ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANT ACTIVITIES OF *Searsia tripartita* (UCRIA) MOFFET GROWING IN AHAGGAR (ALGERIA)

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Abstract. This study aims at assaying the antioxidant activities of *Searsia tripartita* (Ucria) Moffet growing in Ahaggar (Algeria) as a continuation of our previous work on the ecophysiology characteristic of this shrub. The enzymatic antioxidant activities of catalase (CAT) and Ascorbate peroxydase (APX) were evaluated. The total phenolic contents of this plant were measured by the Folin–Ciocalteu method and the radical scavenging activity was determined by using DPPH assay (2,2 Diphenyl 1-Picryl Hydrazyl). The shrub was found to have a high antioxidant capacities and could be potential rich sources of natural antioxidants. Then, the antiradical activity (DPPH scavenging) IC50 was 153.69±0.428 µg antioxidant.mL while phenolic compound values range from 23.923 to 52.926 mg GAE·g^{-1} DW. Moreover, high values of antioxidant enzyme activities were recorded respectively for catalase and ascorbate peroxidase: 136.59 ± 3.412 and 135.03 ± 5.712 µmol·min^{-1}·mg^{-1} proteins. Our experiments indicated that this shrub is well adapted to the arid climate of the Ahaggar region by facing the oxidative stress through a high production of antioxidants. In conclusion, the species *Searsia tripartita* seems to adopt a drought-tolerance type strategy towards aridity oxidative stress.

Key words: *Searsia tripartita*; Ahaggar; ardity; phenolic compounds; antioxidant activities.

INTRODUCTION

Searsia tripartita (Ucria) Moffet (called African sumac and Tahounek in tamahaq) is a multi-purpose species of pastoral, ecological and medicinal interest. *Rhus tripartita* (Ucria) Grande is a synonymous name of this species. In Algeria, it considered as an autochthon xerophytic species of semi-arid and arid regions, particularly in the Sahara especially in the mountains of the Ahaggar that it is found grouping with *Periploca laevigata* and *Myrtus nivellei* [5]. On the one hand, according to Jenks and Hasegawa's classification of desert and semi-arid plants, *S. tripartita* a species adapted to the desert that is not limited by water availability and has provisions to reduce water loss [6]. The branches of this species are tightly packed, with small, sometimes curled and sclerotized leaves; not only reduces transpiration, but also protects the plant from excessive sunlight. The strategy therefore adopted by this shrub is drought tolerance with a high concentration of osmolytes solutes and an ability to maintain its membrane integrity [6].

Aridity is a negative climatic factor, which causes long-term stress, manifested by simultaneous drought accompanied by an increasing annual mean temperature [37]. Then, it is well documented that drought has at least oxidative damage in plants. This phenomenon is caused by reactive oxygen derivatives (RODs), which can react with a wide variety of biomolecules causing irreversible damage and leading to necrosis and cell death [24, 26]. The reduced form of oxygen is the result of the addition of one, two or three electrons to form a superoxide radical (O^{-2}), hydrogen peroxide (H_2O_2) or a hydroxyl radical (OH^-). These forms are extremely reactive and can oxidize biological molecules [3]. To deal with these compounds, the plant has developed an enzymatic and non-enzymatic defense system: the antioxidants [7]. Several enzymes are involved in these reactions. It is mainly the enzymes: superoxide dismutases (SOD), ascorbate peroxidases (APX), glutathione reductase (GR) and catalases (CAT) that participate in the ascorbate - glutathion cycle [27].

Phenols are a major group of antioxidant phytochemicals, they have profound importance due to their biological and free radical scavenging activities. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers [14]. They are crucial for plants growth and reproduction, and produced as a response to environmental factors (light, chilling, pollution etc) and to defend injured plants [35]. They could therefore be a natural source of antioxidants since they are ubiquitous in plants [5] and are largely influenced by many factors, such as biotic and abiotic stress [33].

