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Antioxidant and anti-inflammatory activities evaluation of *Coriaria myrtifolia* from the North of Morocco

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Abstract

The present study has examined the chemical composition and evaluated the pharmacological activities of the ethyl acetate extract of *Coriaria myrtifolia*. The antioxidant activity was determined by using the diphenyl-picryl-hydrazyl (DPPH) test, and the anti-inflammatory activity was evaluated by using the plantar edema model induced in rabbits by carrageenan. The extract has revealed a significant free-radical scavenging capacity. The plant IC_{50} value of 0.016 mg/ml was found less than stated with the Butylated hydroxyl-toluene (BHT) standard (0.025 mg/ml). Administration of the ethyl acetate extract at a dose of 0.013mg/kg wb inhibits completely the inflammation. These results have indicated that the *C. myrtifolia* leaves contains bioactive compounds and have a high antioxidant activity as well as interesting anti-inflammatory properties, suggesting the usefulness of their extracts to prevent oxidative and inflammatory processes.

Introduction

Given the therapeutic limitations of chemical drugs, the development of medicinal plants research has been oriented towards phyto-pharmaceuticals. This development is a crucial step in promoting the growth of an entire sector, which is interested not only to therapy needs, but also to food industry, cosmetics and perfumery. In fact, antioxidant properties of plant extracts are largely examined (Gardelli *et al*., 2008; Himaja *et al.*, 2010; El Hajaji *et al.*, 2010; Mohammedi and Atik, 2011; Boudkhili *et al*., 2012). However, synthetic antioxidants used to delay lipid oxidation have proven to be responsible for side effects. Oxidative stress, which occurs when there is an imbalance between the production of free radicals and antioxidant enzymes, is related to the appearance of Alzheimer's disease, arteriosclerosis and cancer diseases. The best way to prevent the stress that harms and destroys cells is to seek alternative antioxidant compounds. The bioactive molecules of extracts from various plant sources such as rosemary, sage, oats, tea, garlic, olives, ginger and a range wide of plants from all over the world continue to have significant interest as a supplement in complementary medicine.

Previous studies showed that bioactive substances derived from various plant species have an important anti-inflammatory effect (Amezouar, 2013; Hui-Seong Kim, 2013; El Mansouri *et al*., 2014). The plants provide therefore a good source of bioactive molecules. *Coriaria myrtifolia* belonging to the family of Coriariaceae is commonly known in Morocco as Arwaz or rewiza. It is a shrub measuring 2 to 3 meters high, very popular in the Mediterranean region (France, Italy, Spain, Portugal, Algeria and Morocco). Other similar species exist in Central and East Asia, in New Zealand (*Coriaria sarmentosa* or *C. tutu*) and in Western America (Rimbaud *et al.*, 1943). The chemical composition of the leaf (rich in tannins) has made it a tanning substance able to convert animal skin into leather. *Coriaria myrtifolia*'s use in Mediterranean countries is therefore limited to tanning. It is necessary to extend research to its pharmacological properties in order to take more advantage of this plant. This study aims to evaluate the antioxidant and anti-inflammatory activities of *Coriaria myrtifolia* ethyl acetate extract. This investigation was selected using the findings of a preliminary study conducted on its antibacterial activity (Hafsé *et al.*, 2015).

Materials and Methods

Plant materials

Coriaria myrtifolia was collected from Bab Berred in the north of Morocco (Altitude 1290 m, 35° 00' 979'' N, 004° 58' 092''W). The plant identified by

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Professor Abdellah Farah from the National Institute of Medicinal and Aromatic Plants (Taounate) was placed in its herbarium. The sample was dried in the open air. Once dried, the leaves were separated from stems and ground to a final particle size that enables complete dissolution

Preparation of the extracts

Preparation of extract was performed using a Soxhlet apparatus, 45 g of *Coriaria myrtifolia* powder was previously prepared; it was first defatted with 200 ml of hexane. The extraction was then performed by adding 200 ml of ethyl acetate (EA). Finally, the extract was recovered after evaporating the solvent under vacuum using a rotary evaporator (90 rpm, 40°C).

Phytochemical screening

The revelation of certain chemical families of the ethyl acetate extract of *Coriaria myrtifolia* was performed by the phytochemical screening according to Harborne (1984).

Evaluation of antioxidant activity

Antioxidants as proton donors are able to reduce the radical's absorbance. The extract antioxidant capacity is thus determined by evaluating the percentage inhibition of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical absorbance at 517nm. The DPPH is a stable organic radical at room temperature and has a purple color.

