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ANTI-INFLAMMATORY, ANTI-OXIDANT AND ANTIBACTERIAL ACTIVITY OF THE METHANOL EXTRACT OF *AMMODICUS LEUCOTRICUS*

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ABSTRACT

People have always been interested in plants for treatment, because of the high cost of modern medicine. This work was carried out to study the antioxidant, the aerial parts of Ammodicus leucotricus' methanolic extract contain anti-inflammatory and antibacterial properties.

Antioxidant activity was tested by the DPPH method while the HRBC technique (human red blood cell membrane stabilization) had anti-inflammatory effects. The results obtained show an excellent anti-inflammatory activity, a good inhibition of bacteria, and a moderate antioxidant activity, the antibacterial activity was evaluated against bacterial strains (Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli). The plant shows high activity against all types of bacteria. The obtained results of the antibacterial application appear to be very promising materials for the treatment of diseases caused by bacteria

Keywords: *Ammodicus leucotricus.* Anti-inflammatory, Antioxidant and Antibacterial.

1. INTRODUCTION

People have always been interested in plants to treat and cure all kinds of diseases. About 65-80% of the world's population rely due to the exorbitant expense of contemporary therapies, rely mostly on traditional medicine to address their healthcare needs1.The therapeutic virtues of plants are a result of substances created by the plants themselves, which are referred to as secondary metabolites. These metabolites include polyphenols. Which have antibacterial effects, as they safeguard plants against growths, microbes, creatures and, surprisingly, different plants[2]. They also have a few organic exercises that are straightforwardly connected with human wellbeing. They are utilized in chemotherapy and in the therapy of disease[3].

Algeria, given its privileged biogeographic position and its extension between the Mediterranean and sub-Saharan Africa, is considered among the countries known for their floristic diversity[4], to which is added a secular tradition of traditional use of plants. There are 3000 species of plants of which 15% are endemic. This important number of medicinal plants includes thousands of species of various interests and constitutes a particular axis of scientific research[5].

The present research focused on the study of the medicinal and local plant, namely, *Ammodicus leucotricus*

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2. MATERIALS AND METHODS

2.1. Plant material and extraction

The plant of A*mmodicus leucotricus* was collected from the region of Abadla of Bechar (Algeria) in the month of Mars 2021. The plant was identified by Professor Mohamed Terras teaching in the Department of Biology, University of Saida, Algeria. The aerial part of the plant was air dried and then reduced to powder. Five hundred (500) grams of powder were cold macerated in 2.5 L of methanol for 72 hours and shaken regularly. The solution was then decanted and the extract filtered from the solution and evaporated to dryness at 40°C.

2.2. Determination of Total Phenolic Content

To calculate the level of total polyphenols, the Folin-Cioalteu strategy was utilized6. The examples (0.2 ml) were blended in with 1 ml of Folin-Ciocalteu reagent created with 10 ml of deionized water. After 4 minutes of rest at 25°C, 0.2 ml of saturated solution was added with sodium carbonate (75 mg/ml). The blended arrangements were permitted to represent 120 minutes before the absorbance was estimated at 765 nm. Gallic corrosive was utilized as the norm for the adjustment bend. Absolute phenolic content was communicated as mg gallic corrosive identical per gram of concentrate (mg GAE/GE).

2.3. Determination of total flavonoids contents

According to the experimental protocol of[7], 1 mL of the methanolic arrangement of the concentrate was added to 1 mL of 2% AlCl3 in methanol. Following 10 minutes, the absorbance at 430 not set in stone with quercetin as standard. The outcomes got were communicated in mg quercetin comparable per gram of concentrate (mg QE/GE).

2.4. DPPH Assay

According to the experimental protocol of[8], the limit of the concentrate was estimated by blanching the variety answer for the 1, 1-diphenyl-2picrylhydrazyl (DPPH) revolutionary. One milliliter of concentrate at various focuses was added to 0.5 ml of DPPH methanol arrangement. The mixtures were mixed and kept in the dark at laboratory temperature. The absorbance of the chose arrangements was estimated at 517 nm. This enemy of radiation action was communicated as IC50 (micrograms per milliliter). The ability to trap the DPPH revolutionary was determined utilizing the accompanying condition[9]:

DPPH rummaging impact (%) = [(A0 - A1)/A0]

Where:

A0: the absorbance of the control at 30 minutes, A1: is the absorbance of the example at 30 minutes. BHT was utilized as standard.

2.5. Anti-inflammatory activity

The evaluation of the anti-inflammatory activity in vitro by the method of stabilization of the membrane of human red blood cells (HRBC) described by[10] was used to evaluate the anti-inflammatory effect in vivo of the hexanic extract. The standard is the adjustment of the film of human red platelets by hypotonicity-initiated layer lysis and for this purpose 10 ml of human blood centrifuged at 3000 rpm for10 minutes and washed with an equivalent volume of ordinary saline. The volume of blood was estimated and reconstituted as a 10% v/v suspension with saline.