Searsia tripartita (Ucria) Moffet is a vital member of the Anacardiaceae family, particularly in arid climates and in the balance and maintenance of many arid and desert ecosystems, thanks to its tolerance to drought and its highly developed root system [5]. However, the success of the growth and adaptation phases requires a good knowledge of its biochemical and physiological characteristics. Thus, catalase (CAT) and ascorbate peroxidase (APX) activities were evaluated in the leaves of *Searsia tripartita* growing in Ahaggar (Algerian Sahara) and the results were interpreted in relation to aridity in a continuation of our previous work on the ecophysiology characteristic of this shrub [6]. Other potential sources of antioxidant compounds as total phenolic contents have been

searched added to radical scavenging activity investigation.

MATERIALS AND METHODS

The leaves of six wild ucria shrubs are collected during december 2018, from Ilamane region in Tamanrasset (Fig. 1), an arid area from Algerian Sahara. It is located in the National Culturel Parc of Ahaggar in the south of Algeria (22°47'6.00" N, 5°31'22.01" E).

Enzymatic activities are measured by spectrophotometry. To slow down other biochemical processes after extraction, all operations are carried out at 4° C.

Total proteins are first extracted since enzymes are proteins. For this purpose, 100 mg of leaf is cold grinded in 1 mL of extraction buffer (Tris-HCl pH 8.1). Then, a centrifugation is carried out at 12000 rpm for 20 minutes at 4° C. The supernatant containing the total proteins is recovered. For samples intended for the analysis of total soluble ascorbate peroxidase, 5 mM of ascorbate is added to the protein extraction medium because the enzyme is labile in the absence of its electron donor.

The catalase (CAT) activity is determined by following the decomposition of H_2O_2 at 240 nm ($\varepsilon = 36\ M^{-1}\ .\ cm^{-1}$) as described previously [2]. The reaction medium consists of potassium phosphate buffer (KH_2PO_4/K_2HPO_4) at 100 mM pH 7 (2 mL) and protein extract containing the enzyme (200 µL). To start the reaction, 30 mM of H_2O_2 Is added. The activity is expressed in nmoles of H_2O_2 degraded per minute and per mg protein. The conversion of the initial velocity (change in absorbance at 240 nm) to the specific activity of catalase is expressed as follows:

Activity (µmol $H_2O_2 \cdot min^{-1} \cdot mg^{-1}$ .prot) =ΔOD /min. reaction flight / (36·mg protein) [36 = ε]

The principle of ascorbate peroxidase activity (APX) measurement is based on the properties of this enzyme to oxidize ascorbate in the presence of H_2O_2.

Ascorbate + H_2O_2 → Monodehydroascorbate + $2H_2O$

This activity is determined by oxidation of ascorbate ($\varepsilon = 2.88\ M^{-1} \cdot cm^{-1}$) to deshydroascorbate at 290 nm using the method described before [23]. The reaction medium (1 mL) consists of 50 mM phosphate buffer pH 7.0, 0.5 mM ascorbate, 0.1 mM H_2O_2, 0.1 mM EDTA and 30 µg protein. The reaction is initiated by the addition of H_2O_2 and is followed for 30 sec. The measurement of non-enzymatic oxidation of ascorbate (= reaction medium without extract) is used as a correction for the measurements. The APX activity is determined according to the following equation:

Activity (µmoles. $min^{-1} mg^{-1}$ prot) = Δ OD min^{-1}·1000 / (2.88·mg protein) [2.88 = ε]

Total phenolic content was determined as described by Singleton and Rossi [31] slightly modified. 0.5 mL of Folin–Ciocalteu reagent and 0.5 mL of Na_2CO_3 (7.5 %) was added to 0.200 mL of methanolic plant extract. The absorbance was measured at 760 nm after an incubation for 90 min in the dark. The result was expressed as mg gallic acid equivalents per g dry residue (mg GAE/g DW).

