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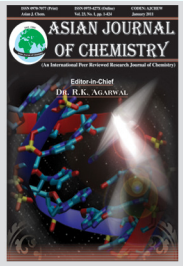

Antioxidant Activity of Four Algerian Plants: *Cistus ladaniferus*, *Crataegus oxyacantha*, *Lavandula stoechas* and *Smyrnium olusatrum*

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The crude extracts of four Algerian plants were screened for their antioxidant properties using free radical method and ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using 2,2'-diphenyl picrylhydrazyl. Ascorbic acid and *Crataegus oxyacantha* extract showed strong antioxidant activity with the DPPH method according to their EC_{50}. This activity was classified in order: ascorbic acid (1.04 μg mL^{-1}) > *Crataegus oxyacantha* extracts (2.75 μg mL^{-1}) > *Lavandula stoechas* extracts (5.29 μg mL^{-1}) > *Smyrnium olusatrum* extracts (9.68 μg mL^{-1}) > *Cistus ladaniferus* extracts (11.42 μg mL^{-1}).

Key Words: Medicinal plants, Free radicals, Antioxidant activity, DPPH.

INTRODUCTION

Free-radicals are generated continuously in the body due to metabolism and disease[1]. A free-radical is an atom, molecule or compound that is highly unstable. One chemical element frequently involved in free-radical formation is oxygen, it has been esteemed that only *ca.* 2 to 3 % of the O_2 consumed by the respiratory chain is converted to ROS[2,3].

In vivo, some of these ROS, including superoxide radicals, hydroxyl radicals and hydrogen peroxide play positive roles in cell physiology. However, they may also cause great damage to cell membranes and DNA, oxidation that causes membrane lipid peroxidation, decreased membrane fluidity and DNA mutations leading to cancer degenerative[4-7]. Free-radical mediated oxidation stress implicated in many age-related neurodegenerative disorders with concomitant neuronal dysfunction and death[8,9]. Oxidation is also responsible for deterioration of various organic materials ranging from biologically important materials (*e.g.*, lipids, foods and oils) to the industrially important ones (*e.g.*, rubber and lubricants)[10].

In order to protect themselves against free-radicals, mammalian cells are endowed with endogenous and exogenous defence mechanisms. Endogenous defences are superoxide dismutase, catalase, glutathione peroxidase/reductase. In addition to antioxidant enzymes, non enzymatic molecules, including thioredoxin, thiols and disulfide bonding play important roles in antioxidant defence systems. Exogenous defence are obtained from food, vitamins C and E, β-carotene, ascorbic acid and such micronutrient elements as zinc and selenium, but these defence systems are not sufficient in critical situations where the production of free-radicals significantly increases[11,12]. The resulting state which is characterized by a disturbance in the balance between ROS production on one hand and ROS removal and repair of damage complex molecules on the other is called oxidative stress[13].

Antioxidant drugs for the prevention of various diseases like diabetes, atherosclerosis, Alzheimer's disease and cancer have appeared during the last three decades[14]. The preservative effect of many plant species and herbs suggests the presence of anti-oxidative constituents[15]. This has attracted a great deal of research interest in natural antioxidants. Many medicinal plants contain large amounts of antioxidants other than vitamin C, vitamin E and carotenoids. Subsequently, a world wide trend towards the use of natural phytochemicals present in herbs, fruits and vegetables has increased[16-19].

The antioxidant characteristics of plant derived materials can be attributed to their content of polyphenols, a widely distributed group of substances in the plant kingdom, with more than 8000 phenolic structures currently known. They arise from two main synthetic pathways *i.e.*, the shikimate and the acetate pathway[20,21]. Flavonoids are most abundant distributed as polyphenols in plants kingdom and their structures are diversified by oxidation, alkylation and glycosylation[22]. Compounds most widely occurred in the nature are flavonols, anthocyanidines and isoflavanones, flavanones, flavanols, anthocyanidines and proanthocyanidines[21].

