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Evaluation of *in vitro* anti-inflammatory activity of *Helichrysum italicum* (Roth) G. Don essential oil

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ABSTRACT

The present experiment was conducted to assess the anti-inflammatory property of essential oil of the aerial parts of *Helichrysum italicum* (Roth) G. Don against the denaturation of protein *in vitro*. The test oil, at different concentrations, was incubated with egg albumin under controlled experimental conditions and subjected to determination of absorbance to evaluate the anti-inflammatory property. Diclofenac sodium was used as the reference drug. The present results exhibited a concentration dependent inhibition of protein (albumin) denaturation by the test oil. The outcome of diclofenac sodium was found to be less effective when compared with the test oil. From the present findings it can be concluded that the *Helichrysum italicum* (Roth) G. Don essential oil possessed marked anti-inflammatory effect against the denaturation of protein *in vitro*. This effect could be either due to Polyphenolic content or to the synergistic activity rather than a single compound.

Keywords: Anti-inflammatory, *Helichrysum italicum* (Roth) G. Don, essential oil, protein denaturation, diclofenac sodium.

INTRODUCTION

Inflammation is a pathophysiological response of living tissue to injury that leads to the local accumulation of plasmatic fluid and blood cells. Even though it is a defense mechanism that helps body to protect it-self against infections, burns, toxic chemicals, allergens or other noxious stimuli; the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases [1].

It is a very common symptom of many chronic diseases such as arthritis, osteoarthritis, inflammatory bowel disease, and chronic asthma which put enormous burden on the economy of the countries. The prevalence of inflammatory diseases is on rise across the world, mostly affecting elderly population [2].

Drugs that are currently used for the management of inflammatory are non-steroidal anti-inflammatory drugs and corticosteroids. All these drugs carry potential toxic effects. One study suggests that risk of gastrointestinal bleeding was significantly associated with acute use of non-steroidal anti-inflammatory drugs like regular dose aspirin, diclofenac, ketorolac, naproxen or nimesulide. Piroxicam increased the risk of bleeding in both acute and chronic therapy [3]

Apart from the synthetic drugs, the number of plants has significant potential to inhibit inflammatory diseases, because it contains a multitude of different molecules that act synergistic all on targets of the complex physiological pathways and have found widely used because of several reasons such as awareness of plant-based remedies, the lower cost of phyto-therapy, the concern with the possible side effects of allopathic medicine, phyto-constituents are having low animal and human toxicity and have a higher bioavailability and therefore higher

protective efficacy than synthetic drugs [4]. Hence it is a subject of time to invent and evaluate more and more herbal drugs with high therapeutic activity, bioavailability and less toxicity.

The genus *Helichrysum*, belonging to the family of *Asteraceae*, consists of a few hundred species widespread throughout the world. In antiquity, their flowers commonly known as gold-everlasting or eternal flowers were used to make the wreaths to crown idols. *Helichrysum italicum* (Roth) G. Don (everlasting) is a typical Mediterranean plant [5].

In fact, the essential oil is present in all green parts of the plant. Everlasting and its extracts are used in popular medicine in the Mediterranean region. This plant is known for its anti-inflammatory, anti-allergic, and antimicrobial activity [6]. In our previous study, we have reported chemical composition and antimicrobial activity of *Helichrysum italicum* (Roth) G. Don essential oil. The present experiment was intended to assess the anti-inflammatory property essential oil of the aerial parts of *Helichrysum italicum* (Roth) G. Don against the denaturation of protein in vitro.

MATERIALS AND METHODS

Plant Material

Aerial parts of *Helichrysum italicum* (Roth) G. Don were collected in June 2013 during the flowering period from Béjaia (North Algeria). After being dried out at the laboratory temperature and obscurity, the plant material was cut to small pieces with a universal knife.

Extraction of the essential oil

A 100g of the air-dried aerial parts of plant was subjected to hydrodistillation for the duration of 3 hours with 500ml distilled water using a Clevenger-type apparatus in laboratory of Natural Resources Valorization, University Ferhat Abbas Setif 1, Algeria. The oil obtained yielded 0.44% was collected and stored in a screw capped glass vials in a refrigerator at 4–5°C prior to analysis.

