contribution to a study of biological applications; anti-inflammatory, antifungal, antioxidant of methanoic extracts of *Moricandia suffruticosa* L.

ABSTRACT

In recent years, several reasons have led to the re-establishment of the use of medicinal plants. First, they are less expensive than synthetic drugs, and second, they come at a time when they come at a time when the public is disillusioned with modern medicine.

This study was done to study antioxidant activities, antiinflamatory and antifungal in vitro methanol extract obtained from the aerial parts of *Moricandia suffruticosa* L, plant was harvested in the region of Tamanrasset - Algeria (south of the Algerian Sahara) ; The antioxidant properties studied were tested by the mechanism of the sweeping activity of DPPH radicals, Anti-inflammatory activity was assessed using the human red blood cell membrane stabilization (HRBC) method. Antimicrobial activity was tested with three fungal strains (Microsporium audouini, Microsporium gypseum and Trichophyton rubrum). The results show a perfect anti-inflammatory activity and a good inhibition on the various fungi on the other hand a moderate antioxidant activity.

The methanoic extrac studied has the potential to be used as an antimicrobial agent against the fungi tested.

Keywords :*Moricandia suffruticosa*L, antioxidant, anti-inflammatory, antimicrobial, methanol extract.

INTRODUCTION

The phenomenon of fungal resistance has become a critical problem in the treatment of many diseases, hence the interest of medicinal plants. Currently, it is the most widely used form of medicine in the world; the use of herbal remedies as well as the search for new bioactive substances is one of the greatest concerns of scientists (1).

Moricandia suffruticosa L. (M. Suffruticosa L.), considered under the name of «Krombe» in Algeria, Perennial plant with stems, most clearly cordiform, amplxicaul at the base. Seeds, in reality, unisex or subbisériées M. Suffruticosa is an endemic subspecies, located in the Algerian Sahara: (2)(3).

In this article, we tried to examine the antifungal activities of the vital oil of Mr. Suffruticosa L. On some pathogens to enrich the Algerian pharmacopoeia and keep this endemic species.

MATERIALS AND METHODS

Plant material

The plant was harvested in the region of Tamanrasset - Algeria (south of the Algerian Sahara) during the spring season; identified by Professor Laouar Hocine "laboratory of plant valorization" A random sampling of the aerial parts of M. suffruticosa L was used, were harvested

Preparation of the methanolic extract

The aerial parts were powdered and macerated in 80% methanol for 24, 48 and 72 hours at laboratory temperature (1:10 w/v, 10 g dried herb). After maceration, the extracts were collected, filtered and evaporated to dryness under vacuum [4]. The dry extract was stored at -18 °C for further use.

Determination of Total Phenolic Content

To determine total polyphenols, the Foline Cioalteu method was used [5].The samples(0.2 mL were mixed with 1 ml of Folin-Ciocalteu reagent produced with 10 ml of deionized water. After 4 minutes of rest of the 25°C solutions, 0.2 mL of saturated sodium carbonate

solution (75 mg/mL) was added. The mixed solutions were left at rest for 120 minutes before the absorbance was measured at 765 nm. Gallic acid was used as a standard for the calibration curve. Total phenolic content was expressed in mg gallic acid equivalent per gram of extract (mg EAG/GE).

Determination of total flavonoids contents

According to the method described by **[6]**, 1 mL of the methanol solution of the extract was added to 1 mL of AlCl3 at 2% in the methanol. Absorbance was determined at 430 nm after 10 minutes. Quercetin was used as a standard. Results were expressed in mg quercetin equivalent per gram of extract (mg EQ/GE).

DPPH Assay

according to the method of Hanato et al., (1998)**[7]**, the capacity of the extract was measured by the bleaching of the colour solution for the 1, 1-diphenyl-2picrylhydrazyl (DPPH) radical. One millilitre of extract at different concentrations was added to 0.5 mL of methanol DPPH solution. The mixtures were shaken vigorously and left at laboratory temperature for 30 minutes in the dark. The absorbance of the resulting solutions was measured at 517 nm. The antiradial activity was expressed in IC50 (micrograms per millilitre).The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = [(A0 – A1)/ A0]

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 **[8]**.