Epidemiological studies have shown that the consumption of foods and beverages rich in phenolic content can reduce the risk of heart disease[23]. *Lavandula stoechas, Smyrnium olusatrum, Crataegus oxyacantha* and *Cistus ladaniferus* are widely distributed and known in a great number of Mediterranean countries. But in Algeria, no documentation on polyphenol phytochemicals composition and any studies on antioxidant power are known. In the present study, the *in vitro* antioxidant properties of four Algerian medicinal plants were investigated. In this report, we studied the crude methanolic extracts of these plants for their potential antioxidant activity with the 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging method.

EXPERIMENTAL

All plants were collected from Tlemcen mounts (Algeria). *L. stoechas, S. olusatrum* and *C. oxyacantha* collected from Oum el Alou area, *C. ladaniferus* from Sidi M'hamed village. The selected plant parts were thoroughly rinsed in water and kept to dry into proper and darkness place.

Extraction: For the preparation of all extracts, 100 g of dried powder biomass were extracted with 500 mL of 70 % aqueous methanol at 25 °C during 24 h without agitation and in the dark. Then to separate the liquid extract from the solids, the mixture of solvent and solids was filtered primarily *via* Whatman No. 1 paper, secondary through a 0.45 µm filter. To obtain dry extracts the filtrates were submitted under reduced pressure to remove methanol using a laborota 4000-rotary evaporator (50 °C). All crude extracts were stored at -20 °C until used.

Phytochemical analysis: Quantitative investigation of the constituents of the crude extracts was realized by thin layer chromatography[24].

DPPH radical-scavenging assay: The DPPH assay was done according to the method of Leitão *et al.*[25] with some modification for all extracts. Briefly, a 0.3 mM DPPH solution was prepared by dissolving DPPH in methanol and 1 mL of this solution was added to 2.5 mL of extracts solutions at different concentrations. The mixture was vigorously vortexed and the absorbance was measured at 518 nm after 0.5 h of reaction at room temperature using a 6405UV/Vis spectrophotometer. The antioxidant activity was calculated as a percentage of DPPH decolouration using the following equation:

$$AA(\%) = \frac{(Abs_T - Abs_E)}{Abs_T} \times 100$$

here, AA = antioxidant activity; Abs_T = control absorbance; Abs_E = sample test absorbance.

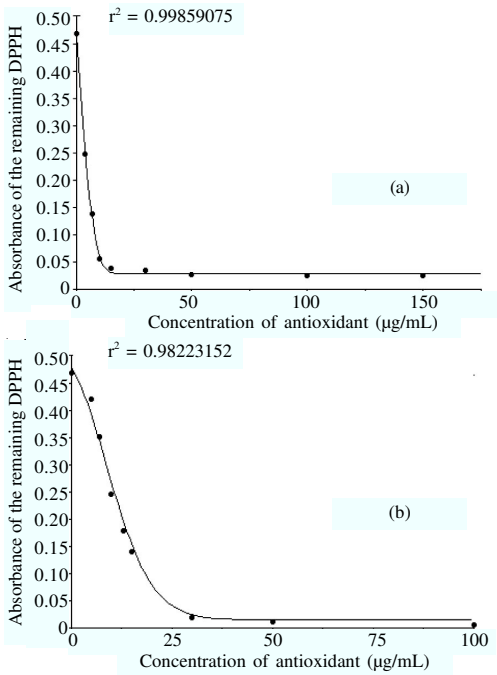
Hydrogen donating ability was expressed as EC_{50}; the amount of sample that is needed for 50 % discolouration of DPPH. The lower value expressed higher antioxidant activity.

Statistical analysis: The results were expressed as mean ± SD. All statistical comparisons were made by means of student's t-test and a *p* value less than 0.05 was regarded as significant.

RESULTS AND DISCUSSION

Because free-radicals may be involved in diverse human diseases, there is evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the preventive biochemical functions of natural antioxidants contained in spices, herbs and medicinal plants[26,27]. For this reason, we were interested to study the antioxidant activity of four abundant and spontaneous plants of Algeria *i.e.*, *Lavandula stoechas, Cistus ladaniferus, Smyrnium olusatrum* and *Crataegus oxyacantha*. Methanolic extracts of these plants were evaluated for their free-radical scavenging activity using the DPPH radical assay. Scavenge of DPPH$^{\bullet}$ can be observed by the decrease in absorbance at 518 nm.