Drugs and chemicals

Diclofenac sodium was obtained from Group SAIDAL, Algeria. Distilled water from all glass still was used throughout the present study.

Anti-inflammatory bioassay in vitro

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of the essential oil of *Helichrysum italicum* (Roth) G. Don so that final concentrations become (35.2, 70.4, 140.8, 281.6, 563.2, 1126.4 µg/ml). Similar volume of distilled water served as control. Then the mixtures were incubated at 37±2°C in an incubator for 15 minutes and then heated at 70°C for 5 minutes. After cooling down, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the final concentration of (78.125, 156.25, 312.5, 625, 1250, 2500 µg/ml) was used as reference drug and treated similarly for determination of absorbance [7]. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times [\text{abs}_t / \text{abs}_c - 1]$$

Where, abs_t = absorbance of test sample, abs_c = absorbance of control.

The oil /drug concentration for 50% inhibition (IC₅₀) was determined from the dose response curve by plotting percentage inhibition with respect to control against treatment concentration.

Statistical analysis

Statistical analysis was done using one way analysis using ANOVA followed Dunnett's test. P<0.05 were considered as significant. Values are expressed as Mean±SD (n= 3).

RESULTS AND DISCUSSION

The present investigation reports the in vitro bioassay of anti-inflammatory effect of *Helichrysum italicum* (Roth) G. Don essential oil, against denaturation of egg albumin. The results were summarized in Table 01 and 02 and IC₅₀ values summarized in Table 03. However, the diclofenac sodium was found to be less effective when compared with *Helichrysum italicum* (Roth) G. Don essential oil. This was consequently confirmed by comparing their IC₅₀ values. *Helichrysum italicum* (Roth) G. Don essential oil possessed IC₅₀ value 296 µg/mL whereas that of diclofenac sodium was found to be 590 µg/mL.

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of tissue proteins is one of the well-documented causes of inflammation[8].

The increments in absorbance of test sample with respect to control indicated stabilization of protein i.e., inhibition of protein (albumin) denaturation or anti-denaturation effect by the test extract and the reference drug diclofenac sodium[9]. Polyphenols are well known natural products known to possess several notable biological properties[10]. Three phenols (thymol, eugenol and 3-isopropylphenol) and twenty seven volatile carboxylic acids were identified by Josip Mastelić *et al.*, [5] of *Helichrysum italicum* (Roth) G. Don essential oil.

Polyphenols are well known natural products to possess several notable biological properties[10]. In the present study, the *in vitro* anti-inflammatory activity of *Helichrysum italicum* (Roth) G. Don essential oil can be attributed to its polyphenols content. The effect may be due to the synergistic effect rather than single constituent. It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat treated albumin at the physiological pH (pH: 6.2-6.5)[11].

Table1. Effect of *Helichrysum italicum* (Roth) G. Don essential oil against protein denaturation.

Concentration (µg/ml)	% Inhibition
Control	-
35.2	7.48
70.4	20.3
140.8	32.74
281.6	47.46
563.2	55.62
1126.4	94.86

Table2. Effect of diclofenac sodium against protein denaturation.

Concentration (µg/ml)	% Inhibition
Control	-
78.125	13.11
156.25	13.24
312.5	26.48
625	52.96
1250	205.92
2500	211.84

Table3. IC50 values of *Helichrysum italicum* (Roth) G. Don essential oil and diclofenac sodium against protein denaturation.

Treatments	IC50 values (µg/ml)
<i>Helichrysum italicum</i> essential oil	296
Diclofenac sodium	590

CONCLUSION

In sum, from the results achieved in the present preliminary study it can be concluded that *Helichrysum italicum* (Roth) G. Don essential oil possessed marked *in vitro* anti-inflammatory effect against the denaturation of protein. Further definitive studies are necessary to ascertain the mechanisms and constituents behind its anti-inflammatory actions.

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