Activité anti-inflammatoire :

Estimation of in vitro anti-inflammatory activity using the human red blood cell(HRBC) membrane stabilization method described by Sadique et al. (1989)**[9]**was used to assess the in vivo anti-inflammatory effect of hexane extract. The principle involved is the stabilization of the membrane of human red blood cells by membrane lysis induced by hypotonicity

To prepare the HRBC suspension, fresh completely humanblood(10mL)wascollectedandtransferredintothecentrifuge tubes. These lasts were centrifuged at 3000 rpmfor10minutesthriceandwashedwithequalvolumeofnormal saline each time. The volume of blood was measuredandreconstitutedas10%v/vsuspensionwithnormalsaline.

The principle involved here was stabilization of human redblood cell membrane by hypo tonicity induced membranelysis.Themixturecontain1mLphosphatebuffer(pH7.4, 0.15 M), 2 mL hypo saline (0.36 %), 0.5 mL HRBC suspension(10 % v/v) and 0.5 mL ofplant extract or standard drug(diclofenac sodium) at various concentrations (10, 50, 100,250, 500µg/mL). The control was distilled water instead ofhyposaline toproduce 100%haemolysis.

The mixtures were incubated at 37 °C for 30 minutes andcentrifuged at 2500 rpm for 5 minutes. The absorbance ofhaemoglobin content in the suspensions were estimated at560 nm. The percentage of haemolysis of HRBC membranecanbe calculatedasfollows:

Haemolysis (%) = (Optical density of Test sample / OpticaldensityofControl)×100

The microorganisms tested

Three fungi are used in this study: *Microsporium audouini*, *Microsporium gypseum* and *Trichophyton rubrum* from patient samples (skin) at the Bendadis Hospital-University Hospital (CHU) in Constantine (Algeria)

Treatments

Procedure of Mushrooms direct contact

In order to verify the antifungal activity, the fungal mycelial growth was measured by direct contact with the methanol extract of Moricandia suffruticosa . The tests were performed on a Petri dish by melting the SABOURAUD culture medium (20 mL) with antibiotics and

cycloheximide and then allowing it to cool. It is important to know the volume of the stock solution to reach the desired concentrations.

Estimation of mycelial growth

The estimation of mycelial growth wasdone according the method described by Rapilly (1968)**[10]**, which depends on measuring the linear and diametrical growth of the colonies using the following formula:

$L = D - d / 2$

L: mycelial growth; D: the diameter of the colony; D: the diameter of the explant.

The rate of inhibition of growth

The rate of inhibition of mycelial growth is expressed as a percentage (%) of control mycelial growth (zero oil concentration) according to the formula described by Leroux and Credet **[11].**

Inhibition rate (%) = (L-I / L) x 100

L is the mycelial growth of the control; I is the mycelial growth of the fungus undergoing treatment.

Statistical analysis

The data analysis was performed using Microsoft Office Excel 2007 for the classification of raw data and for the development of graphs and using stat box version 6.0 for the ANOVA analysis and the Newman-Keuls test.

RESULTS AND DISCUSSION

The results obtained by the extraction method have a very low yield containing 3,191± 0,629 mg EAG/GE of polyphenols and 3,443± 0,09 mg QG/GE of flavonoids.

The DPPH radical has been widely used as a model system for studying trapping several natural compounds (Huang et al. 2004). The results are shown in Fig 1 obtained.