The results (Figs. 1 and 2) showed that the decrease in absorbance of the DPPH$^{\bullet}$ was due to its reduction by different antioxidants. All plant extracts and standard antioxidant reduced DPPH$^{\bullet}$ significantly. Absorbance decrease as a result of a colour change from purple to yellow as the radical was scavenged by antioxidant through donation of hydrogen to form the stable DPPH-H. With higher concentrations, the bleaching ability of the DPPH$^{\bullet}$ solution was nearly complete. Ascorbic acid known to acts as antioxidant[28] was more active in comparison with different extracts, especially more active compared to *S. olusatrum* and *C. ladaniferus*. It was possible to observe nearly complete free-radical scavenging effect (Fig. 1), with ascorbic acid. This showed at the concentration of 5 µg mL^{-1}, followed by crude extract from *C. oxyacantha* at the concentration of 10 µg mL^{-1}, then crude extract of *L. stoechas* and *S. olusatrum* (15 µg mL^{-1}) and in the last comes crude extract of *C. ladaniferus* where the reaction of reduction was nearly complete at 25 µg mL^{-1}. The kinetic classification, according to the time at the steady state has been reported as follows: rapid < 5 min, intermediate 5-30 min and slow > 30 min[29]. Fig. 2 shows the lowering of the quantity of the DPPH$^{\bullet}$ in time. The disparition of DPPH free-radical form is the result of the reduction reaction with the antioxidant substance to form DPPH-H. All natural extracts like synthetic antioxidant (ascorbic acid) reached a plateau in a time lower than 5 min and thus the reactions are rapid.



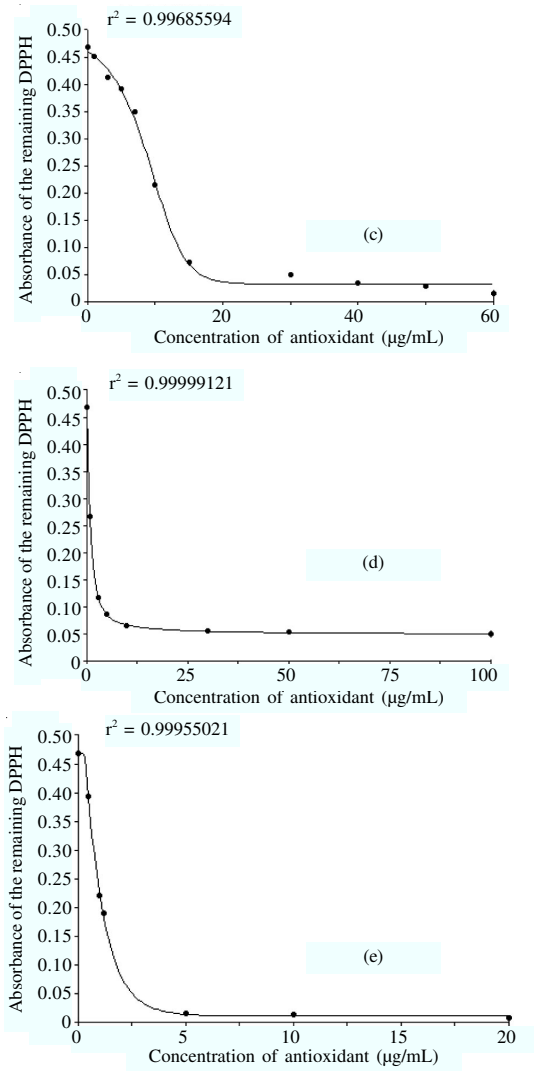


Fig. 1. Antioxidant capacities of plant extracts determined by DPPH• method (a) *Lavandula stoecas*, (b) *Cistus ladaniferus*, (c) *Smyrnium olusatrum*, (d) *Crataegus oxyacantha* and (e) antioxidant synthetic (ascorbic acid)