The rule here was adjustment of the human red platelet layer by hypotonicity-actuated film lysis. The solution contained 2 mL of hypo saline (0.36%), 1mL of phosphate buffer (pH7.4,0.15 M), 0.5 mL of HRBC suspension (10% v/v), and 0.5 mL of plant concentrate or reference arrangement (diclofenac sodium) at a few focuses (10, 50, 100,250, 500 µg/ml). The control was refined water. The arrangements were brooded at 37°C for 30 minutes and centrifuged at 2500 rpm for 5 minutes. The absorbance of hemoglobin determined as follows

Hemolysis (%) = (optical thickness of test/optical thickness of control) ×100

2.6. Antibacterial activity

Evaluation of the antibacterial activity against several bacterial strains (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis*, and *Bacillus subtilis*) as per the convention of [11]. 0.5 ml of societies were weakened in 20 ml of Muller Hinton fluid. The last option, evidently strong, was softened by warming and afterward cooled prior to being carried into contact with the bacterial suspension. The combination was put in 90 mm Petri dishes. 7 mm width wells were diving in the Muller Hinton agar filled sterile Petri dishes. 30 mg of the extract were poured into the wells. Then, the antibacterial activity is determined after incubation by the zones of inhibition of each sample around the spots at regular intervals after 24 hours of incubation, at 37 °C, were noted macroscopically[12].

2.7. Statistical analysis

The information examination was performed utilizing Microsoft Office Succeed 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.

3. RESULTS AND DISCUSSION

3.1. Plant extraction

The results obtained by the extraction method have a very low yield containing 4,01± 0,29 mg EAG/GE of polyphenols and 2,413± 0,05 mg QG/GE of flavonoids.

3.2. Antioxidant activity

The DPPH radical has been widely used as a model system for studyingtrapping several natural compounds[13].Low antioxidant activity was observed in ourextract (IC50=82.26 ± 19.162 µg/mL) against BBW (11.76± 0.46 µg/mL).BHT remains the most effective antioxidant in evaluation with the methanolic extract (Figure 1).

3.3. Anti-inflammatory activity

The anti-inflammatory activity of *Ammodicus leucotricus* extract was proven by the human red blood cell membrane stabilization test. The results indicate that erythrocyte membranes resisted lysis caused by hypotonic solution at different concentrations. The outcomes uncover that human erythrocyte layers were safeguarded against hypotonic arrangement instigated lysis at various concentrate focuses, including the highest, the protective effect is superior to that of sodium diclofenac at a concentration of 200 µg/ml and above. Membrane stabilizing attributes have been recognized for their interposing power with the arrival of phospholipases that initiate the foundation of provocative arbiters[14].During the incendiary response, lysosomal catalysts and hydrolytic parts are delivered by phagocytes into the extracellular space, which causes organelle and tissue damage and also promotes several disorders[15]. Therefore, the extract has anti-inflammatory properties.

3.4. Antibacterial activity

The antibacterial power of the extract was tested on gram-negative (*Escherichia coli, Pseudomonas aeruginosa*), and gram-positive (*Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis*) bacteria. The inhibition halos are displayed in Table 1.

Studies on the mechanisms of action of this activity are negligible. To date, no study allows us to have a clear and precise idea on the mechanism of action of methanolic extracts.Given the complexity of their chemical components, this mechanism of action is relatively complex and difficult to determine from a molecular point of view. It is very likely that each of the constituents of the extracts has its own mode of action, but in general, their action occurs in three phases:

- destruction of the bacterial wall, leading to an increase in porosity, then the escape of cellular components.
- Decrease of the intra-cellular pH, preventing the production of cellular energy and the formation of new structural components.
- Destruction of the DNA, leading to the death of the bacterial cell.

The mode of action of methanolic extracts also depends on the type of microorganisms[16]. Our study extract could become a natural antimicrobial agent and has potential in the food and pharmaceutical industries.Variations in chemical composition can certainly account for the discrepancies found in the antimicrobial activity of extracts from identical or different plant species. The ideal viability of a concentrate may not be because of a solitary dynamic part, but to the result of the synergy of the various components of the extract[17].Several works have highlighted the high sensitivity of Gram (+) bacteria compared to Gram (-)[18,19],This phenomenon may be due to the nature of the outer layers of Gram (-) and Gram (+) bacteria. This work is not in agreement with our results which showed resistance of E. coli against the extracts studied.Studies have suggested that polyphenols and flavonoids are characterized by antimicrobial properties[20]**.** However, the antibacterial tests carried out on our EAOF extracts reveal the presence of an inhibitory effect against the growth of the germs studied (Staphylococcus sp, pseudomonas ...etc.).*A. leucotrichus* is a plant used for medicinal and culinary purposes by indigenous populations. Its main uses are against: stomach ache, indigestion, diarrhea, vomiting, spasms and colic, intestinal worms and constipation[21,22,23].