The radical scavenging activity was determined by using DPPH (2,2 Diphenyl 1-Picryl Hydrazyl) assay according to Popovici et al. [25]. The 63.4 µM DPPH solution (25 mg in 100 mL 90% methanol) is prepared 1-2 hours in advance. Volumes of 0.1 mL of the plant extracts were thoroughly mixed with the DPPH solution. The measurement of antioxidant efficiency was exprimed by the decrease of the blue coloration at 515 nm.

DPPH scavenging activity is expressed by the concentration of the sample required to scavenge 50 % of free radicals (IC50 value) present in the test solution.

The data were subjected to statistical analysis using the Microsoft Excel 2010 program. All values are the mean ± SE (standard error) of three replicates of a single sample. The obtained data have been submitted to ANOVA using the Statistical Analysis System (XLSTAT) version 2016. 02. When the F value of this analysis is significant at the 5% ($P<0.05$) threshold, the averages are compared using the LCD test.

It should be remembered that there is no control because the study was carried out on a spontaneous species *in situ* and not under controlled conditions.

Figure. 1. Geographical location of the shrub *Searsia tripartita* (Ucria) Moffet in the rocky mountains of ILAMANE in the Ahaggar National and Cultural Park (Algeria, Google Maps, 2020)

RESULTS

To cope with the harmful effects of dehydration such as increased production of ROS, the plant uses enzymatic and non-enzymatic antioxidant systems. We studied this latter categories on *S. tripartita* to explain its adaptation to arid climate of Ahaggar.

According to our results, the values of catalase vary between 112.84 and 135.037 µmol H_2O_2 degraded·mg^{-1}·Prot·mn^{-1} in shrubs 2 and 1 respectively, while those of ascorbate peroxidase vary between 121.153 and 136.593 µmol AA oxidized·mg^{-1}·Prot·mn^{-1} respectively in shrubs 3 and 1 (Fig. 2). The analysis of variance (Table 1 and 2) also indicated a non-significant difference for ascorbate peroxidase and catalase activities (P>0.05).

The contents of total phenolic compounds, in our case, vary between 11.49 and 52.93 mg GAEg^{-1} DW respectively in shrubs 1 and 5 (Table 3). The analysis of variance (Table 4) also indicated a significant difference in the phenolic compounds (P<0.05).

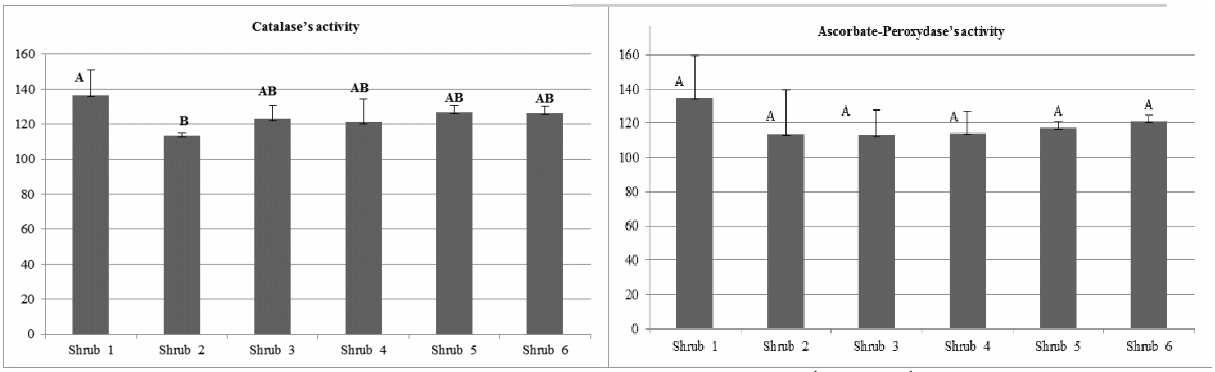

Figure. 2. Catalase (Cat) and Ascorbate peroxidase (APX) activities (µmol H_2O_2 degraded · mg^{-1} · Prot · mn^{-1}) of *Searsia tripartita* (Ucria) Moffet leaves from the Ilamane region (Ahaggar, Algeria)