Different extracts from 4 Algerian selected plants showed good free-radical scavenging activity depending on the concentration and phenolic compounds. In comparison with the antioxidant activity of the synthetic antioxidant such as ascorbic acid using a DPPH free-radical, *L. stoechas* and *C. ladaniferus* leaf extracts, *S. olusatrum* aerial part extract and *C. oxyacantha* leaf and flowers extract have been found to contain a wide range of phenolic compounds (Table-1) which may act as antioxidants. Many flavonoïds and polyphenols can exhibit antioxidant activity as their extensive conjugated π-electron systems allow ready donation of electrons or hydrogen atom, from the hydroxyl moieties to free-radicals[30]. Brand-Williams *et al.*[31] reported that the antioxidant activity of phenolic compounds

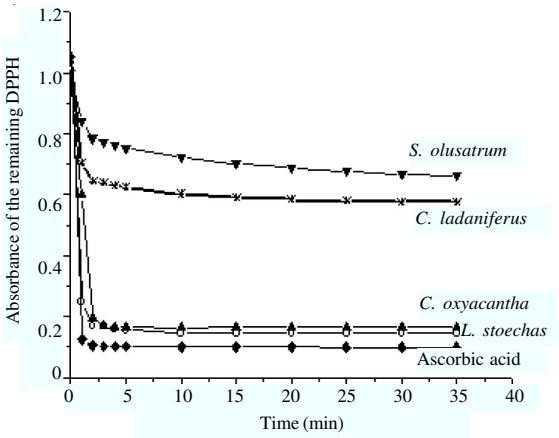


Fig. 2. A time course of DPPH scavenging by the same concentration of different natural and synthetic antioxidants, measured immediately after mixing and after each minute of incubation

is determined by their molecular structure and more especially by the position and the degree of hydroxylation of the ring structure.

The antioxidant activity conventionally used to indicate the ability of antioxidant to scavenge some radicals and the concentration of antioxidant needed to decrease by 50 %, the initial substrate concentration is a parameter widely used to measure the antioxidant power[32-34]. This parameter called EC_{50} reflects the depletion of 50 % radical scavenging activity was calculated for each type of sample. EC_{50} values were obtained from the plot of the absorbance against the concentration and are summarized in Table-1. The lower EC_{50}, the higher was the antioxidant power. In present study, all plant extracts exhibited efficient antioxidant properties due to their phenolic phytoconstituents. According to their EC_{50} values, the classification order for the tested antioxidants was: ascorbic acid (1.04 µg mL^{-1}) > *C. oxyacantha* crude extracts (2.75 µg mL^{-1}) > *L. stoechas* crude extracts (5.29 µg mL^{-1}) > *S. olusatrum* crude extracts (9.68 µg mL^{-1}) > *C. ladaniferus* crude extracts (11.42 µg mL^{-1}).

Compared to reference synthetic antioxidant, ascorbic acid showed a higher antioxidant than all natural extracts. However, *C. oxyacantha* leaf and flowers extracts were the most efficient due to their lowest EC_{50} values among all selected natural plant extracts. The significant antioxidant activity of *C. oxyacantha* is related to the high content of the flavonoïds, in particular the oligomers proanthocyanidins[35].

Conclusion

All plant extracts showed good free-radicals scavenging activity. The crude extract of *C. oxyacantha* provided a stronger antioxidant activity assayed by 2,2-diphenyl-1-picrylhydrazyl free radical. This extract showed a prominent role as antioxidant. Therefore supplementing a balanced diet with *C. oxyacantha*

TABLE-1
ANTIOXIDANT ACTIVITY OF NATURAL EXTRACTS EXPRESSED BY EC_{50}

Antioxidant	Part used	Yield (%)	Major compounds detected	EC_{50} (µg/mL) ± SD
C. ladaniferus extract	Leaves	3.6	Flavones, flavonols,phenolic acids	11.42 ± 0.5819
C. oxyacantha extract	Leaves, flowers	4.6	Flavonols, phenolic acids	2.75 ± 0.3087
L. stoechas extract	Leaves	2.4	Anthocyanidin 3-glycosides, flavonols	5.29 ± 0.2766
S. olusatrum extract	Leaves, flowers, stem	1.1	Anthocyanidin 3-5-diglycosides, phenolic acids	9.68 ± 0.2187
Ascorbic acid	–	–	–	1.04 ± 0.1361

leaf and flowers extracts may provide health-prevention against free-radicals effects.

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