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In Vitro Antioxidant and Anti-Inflammatory Activities Valorisation of Methanol Extracts of *Orchis maculata* L. *subsp baborica* M. Et W and *Ophrys subfusca* (Rchb.) Batt.

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Abstract— *Orchidaceae is regarded as the largest family of plant kingdom comprising a many species with therapeutic values. Orchis maculata L. subsp baborica M. Et W and Ophrys subfusca (Rchb.) Batt., are two orchids growth in Setif – Algeria tested for their antioxidant and anti-inflamatory capacities. The antioxidant investigation has been carried out by radical scavenging activity (DPPH) and anti-inflamatory activity with the Human Red Blood Cell (HRBC) membrane stabilization method. Result show an excellent antioxdant and anti-inflamatory capacities for Ophrys subfusca (Rchb.) Batt and an important value for Orchis maculata L. subsp baborica M. Et W.*

Keywords— *Extract; Orchis; Ophrys; Antioxidant; anti-inflammatory.*

I. INTRODUCTION

Orchidaceae is regarded as the largest family of plant kingdom comprising 25,000-35,000 species [1] [2].

The flora of Algeria quote around 50 orchid taxa: Battandieri & Trabut [3] describe 10 genera and 44 species. Maire [4] provides for Algeria 14 genera and 48 species (55 taxa individualizing each subspecies). Finally Quézel and Santa [5] described 48 species (52 species and subspecies) and 14 genera.

According to Quezel and Santa. (1962), *Orchis maculata* L. *subsp baborica* M. Et W is a plant of 20 – 50 cm high. Stem with 5 – 6 leaves mackled with a dark purple, the interior short and large. A cylindrical spike reach 15 × 3,5 cm, a big bright lilac flowers or purplish white. Labellum mackled or lineoled of purple. This species growth in mountain humid area [5].

However, *Ophrys subfusca* (Rchb.) Batt, is a plant with variable high (5 – 50 cm). Flowers in slack spike and generally pauciflora. Labellum with narrow yellow borders (1 – 2 mm). This species can be found in brushwood, pasture and forest [5].

Many Orchids species has been used in old traditional medicine for their therapeutic value as anti-inflammatory, diuretic, anti-diabetic, and also to treat some liver disorders contain chemical substances of pharmacological use [6-8]. Medicinal orchids belong mainly to genera: *Anoctochilus, Bletilla, Calanthe, Coelogyne, Cymbidium, Cypipedium, Dendrobium, Ephemerantha, Eria, Galeola, Gastrodia, Gymnadenia, Habenaria, Ludisia, Luisia, Nevilia and Thunia* [9]. Recently, more species belonging to different genera have been reported to have medicinal properties and in future more will be added in the list [10], [11].

Fig. 1. *Orchis maculata* L. *subsp baborica* M. Et W in left side and *Ophrys subfusca* (Rchb.) Batt in right side.

The aims of this study is to evaluate medicinal proprieties of two orchids growth in Setif, Algeria by testing their antioxidant and anti-inflammatory capacities.

II. MATERIALS AND METHODS

Plant Material

The random sampling were used during the harvesting, the areal parts of *Orchis maculata* L. *subsp baborica* M. Et W were taken from the mountain of Megriss (**X:** 5° 18’ 20” Y**:** 36° 18' 30” and **X’:** 5° 24' 7” Y’:36° 21’ 54”). However, *Ophrys subfusca* (Rchb.) Batt was harvested from the massif of Boutaleb (**X_1 E:** 5° 30' 2.46” **Y_1 N:** 35° 44’ 41.74" ; **X2 E:** 5° 25' 17.69” **Y2 N:** 35° 50’ 1.85"; **X3 E:** 5° 8' 35,98” **Y3 N:** 35° 43’ 12.47"; **X4 E:** 5° 18' 45.10” **Y4 N:** 35° 41’ 3.56"). Determined by Dr. Nouioua Wafa in Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Natural Life and Sciences, University Ferhat Abbas Setif, Algeria.

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Preparation of Methanol Extracts

The areal parts of the chosen species 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 [12]. The dry extracts were stored at a temperature of -18 °C for later use.

Determination of Total Phenolic Content

For total polyphenol determination, the Foline Ciocalteu method was used [13]. The samples (0.2 mL) were mixed with 1 mL of the Folin-Ciocalteu reagent previously diluted with 10 mL of deionized water. 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 absorbances at 765 nm were measured. Gallic acid was used as a standard for the calibration curve. The total phenolic compounds content was expressed as mg equivalent of Gallic acid per gram of extract (mg EAG/GE)

Determination of Total Flavonoids Contents

The flavonoids content in our extracts were estimated by the Aluminium chloride solution according to the method described by Bahorun et al., (1996) [14]. Briefly, 1 mL of the methanol solution of the extracts were added to 1 mL of 2 % $AlCl_3$ in methanol. After 10 minutes, the absorbances were determined at 430 nm. Quercetin (0 – 40 µg/ml) were 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) [15]. One millilitre of the extracts 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 absorbances of the resulting solutions were then measured at 517 nm. The antiradical activity was expressed as IC_{50} (micrograms per millilitre). The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1)/ A_0]\times 100$$

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 [16].