Table 1. Variance's analysis of catalase's activity

Source	DDL	Sum of squares	Average of squares	F	Pr > F
Model	5	862.639	172.528	2.165	0.127
Error	12	956.087	79.674	-	-
Total corrected	17	1818.726	-	-	-

Table 2. Variance's analysis of ascorbate-peroxydase's activity

Source	DDL	Sum of squares	Average of squares	F	Pr > F
Model	5	1059.903	211.981	0.768	0.590
Error	12	3312.847	276.071	-	-
Total corrected	17	4372.750	-	-	-

Table 3. Total phenolic compound contents of *Searsia tripartita* growing in an arid area of Algeria (Ahaggar)

Samples	Total phenolic compounds (mg GAE·g^{-1} DR)
Shrub 1	11.487±0.675d
Shrub 2	37.550±0.547b
Shrub 3	52.373±1.853a
Shrub 4	27.013±0.439c
Shrub 5	52.926±0.805a
Shrub 6	23.923±0.703c

Table 4. Variance's analysis of total phenolic compounds

Source	DDL	Sum of squares	Average of squares	F	Pr > F
Model	5	4096.000	819.200	38.767	< 0.0001
Error	12	253.576	21.131	-	-
Total corrected	17	4349.575	-	-	-

Table 5. Trapping activity of the DPPH radical of *Searsia tripartita* growing in an arid zone of Algeria (Ahaggar)

Samples	IC 50% (µg·mL^{-1})
Shrub 1	153.69±0.428b
Shrub 2	123.75±1.737b
Shrub 3	153.77±3.206b
Shrub 4	153.797±2.205b
Shrub 5	137.843±4.462a
Shrub 6	141.507±2.964b

Table 6. Variance's analysis of DPPH trapping activity (%RSA)

Source	DDL	Sum of squares	Average of squares	F	Pr > F
Model	5	504.770	100.954	7.626	0.002
Error	12	158.854	13.238	-	-
Total corrected	17	663.624	-	-	-

Table 7. Variance's analysis of IC50

Source	DDL	Sum of squares	Average of squares	F	Pr > F
Model	5	5235.089	1047.018	0.942	0.489
Error	12	13342.652	1111.888	-	-
Total corrected	17	18577.740	-	-	-

Table 5 shows the concentration of each plant extract required to inhibit 50% of DPPH (IC50 value).The values of IC50 measured in *S. tripartita* vary from 123.75 and 153.8 $\mu g \cdot mL^{-1}$ knowing that this concentration is inversely proportional to the anti-free radical activity. The analysis of variance (Table 6) also indicated an insignificant difference in DPPH radical trapping activity ($P>0.05$) which is inversely highly significant for IC50 values (Table 7).

DISCUSSION

In times of stress, the plant faces oxidative damage, and to cope with this, it produces antioxidant enzymes and other metabolites to ensure its survival [12, 20]. Aridity can induce oxidative stress, which would involve activation of key enzymes in the antioxidant defense system of *S.tripartita*. In this study, the effect of oxidative aridity stress on *S.tripartita* was evaluated by quantifying the activities of catalase (CAT) and total ascorbate peroxidase (APX), two of the most important enzymes involved in the antioxidant defense system.

In view of the high values recorded for the species studied, this appears to be the case. Indeed, the actrivity of CAT is greater than that observed under moderate water stress in tobacco, *Nicotiana sylvestris* [9] or *Amaranthus tricolor* [29] for which a value of about 95 µmol $H_2O_2 \cdot mg^{-1} \cdot prot \cdot mn^{-1}$ and 65 µmol $H_2O_2 \cdot mg^{-1} \cdot prot \cdot mn^{-1}$ respectively is recorded. Also, it was found that several drought stress, induced a double catalase's activity than in control in *Amaranthus tricolor* [26]. The same is true for APX, whose activity is much higher than that of *Gypsophila aucheri* (halophyte), whose value is only 1.5 $\mu mol.mg^{-1}.prot.mn^{-1}$ under water stress [11]. Catalase removes H_2O_2 [30] by converting it into water and molecular oxygen [13]. The APX appears to be preferred to catalase in the removal of H_2O_2 since it has a higher affinity for ROS [18], which may explain its slightly higher content compared to the catalase recorded in our study. These observations are consistent with the mitigation of oxidative stress damage through the activation of antioxidant enzymes. Indeed, the induction of antioxidant enzyme activity is a general strategy adopted by the plant to overcome oxidative stress imposed by the environment [20, 36].

