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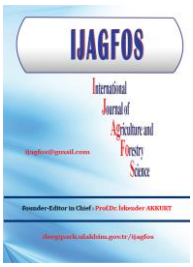

Research Article

Analgesic and antioxidant activities of the flavonic extract of *Globularia alypum* L.#

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Abstract: *Globularia alypum* L. is an aromatic and medicinal plant belonging to the Globulariaceae family widely used for its therapeutics virtues. The present study evaluates flavonic extract from aerial part of *Globularia alypum* L for its analgesic activity by using acetic acid induced writhing test and for its antioxidant capacity using DPPH free radical scavenging assay method. The results had shown that *Globularia alypum* L. was found to exhibit higher analgesic activity against acetic acid induced pain in mice and reduces the number of writhing induced by acetic acid with a percentage analgesic activity was 95.5% compared to the effect of the standard paracetamol (91%). Extract also exhibited excellent antioxidant activity in DPPH radical scavenging activity. In this study, we conclude that the extract of plant possess analgesic activity and provides the traditional uses of Globularia alypum L as analgesic.

1. Introduction

According to the International Association for the Study of Pain (IASP), pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" [1]. Opoids and non-steroidal anti-inflammatory drugs (NSAIDs) are analgesics used to treat or reduce pain. However, these drugs cause serious side effects. NSAIDs usually cause gastrointestinal disorders and opioids analgesics are associated with a lot of side effects ranging from constipation, dry mouth, vomiting, nausea and respiratory depression. Due these drawbacks of analgesics drugs, there is a important need for the search for bioactive compounds from medicinal plants for use as alternative analgesics with a few or no side effects [2-4]. *Globularia alypum* L. locally named''Tasselgha''is an aromatic and medicinal plant belonging to the Globulariacea family, commonly used in Algerian folkloric medicine. It is a perennial shrub which is found throughout the Mediterranean area, known for their antimicrobial, antitumerous [5], anti-inflammatory, antidiabetic and cardiovascular diseases [6]. Recently, ethnobotanical investigation on plants in Algeria had shown other ethnomedicinal uses of *Globularia alypum* L., which was used to gynecological diseases, blood purification, antiseptic, antifungal and respiratory system diseases [7]. This specie is also considered to treat various cancerous lesions for stomach, colon, rectum and osophageous [6]. Polyphenols are an important class of secondary metabolites of plant possessing a wide range of biological effect such as antioxidant, antimicrobial, anti-inflammatory and vasodilatory actions [8]. Actually, many researches are focused on natural antioxidants from fruits and medicinal plants because of their interest for human health. Several herbs were found to exhibit antioxidant properties, which are mainly attributed to a variety of active

natural antioxidants including flavonoids, alkaloids, anthocyanins, terpenoids and vitamins [9]. The aim of the present study is to estimate of total phenol and flavonoids content and to evaluate the analgesic and antioxidant activities of the flavonic extract of *Globularia alypum*L.

2. Material and methods

2.1. Plant material

The sample was collected from a mountainous region of Hammam Melouane situated at 36°29'00'' North, 3°03'00'' East, located approximately at 40 Km from south Algiers in North Algeria. Plant material has been identified and authenticated by botanic laboratory of EL Hamma (Algiers-Algeria) according to the Algerian flora and voucher specimen. The plant material was dried at room temperature and was ground further to obtain a fine powder, and then stored at ambient temperature in a dry and dark place until being used.

2.2. Extraction and isolation

*Globularia alypum*L.powder(100g) was extracted by maceration with ethanol/water (50%) at room temperature, in the dark for 24h. This procedure was repeated three times. After filtration, the residue was discarded and the solvent was evaporated to obtain the crude extract (CE) of *Globularia alypum*L.[10,11]. The crude extract was mixed with boiling distilled water and a decantation at room temperature for 12h was carried. The aqueous solution obtained was partitioned into three phases by various organic solvents: diethyl ether, ethyl acetate and n-butanol. The extraction series has yielded three fractions: diethyl ether extract (DEA), ethyl acetate extract (EAE) and n-butanolic extract (BuOH-E). All the solvent were removed by evaporation under vacuum and the extract were weighed and stored until use.

2.3. Total phenolic content

The total phenolic in each fraction of extract was determined with Folin-Ciocalteu reagent assay [12]. The results are expressed in mg of gallic acid equivalents per gram of extract (mg GAE/g E).

2.4. Total flavonoids content

The total flavonoid content in each fraction of extracts was determined, using a method based on the formation of a complex flavonoid–aluminium [12]. The total flavonoid content was expressed in microgrammes quercetine equivalent (QE) per g of extract (µg QE/g E).