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 10 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 mixture 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 extracts or standard drug (diclofenac sodium) at various concentrations (50, 100, 250, 500, 1000, 2000 µg/mL). 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:

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

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

$$\text{Protection (\%)} = 100 - [(\text{Optical density of Test sample} / \text{Optical density of Control}) \times 100]$$ [17]

Statistical Analysis

Results were expressed as the mean ± standard deviation. Data was statistically analysed using one-way ANOVA and Newman-Keuls Multiple Comparison to determine whether there were any significant with the criterion of P values < 0.05 between methanol extracts of the two orchids species and standards, using Graphpad prism 5 Demo Software.

III. Results and Discussions

Phytochemical quantification was performed for the two extracts, which revealed an important extracts yields and the presence of phenols and flavonoids in low concentrations (Table I).

TABLE I. Yield, polyphenols and flavonoids quantification of the two orchids species methanol extracts.

	Yield (%)	**Poly phenols (mg EAG/GE)**	**Flavonoids (mg EQ/GE).**
Ophrys subfusca	18.78	18,00±0,34	4,10±0,20
Orchis maculata	26.44	8,66±0,12	2,73±0,19

Radical scavenging potential of extracts at different concentration was tested by DPPH method (figure 2). The percentage of scavenging activity and IC_{50} value were found to be 77,93± 0,93 % and 10,00±3,19 µg/mL for *Ophrys subfusca*, 79,99±1,03% and 56,30±0,36 µg/mL*** for *Orchis maculata* against 99,50±0,01% and 8,76±,69µg/mL for BHT .

Although some studies have demonstrated a correlation between phenolic content and antioxidant capacity [18]. Neverless, there is no correlation between polyphenols quantities and antioxidant capacities in the two species. Bajpai et al. (2005) [19] reported no correlation between total phenolic content and antioxidant capacities of a number of medicinal plant extracts. This phenomenon was probably due to the following factors: the antioxidant capacity observed was not solely from the phenolic contents, but could possibly be due to the presence of some other phytochemicals such as ascorbic acid, tocopherol and pigments as well as the

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synergistic effects among them, which also contribute to the total antioxidant capacity [20].

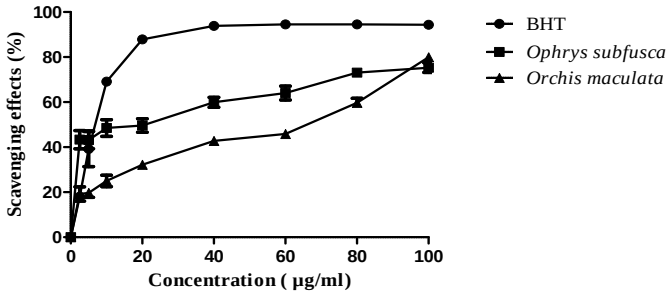

Fig. 2. Scavenging effect of standard and the two orchids species methanol extracts.

On the other hand, total phenolic content determined according to the Folin- Ciocalteu method is not an absolute measurement of the amount of phenolic materials. Different types of phenolic compounds have different antioxidant activities, which is dependent on their structure. The extracts possibly contain different type of phenolic compounds, which have different antioxidant capacities [20].

Methanol extract of the two orchids significantly ($p \leq 0.001$) inhibited lysis induced by water. This is confirmed by the high percentage inhibition of haemolysis obtained for doses from 10, to 500 µg/mL (figure 3). However, Declofenac Sodium was weaker than the tested extracts even at high concentrations

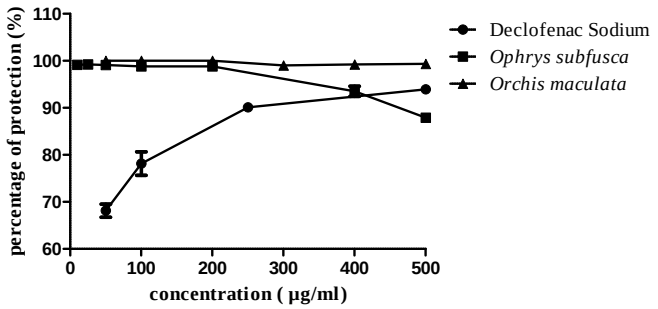

Fig. 3. The percentage inhibition of hypotonicity induced haemolysis of HRBCs (%) of standard and the two orchids species methanol extracts.

Compounds with membrane stabilizing properties are well known for their ability to interfere with release of phospholipases that trigger the formation of inflammatory mediators [21]. A possible explanation for the stabilizing activity of the orchids extracts could be an increase in the surface area to volume ratio of the cells, which could be brought about by an expansion of membrane or shrinkage of the cell, and an interaction with membrane proteins [22], [23].

IV. Conclusion

The two orchids species demonstrated excellent medicinal properties. In the antioxidant activity, no correlation were found between polyphenols content and antioxidant capacities, but and an important value were registered especially in the case of *Ophrys subfusca* (Rchb.) Batt. The *in vitro* anti-inflammatory experiment was studied to valorise the possible protection mechanism of erythrocyte during the inflammation process. This activity show an excellent power better than the non-steroidal medicines.

Further investigation concerning the chemical composition, molecules purification and toxicity are recommended.

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