The free-radical scavenging capacity estimation of the ethyl acetate extract of *Coriaria myrtifolia* was hence carried out by DPPH method according to the protocol described by Gardeli *et al.* (2008). An extract concentration ranging from 0.25 to 0.00195 mg/ml was prepared in methanol. 3 ml of a methanol solution of DPPH (0.04%) was added to 3 ml of each extract concentration, the mixture was vigorously shaken and then the tubes were incubated at room temperature for 30 minutes in the dark. Blank was represented by methanol; the negative control was 3 ml of a 0.04% solution of DPPH in methanol and 3 ml of methanol. The positive control was represented by BHT methanol solution. The absorbance was measured at a wave length of 517 nm, and the antioxidant activity estimated using the following equation:

% antioxidant activity = (Abs control - Abs sample / Abs control) x 100

Evaluation of anti-inflammatory activity

Evaluation of Anti-inflammatory activities of *Coriaria myrtifolia* was conducted on rabbits (1.5 to 2 Kg) using plethysmograph principle. Briefly, rabbits were divided into four groups of three animals each. (Group1, control) was given 0.7ml of 1% of carragenan per animal. Groups 2 and 3 received carragenan 30 min after the administration of two concentrations of *C. myrtifolia* extract and group 4 received Diclofenac (300 mg/kg Wb) before carragenan. Evaluation of paw edema volume was recorded 0, 1, 2, 3, 4 and 5 hours after injection. For each treatment group, the volume average was measured by comparing the volume observed (Vt) with the control volume (V0). The percentage edema inflammation was calculated using the following formula:

Inflammation percentage: ((Vt -V0) / V0) / 100

Whereas the percentage edema inhibition was calculated using the following formula:

[(Vt - V0) control - (Vt - V0) Treaty] / (Vt - V0) control *100

Results

Phytochemical screening

The phytochemical screening has revealed the presence of many active compounds in the ethyl acetate extract of *Coriaria myrtifolia* which contains polyphenols, flavonoids, tannins, steroidal glycosides and heterosoïdes triterpenes. While, mucilages, saponosides and cardiac glycosides are absent in this extract (Table 1).

Antioxidant activity

DPPH radical has an intense purple color. Measuring the effectiveness of an antioxidant (ability to bind free radicals and stop chain reaction spreading) is carried out by measuring the decrease of the purple color spectrophotometrically at 517 nm. The absorbance and the percent of antioxidant activity are shown in Table 2. It can be noticed that the free-radical scavenging activity of the ethyl acetate extract was more important when this tested extract was more concentrated, hence this activity is then dose dependent. The calibration curve has shown an IC_{50}value (concentration needed to give 50% reduction of DPPH radical) of 0.016 mg/ml (Figure 1).

For comparison, the antioxidant activity is measured by using the same method for BHT that has been used as a reference standard. This standard is still known as the most effective free radical

Table 1. Revelation results of the principal chemical families present in the ethyl acetate extract of *C. myrtifolia*

Chemical families	Test Results
Polyphenols	+
flavonoids	+
Tannins	+
Steroidal glycosides	+
Triterpenesheterosoïdes	+
Cardiac glycoside	-
saponosides	-
Mucilage	-

Table 2. Optical density values (OD) and inhibition percentage of DPPH by range of extract concentrations

Concentration (mg/ml)	OD	Inhibition percentage (I %)
0,25	0,013	96,37883008
0,125	0,018	94,98607242
0,0625	0,026	92,75766017
0,03125	0,029	91,92200557
0,015625	0,178	50,4178273
0,0078125	0,271	24,51253482
0,00390625	0,322	10,30640669
0,001953125	0,332	7,520891365

scavenger. The calibration curve of BHT has shown an IC_{50} value of 0.025 mg/ml. At the concentration of 0.25mg/ml, extract's percentage inhibition of DPPH was found to be 96.37%.

Anti-inflammatory activity

Results of anti-inflammatory activity of the two extracts concentrations tested and compared to those of Diclofenac (Group 4) and to control (Group1) were reported in Figure 2. The evolution of edema volume after carrageenan injection was time dependent and was divided into two phases: The first phase from 0 to 1 hour (inflammation = 65.62%) and a second phase from 1 to 5 hours.

The ethyl acetate's extract of *C. myrtifolia* was significantly effective in preventing inflammation one hour after its administration at a dose of 0.005 mg/Kg wb (inflammation = 31.47%). It exhibited a complete inhibition at 0.013mg/kg wb, meaning it has an effect at the initial phase of inflammation as compared to indomethacin administration (Group 4).