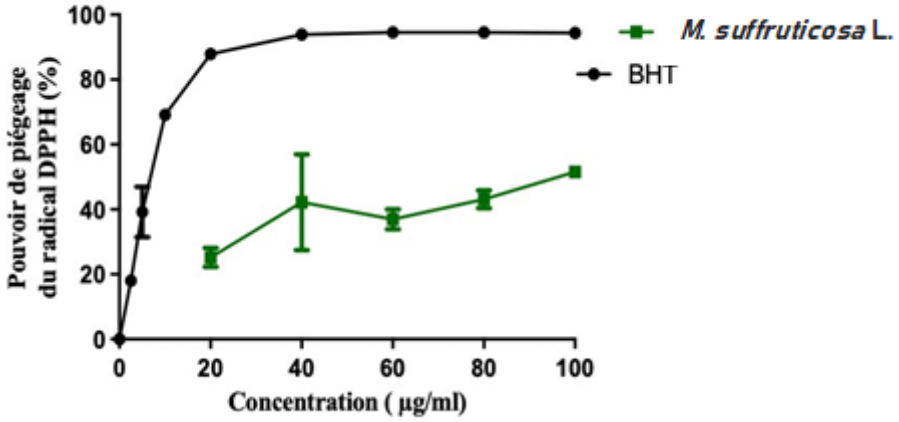

Figure 1: DPPH test of methanol extract of*Moricandia suffruticosa* L.

9Low antioxidant activity was observed in *Moricandia suffruticosa* extract L.(IC50=122.022± 18.162 µg/mL) against BBW (8.76± 0.69 µg/mL).

BHT remains the most effective antioxidant in evaluation with the methanolic extract of *Moricandia suffruticosa*L.

The anti-inflammatory activity of *Moricandia suffruticosa* extract wasconfirmed by erythrocyte membrane stabilization test. Theresults (figure 2) show that human erythrocyte membraneswere protected against hypotonic solution-induced lysis atdifferentconcentrationsofextract,especiallythesmallestones.

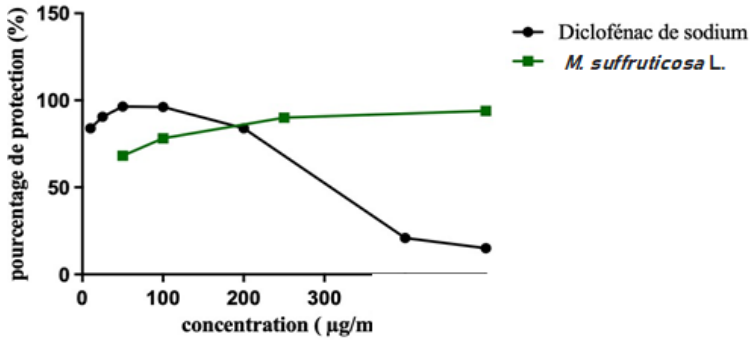

Figure 2: The percentage inhibition of hypotonicity induced haemolysis of HRBCs (%) of standard and methanolextractof*Moricandia suffruticosaB. etR.*

The results show that the membranes of human erythrocytes were protected against lysis induced by hypotonic solutions at different extract concentrations, especially the largest, The protective effect is greater than that of sodium diclofenac at a concentration of 200 µg/ml and above.

Membrane-stabilizing attributes were acknowledged for their power to interpose with release of phospholipases that activate the establishment of inflammatory intercessors **[12]**. During inflammation, lysosomal enzymes and hydrolytic components are released from the phagocytes to the extracellular space, which causes damages of the surrounding organelles and tissues and also assists a variety of disorders **[13]**. Hence, methanol extract of Cyclamen africanum act as an anti-inflammatory agent.

Antifungal Test

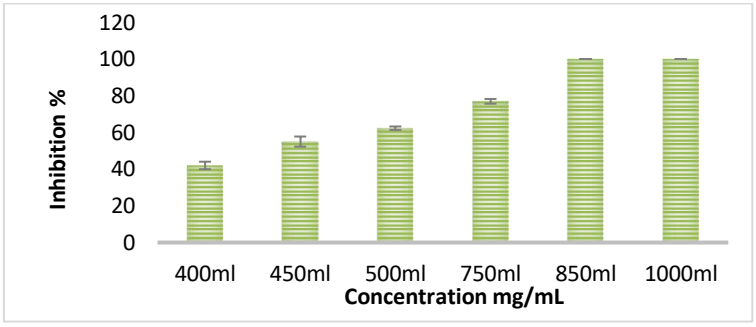

Figure3 : Inhibition rate of Trichophyton Rubrum treated with methanol extract of *Moricandia suffruticosa* L.

