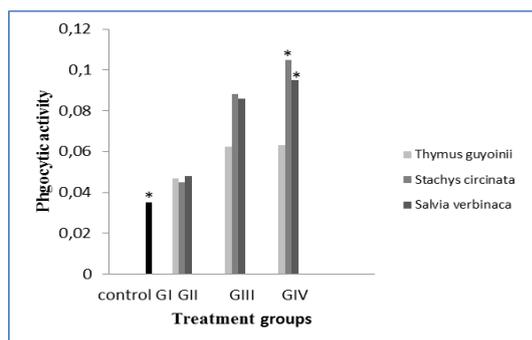


Fig. 4: It shows the phagocytic activity of mice treated with three different extras

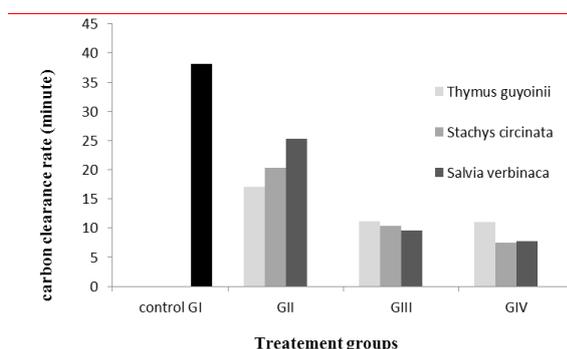


Fig. 5: It shows the carbon clearance rate of mice treated with three different extracts

As shown in the fig. 5, the half time of colloidal carbon were decreased between groups but not significantly and with *Stachyscircinata* was faster when compared with the groups GI, GII, GIII of the extracts *Thymus guyoini* and *Salvia verbenaca*.

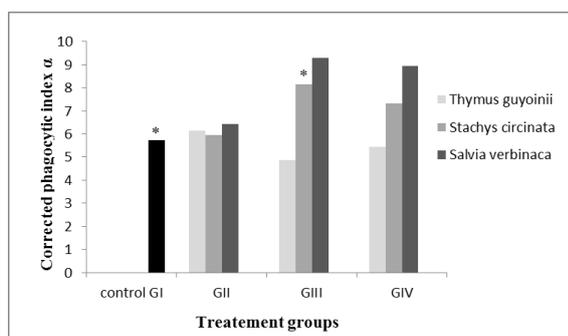


Fig. 6: It shows the corrected phagocytic index alpha of mice treated with three different extracts

The results of this study showed that there is a significant increase of the corrected phagocytic index α at the group III with *Stachys circinata* when it is compared with the control GI $p \leq 0,05$ (fig. 6). And was higher at the GIII with *salvia verbenaca* (0,095±1,71) but not significantly when it is compared with GI, GII and GIV of the two extracts (*Thymus guyounii* and *Stachys circinata*) $p \geq 0,05$.

DISCUSSION

According to these results, we are suggesting that *Stachys circinata* exhibit a better effect in enhancing the immunostimulant activity of the reticuloendothelial system (R. E. S) the presence of this activity was already proven in other species belonging to the same genus such as *Stachys mialhesi* [20]. Also this result agrees with those of ARIBI *et al.*[21]and KEHILI *et al.* [22] who reported that the administration of *Argania spinosa* seeds and *Phoenix dactylifera* respectively in the mouse increased the phagocytic index at different concentrations. In other study of ASSIA *et al.* [23] reported that *Stachys ocymastrum* extract enhanced the phagocytic activity at low concentration (50 mg/kg) by stimulating the reticulum endothelial and then at high concentrations of 100 and 500 mg/kg the extract decreased the phagocytic activity, in a dose dependant manner by inhibiting the reticulum endothelial.

The reticuloendothelial system (R. E. S) is considered as the first line of defense and functionally recognized by its ability to scavenge debris or other foreign matter [24]. The rate of removal of carbon particles, by the sessile intravascular phagocytes in the liver and spleen which are considered to be the main organs in where endothelial cells and macrophages eliminate undesirable substances from circulation is measured by reticuloendothelial phagocytic activity [25].

In this study we noticed that *Thymus gyuonii* which has the highest antioxidant activity showed no significance for immunostimulant activity however *Stachys circinata* which has the lowest antioxidant activity showed a higher significance for immunostimulant activity, the remaining extract of *Salvia verbenaca* showed a moderate significance for both activities. From these results, we are suggesting that this difference is due to the difference in composition of secondary compounds between the three extracts.

CONCLUSION

The overall observations of the present work suggested that *Stachys circinata* held appreciable immunostimulant property which acted by activating the function of the reticuloendothelial system. However, *Thymus guyonii* showed an antioxidant activity three fold higher than the other extracts. The remaining extract of *Salvia verbenaca* showed a moderate significance for both activities.

The current study has provided that the plants studied here can be seen as a potential source of useful drugs. Further studies are going on these plants in order to fig. out other biological activities.

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CONFLICT OF INTERESTS

Declared None

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