The plant is also used for the treatment of allergy symptoms. It is also used against cough, as an emmenagogue and against anorexia[24]. As an infusion, *A. leucotrichus* fruits are used for the treatment of heart palpitations[25]. A recent study by[26] reports that some populations in Morocco use *A. leucotrichus* fruits for the treatment of lung cancer in the form of a powder mixed with honey and administered orally.Local herbalists told us that the plant is also used for treatment of diabetes. Very few phytochemical studies have been reported on *A. leucotrichus* species. Indeed, the study made by[27] on the ethereal fraction of the fruits of this species revealed the presence of several compounds, namely: ammo lactone, limonene, peril aldehyde, hydroxyl perillaldehyde, methyl-

perillate, borneoleangelate and a decalactone. Another study revealed the protective effect of the aqueous extract of the fruits of *A. leucotrichus* fruits against urinary lithiasis tested in vitro. High percentages of inhibition were reported[28]. The plant is used in decoction for the treatment of diabetes, fever and digestive disorders, particularly in children[29]. Specific pain and inflammation models have been developed to detect and evaluate the anti-inflammatory and analgesic potential of *Ammodicus leucotricus* extracts.

The Koster test is considered a model of visceral pain, as acetic acid-induced pain is similar to peritonitis[30]. This test is frequently used to detect substances with a peripheral analgesic effect such as NSAIDs. With this method, our results showed that the extract has an anti-inflammatory effect and the mechanisms involved in the nociceptive reaction are related to arachidonic acid metabolism and prostaglandin biosynthesis by cyclooxygenase[31]. Based on these data, the inhibitory effect of the nociceptive response to this chemical stimulus is probably due to the inhibitory action of the tested extracts on cyclooxygenase and prostaglandin release. We can suggest that our extract acts at the peripheral level.

4. CONCLUSION

The results of this study showed that polyphenol and flavonoids extracted from *Ammodicus leucotricus*., present potential antioxidant, anti-inflammatory and antibacterial activities. These results indicate that our plant 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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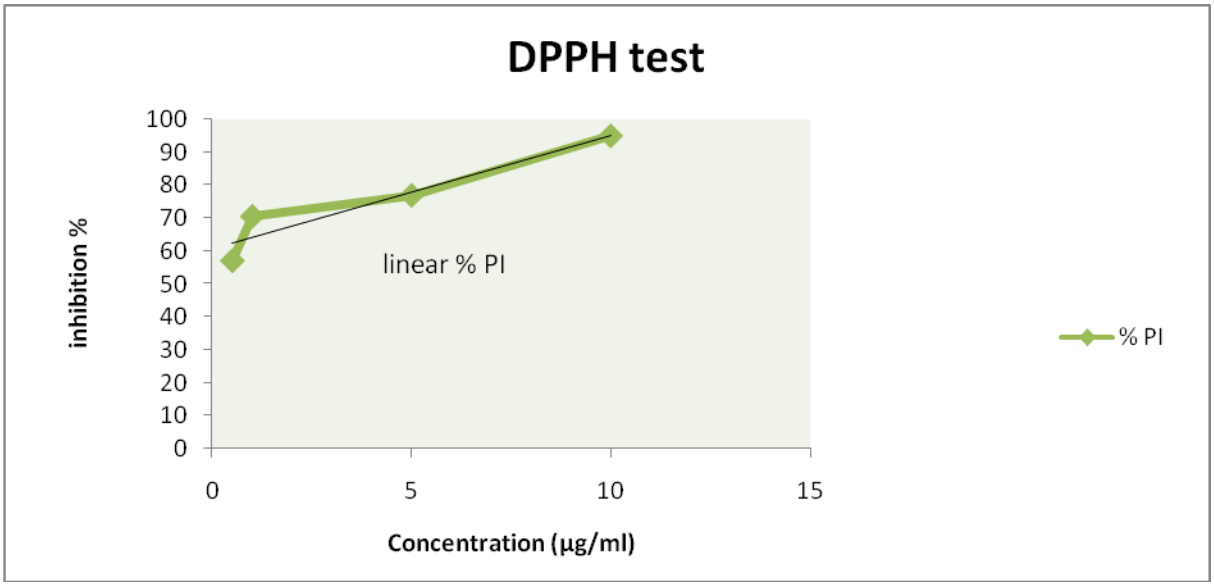
Table 1: Antibacterial test results of methanol extract of Ammodicus leucotricus Inhibition zone size (mm)

	E. coli (a)	*S. aureus* (b)	*E. faecalis* (c)	*B. Subtilis* (d)	*P. Aeruginosa* (e)
Methanol Extract	17	16	19	24	32



IC50=82.26±19.162 µg/ml, BBW (11.76±0.46 µg/ml)

Fig. 1: Variation curve of the percentage of inhibition as a function of the ascorbic acid concentration

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