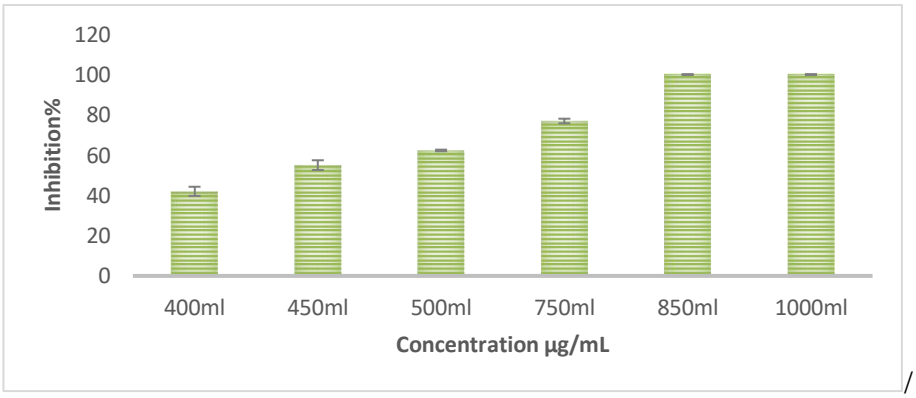

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Figure4 : Inhibition rate of microsporic gypsum treated with methanol extract *Moricandia suffruticosa* L

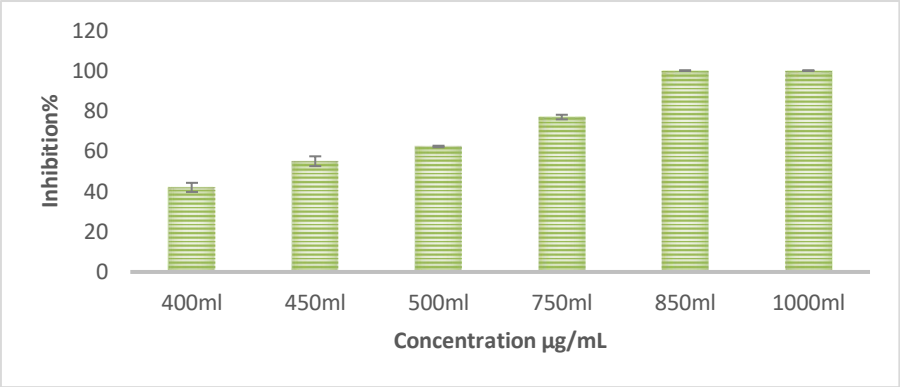

Figure 5: Inhibition rate of Microsporum audouinii treated with methanol extract of *Moricandia suffruticosa* L

The results (Figure 3.4.5) showed that the maximum inhibitory activity of the three pathogenic fungi was observed with methanol extract at 750 µg/mL (excluding 850 and 1000 µg/mL concentrations that have the same 100% inhibition rate). This method has shown efficacy in extracting active compounds that have antifungal properties.

The results of the inhibition tests of the three strains (M. audouinii, M. gypseum and T. rubrum) clearly indicate that the methanol extract tested has an antifungal action. This confirms our hy-pothèse which proposes the inhibitory action of *Moricandia suffruticosa* L.

on dermatophytes. In addition, the observed antialcoholic activity suggests that *Moricandia suffruticosa* L.

contains active compounds on dermatophytes which may be results for drug design.

Exrtait shows that at 500 µg/mL the mycelial growth rate was significantly reduced, and at this level the growth was halved (50%). The 500 µg/mL dose is in fact the lowest inhibitory concentration, which is the lowest concentration with minimal growth.

CONCLUSION

The results of this study showed that polyphenol and flavonoids extracted from *Moricandia suffruticosa*L., present potential antioxidant, antiinflamatory and antifungal activities. These

results indicate that the selective extraction of naturally occurring bioactive molecules such as endemic species, with appropriate techniques, can provide high-activity products that could be used as an alternative to the synthetic molecule with the aim of reducing pollution and healthier and economic sides.

The results serve as a scientific basis for the further development of these extracts into new medicinal and agronomic products.

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