

# In Vitro Antioxidant and Anti-Inflammatory Activities Valorisation of Methanol Extract of *Rosmarinus eriocalyx* Jord. & Fourr.

Gaamoune Sofiane<sup>1</sup>, Nouioua Wafa<sup>2</sup>

<sup>1</sup>National Institute of Agricultural Research – Setif – Algeria

<sup>2</sup>Faculty of Natural Life and Sciences, University Ferhat Abbas Setif, Algeria

**Abstract**— *Rosmarinus eriocalyx* is an aromatic evergreen bush endemic to Algeria used as a condiment to flavour soups and meat and as a traditional remedy. The methanolic extract was tested for their antioxidant and anti-inflammatory capacities. The antioxidant investigation has been carried out by radical scavenging activity (DPPH) and anti-inflammatory activity with the Human Red Blood Cell (HRBC) membrane stabilization method. Results demonstrate a powerful antioxidant and anti-inflammatory activity due to the probable presence of rosmarinic acid, carnosic acid and carnosol that are the main responsible for the noteworthy antioxidant activity observed in the assays.

**Keywords**— DPPH; HRBC; extract; methanol; Rosemary.

## I. INTRODUCTION

Phytochemicals are naturally occurring compounds of plant kingdom, such as medicinal plants, vegetables, fruits, that work with nutrients and fibers to act against diseases or more specifically, provides protection against diseases [1].

Rosemary plants growth worldwide and have been cultivated since a long time ago for its strong antioxidant and antimicrobial activities. This plant species also has many other beneficial activities such as antiviral, anti-inflammatory and anticarcinogenic [2–6] activity. This species is considered one of the most important sources of both volatile and non-volatile bioactive compounds [7], [8].

Botanically, there are three species belong to the *Rosmarinus* genus, which are *R. officinalis*, as well as two endemics, namely *Rosmarinus tomentosus* Hub.-Mor. & Maire, and *Rosmarinus eriocalyx* Jord. & Fourr. (*R. eriocalyx*) [9]. Previously known as *Rosmarinus tournefortii* (Noë ex Jord. & Fourr.) Jahand. & Maire, and described as exclusively endemic in Algeria [10], *R. eriocalyx* is actually defined as an endemic plant in North-West of Africa and Southern Spain [11]. The plant grows widely on rocky ground and pastures in the mountainous areas of eastern Algeria [12], [13]. *R. eriocalyx* differs from *R. officinalis* for its smaller leaves, only 5–15 mm long and less than 2 mm broad, and for a more densely hairy flower stems. In this regard, the epithet ‘*eriocalyx*’ means woolly calyx, alluding to its double hairy calyx, being characterized by one short type and long erect glandular hairs. Another difference with respect to *R. officinalis* is its prostrate growth and lower height (often under 25 cm and never exceeding 1 m tall) [14].

For the purpose, methanol extract was valorised, to evaluate the antioxidant and anti-inflammatory activities potency of polar molecules obtained from the aerial parts of Algerian endemic rosemary, the *R. eriocalyx*.

## II. MATERIALS AND METHODS

### A. Plant Material

The random sampling were used during the harvesting. The areal parts of *R. eriocalyx*. were taken from the massif of Boutaleb (X1 E: 5° 30' 2.46" Y1 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 in Laboratory of National Institute of Agricultural Research – Setif – Algeria.

### B. Preparation of Methanol Extracts

The areal parts of the *R. eriocalyx*., 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 [15]. The dry extracts were stored at a temperature of -18 °C for later use.

### C. Determination of Total Phenolic Content

For total polyphenol determination, the Foline Ciocalteu method was used [16]. 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).

### D. Determination of Total Flavonoids Content

The flavonoids content in our extracts were estimated by the Aluminium chloride solution according to the method described by Bahorun et al., (1996) [17]. Briefly, 1 mL of the methanol solution of the extracts were added to 1 mL of 2 % AlCl<sub>3</sub> 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).

**E. Determination of Total Flavonoids Content**

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) [18]. 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 IC50 (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:

A0: the absorbance of the control at 30 minutes

A1: is the absorbance of the sample at 30 minutes. BHT was used as standard [19].

**F. The Human Red Blood Cell (HRBC) Membrane Stabilization Method**

The flavonoids content in our extracts were estimated by the Aluminium chloride solution according to the method described by Bahorun et al., (1996) [17]. Briefly, 1 mL of the methanol solution of the extracts were added to 1 mL of 2 % AlCl3 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).