Therefore, under unfavorable environmental conditions, the oxidative stress is not considered as symptom of cellular dysfunction but it can represent an important signal for a plant to induce acclimation mechanisms [21]. However, in many cases, the production of ROS is genetically programmed, induced during the course of development and by environmental fluctuations [12]. The induction of antioxidant enzymatic activity is a general strategy adopted by the plant to overcome oxidative stress imposed by the environment [20, 36]. H_2O_2 is eliminated by catalases [30] and the peroxidases [13] by converting it to water and molecular oxygen in the case of catalase. Studies of various crop species have shown that stress-tolerant plants generally have good oxidation control systems [28]. By their protective role, anti-oxidants are therefore essential to the survival and health of the plant [16].

Phenolic compounds, as secondary metabolites, may be considered contributors to the antioxidant capacity of plants [33]. The contents of total phenolic compounds, in our case, vary between 11.49 and 52.93 mg $GAE \cdot g^{-1}$ DW, values lower than those observed by Tlili et al. [32] in the same species harvested in southern Tunisia (91.58 mg $GAE \cdot g^{-1}$ DW). Itidel et al. [17] found an average content of 71.16 mg $GAE \cdot g^{-1}$ DW in the same species harvested under the same arid conditions. It is known that variations in the content of phenolic compounds can be observed in the same species in relation to environmental conditions, plant phenology or physiology and abiotic stresses [38].

Djeridane et al. [10] found that phenolic compounds were a major contributor to the antioxidant activity of 11 Algerian medicinal plants, ranging from 3.13 to 32.32 $mg \cdot g^{-1}$ DW expressed in gallic acid equivalents (GAE), which is within the range of results found in *S. tripartita*, which is also considered as a medicinal plant. Furthermore, the total phenolic compouds of *S.tripartita's* leaves was comparable to the same species growing in the north-eastern Algerian Sahara [15] and that of *Acer campestre* of northeastern Algeria [4].

However, it was recorded a significant difference in the composition of metabolites between different individuals of the same species. Indeed, the samples were taken from shrubs positioned differently, for example, one is oriented towards the sunlight and the other is hidden from the sunlight while others are grouped with other plants, ...etc. These stationary factors may explain the difference between the results found in shrubs within the same species.

Compared with the IC50 of 15.74 $\mu g \cdot mL^{-1}$ measured in the same species harvested in southern

Tunisia [17], this activity is up to 10 times lower in our species. On other hand, the antiradical activity of *Searsia'*s leaves of Ahaggar region was comparable to that of Saudi Arabia [1]. Furthermore, our results revealed a higher antiradical activity than the same species growing in South Sinai, Egypt [22]. It remains low compared to other species such as *Cassia fistula* (25.9 to 98.9 $\mu g \cdot mL^{-1}$) [19], *Caesalpinia pyramidalis* (38.93 $\mu g \cdot mL^{-1}$) or *Sapium glandulosum* (58.55 $\mu g \cdot mL^{-1}$) [8].

The present study indicated that *S. tripartita* is well adapted to the arid climate of the Ahaggar region which can be related to the high enzymatic antioxidant activity (Cat and APX), as well as the high value of the total non-enzymatic antioxidant capacity which is represented by the phenolic compounds but also by glutathione, tocopherols and ascorbate. In conclusion, the species *Searsia tripartita* seems to adopt a drought-tolerance type strategy towards aridity according to Turner's [34] categorization.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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