Discussion

The phytochemical screening of the studied

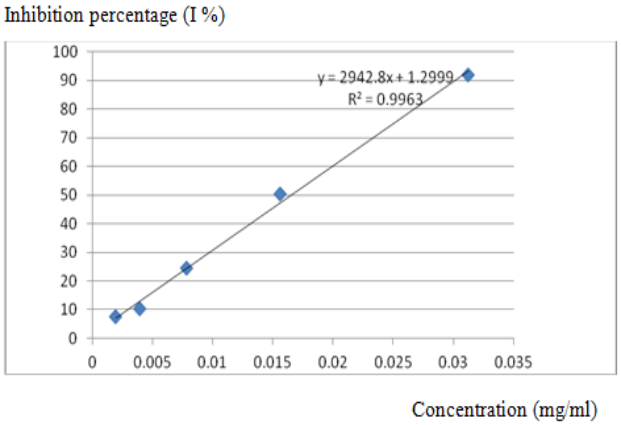

Figure 1. Ethyl acetate extract calibration curve

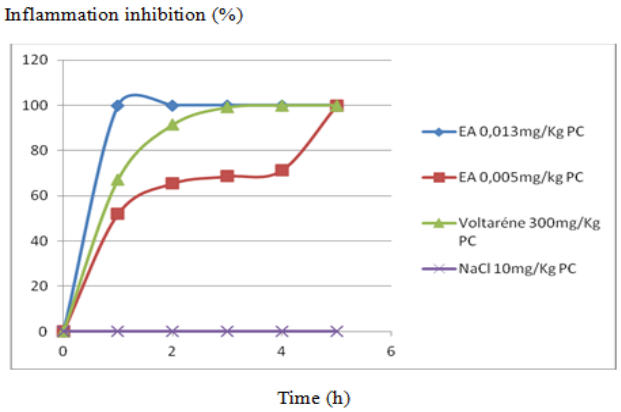

Figure 2. Percentages of the inhibition of inflammation in the tested EA extract. (EA: Ethyl acetate Extract).

ethyl acetate extract showed that *C. myrtifolia* is rich in a lot of active compounds such as polyphenols, flavonoids, tannins, steroid glycosides and triterpenes which confirmed a preliminary study performed on the methanol extract of the same species (Boudkhili *et al.*, 2013). The low IC_{50} value of 0.016 mg/ml obtained for the ethyl acetate's extract of *Coriaria myrtifolia* show its considerable antioxidant activity. Furthermore, the comparison of IC_{50} values of the BHT and the *C. myrtifolia*'s extract has shown that the latter's antioxidant activity was higher than the reference standard.

The Ethyl acetate extract of *C. myrtifolia* have an important inhibition percentage of DPPH (96.37%) showing that it has a higher free-radical scavenging activity than the ethyl acetate extract of *Nigella sativa* whose IC_{50} value is of 0.121 mg/ml (Meziti *et al.*, 2012) and that of ethyl acetate extracts of three Caroub varieties whose IC_{50} value ranged from 410 to 1500 mg/ml (El Hajaji *et al.*, 2010). However, the aqueous and ethyl acetate extracts of *Curcuma zedoaria*, known for its important antioxidant effect, were found more active with DPPH solution (97.9% and 98.95%) at a concentration of 100 µg/ml (Himaja *et al.*, 2010). It is noteworthy that the free-radical scavenging capacity of the ethyl acetate extract of

Coriaria myrtifolia was evaluated for the first time by DPPH method.

The anti-inflammatory activity increase of the tested extract was probably accompanied by an increase in the synthesis of prostaglandins (PGs), mainly prostaglandin E2 (PGE2) involved in the process of pain and inflammation (Posadas *et al*., 2004; Nantel *et al.*, 1999). The significant effective anti-inflammatory activity of ethyl acetate's extract of *C. myrtifolia* just one hour after its administration at 0.005 mg/Kg wb and the complete inflammation inhibition at 0.013mg/kg wb, showed the effect of this extract at the initial phase of inflammation as compared to indomethacin administration (Group 4).

It is important to notice that the ethyl acetate extract of *C. myrtifolia* anti-inflammatory activity is evaluated for the first time. In fact, this activity is probably related to its high content in bioactive compounds such as total polyphenols, flavonoids and tannins. These compound's anti-inflammatory effect was also highlighted by numerous studies (González-Gallego *et al*., 2007; García-Lafuente *et al*., 2009; Sindhu *et al.*, 2012). Indeed, tannins inhibit the actions of cyclooxygenase, phospholipase A2, lipoxygenase and arachidonic acid (Hyun *et al.*, 2004). The anti-inflammatory and antioxidant activities of tannins reported by Giovannelli *et al.* (2000) showed that they might have both a protective and a therapeutic potential in oxidative damage-related pathologies (El Mansouri *et al.*, 2014).

Conclusion

The present study was conducted to evaluate the antioxidant and anti-inflammatory activities and reveal different chemical families of ethyl acetate extract of *Coriaria myrtifolia* collected from the North of Morocco. Bioactive compounds such as tannins, flavonoids and polyphenols were found in the phytochemical screening test. The antioxidant and anti-inflammatory properties of this extract are demonstrated here for the first time, it has an interesting free-radical scavenging capacity, and its anti-inflammatory effect at the dose of 0.013mg/kg B.W is higher than that of the positive control. *Coriaria myrtifolia* performances could be considered as a new natural source of antioxidants and anti-inflammatory agents.

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