**G. Determination of Total Flavonoids Content**

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 (10, 50, 100, 250, 500 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:

$$\text{Protection (\%)} = 100 - [(\text{Optical density of Test sample} / \text{Optical density of Control}) \times 100] \text{ [20].}$$

**H. Statistical Analysis**

Results were expressed as the mean ± standard deviation. Data was statistically analysed using t test of Student as primary test followed by Fisher test with the criterion of P < 0.05 to determine whether there were any significant differences between methanol extract of R. eriocalyx., and standards, using Graphpad prism 5 Demo Software.

**III. RESULTS AND DISCUSSIONS**

The extraction method have revealed high yield, was close to 30,23 % contain 36,95±1,38mg EAG/GE of polyphenols and 6,54±1,65 mg EQ/GE of flavonoids. According to Bendif et al., 2017 [21], this species contain a high level of polyphenols, which was not agree with our finding may due to the method extraction.

Free radical scavenging potential of extract at different concentrations was tested by DPPH method (figure 1).

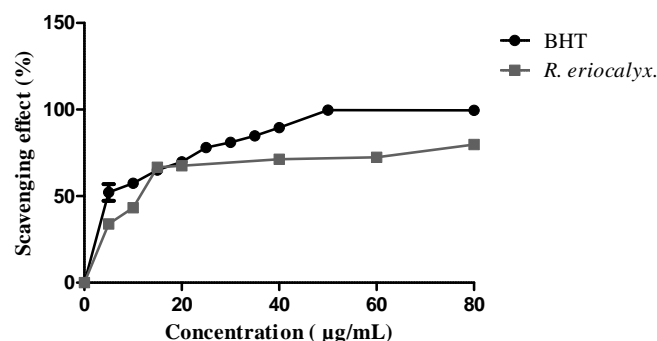


Fig. 1. DPPH radical scavenging activity of methanol extract of R. eriocalyx.

IC50 of the extract reach 6,37±0,95 µg/mL against 8,76±0,69 µg/mL for BHT. The percentage of scavenging activity however, attend 79,48± 3,17 % at the concentration of 80 µg/mL.

In general, the antioxidant activity of plant extracts is due primarily to phenolic compounds and in rosemary extracts, the presence of different groups of phenolic such as diterpenoids, flavonoids and phenolic acids are responsible for the antioxidant properties observed. The major components of R. eriocalyx extracts, i.e. the diterpenes carnosic acid and carnosol, and the hydroxycinnamic acid ester rosmarinic acid are considered the most important antioxidant compounds [22].

Figure 2 demonstrate the anti-inflammatory activity of methanol extract of R. eriocalyx. The results show a level of 96,20±0,28 % of haemolysis inhibition of the erythrocytes produced by extract of R. eriocalyx against 68,13±1,40 % of inhibition of the standard at the same concentration of 50 µg/mL.

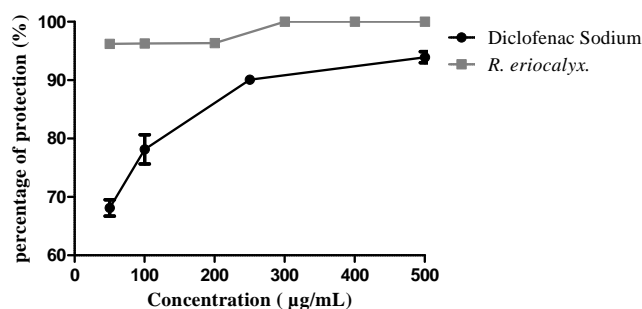


Fig. 2. The percentage inhibition of hypotonicity induced haemolysis of HRBCs (%) of standard and methanol extract of *R. erioocalyx*.

This indicates that this extract possess biological membrane stabilization properties preventing stress-induced destruction of the plasma membrane. Stabilization of lysosomal membrane is important in limiting the anti-inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release [23].

#### IV. CONCLUSION

The methanolic extract of *R. erioocalyx* was able to significantly reduce inflammation at low concentrations and have an ability to scavenge free radicals better than standard.

Phytochemical components of *R. erioocalyx* had been screened and isolated by many researchers; ours perspectives could be conducted in order to test toxicity of each component in order to recommend firms of pharmacy to manufacture these compounds.

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