2.5. DPPH radical scavenging activity assay

The antioxidant activity was evaluated using DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging activity assay according to the method described by Sanchez-Moreno [13]. Fifty microliters of various concentrations of extracts in methanol were added to 1950 µL of DPPH methanolic solution (0.025 g/L). The mixture was vigorously shaken and incubated at room temperature, in darkness for 30 min. The absorbance was measured at 517 nm. The DPPH radical scavenging activity (%) was calulated using the following formula:.

$$DPPH\ scavenging\ effect\ (\%) = (A_{control} - A_{sample} / A_{control}) \times 100 \quad (1)$$

Where:

$A_{control}$: absorbance of the control reaction;

A_{sample}: absorbance of the test compound.

The antioxidant activity of extracts was expressed in terms of concentration required to inhibit 50 % DPPH radical formation (IC50 mg/mL). Ascorbic acid (AA) was used as positive control.

2.6. Analgesic activity measurements

To the best of our knowledge, there are no reports in the literature concerning the analgesic activity of Algerian G*lobularia alypum*L. In our study, the analgesic activity of flavonic extract (butanolic fraction) was performed using acetic acid –induced writhing test [14,15]. Experiments were performed on Fifty four adults albino mice of either sexes weighing 19-21 g. All animals were housed in stainless steel

cage at temperature of 22± 2°C with a 12h light cycle and relative humidity of 50%. They were feed with standard diet and water ab libitum. Mice were selected one day prior to each test, and were divided into four groups of six mice each and then treated as follows: Groupe I served as the control and was treated with 10 mL/kg of vehicle administered through intra-peritoneal injection (i.p). Group II received Paracetamol (i.p) (100 mg/kg), while the group III and IV were treated with flavonic extract (butanolic fraction) (200 mg/Kg and 300 mg/Kg, respectively) by intra-peritoneal route. Thirty minutes later, all animals received 10 mL/kg (i.p.) of 1% acetic acid. After the injection of acetic acid, each animal was isolated in an individual box and abdominal writhings counted during a 10 min period starting 5min after acetic acid injection. Percent inhibition was calculated using the following formula:

Analgesic activity(%)= [(Mean number of writhings (control) – Mean number of writhings (test extract) / Mean number of writhings (control)] X 100
(2)

3. Results and discussion

3.1.Extract yield, total phenolic and flavonoid content

As shown in Table 1, butanolic extract showed the better yield (2.1%), comparing to ethyl acetate extract (0.5%) and diethyl ether extract (0.15%). The amount of total phenolic varied from 84±1.3 to 189 ± 2.25 mg GAE/g extract. The content of flavonoids varied from 1.47± 0.1 to 13.4 ± 0.21 µg EQ/g extract. The amount of total phenolic and flavonoid were higher to data reported in other study (21.54 mg GAE/g dw and 4.54 mg RE /g dw respectively) [16]. This difference can be attributed different factors such as type of cultivar, climate conditions and soil composition that could explain this variability [17]. Moreover, these results showed an important polyphenols content in *Globularia alypum* L.

3.2. Antioxidant assay

DPPH is widely used to test the ability of compounds to act as free radical scavenger or hydrogen donor to evaluate antioxidant activity. This method is based on the reduction of DPPH, a stable free radical [18]. A lower value of IC50 indicates a higher antioxidant activity.

Table1 : *Extract yield, Total phenolic and flavonoid content*

Extracts	Yield (%)	Total phenolic content (mgGAE/g extract)	Total flavonoid content (µg EQ/g extract)
BuOH	2.1	139 ± 1.82	13.4 ± 0.21
EAE	0.5	189 ± 2.25	11.6 ± 0.4
DEA	0.15	84 ± 1.3	1.47 ± 0.10

Table 2: *DPPH radical scavenging activity IC50 (mg/mL) of G.alypumL.*

Samples	IC50 (mg/mL)
Ascorbic acid	0.46
BuOH-E	0.9
EAE	0.57
DEA	10.9

As shown in table 2, the highest DPPH scavenging activity was found with ethyl acetat extract IC50 = 0.57 mg/mL followed by butanolic extract IC50= 0.9 mg/mL. However, the scavenging effect of diethyl ether extract was poor with IC50 value of 10.9 mg/mL. This study determined that extract of aerial part of *Globularia alypum* L. showed a better antioxidant potential by DPPH radical scavenging method when compare to standard ascorbic acid IC 50 value of 0.46 mg/mL . Also, we deduce that extracts possessing higher antioxidant activity also had higher phenols content. According to khlifi [19], polyphenols were reported to have an important role in stabilizing lipid peroxidation and are associated with antioxidant properties due to their redox properties, as reducing agent or hydrogen atom donors.

3.3. Analgesic activity

As shown in table 3, the both dose of flavonic extract (butanolic fraction) and paracetamol pre-treatment animals reduced the painful response produced by injection of acetic acid, manifested as writhings.

Table 3: *Analgesic activity of the flavonic extract(butanolic fraction) of Globularia alypumL. on acetic acid induced writhing method in mice*

Groups	Number of writhings	Inhibition percent (%)
Control	11.7	-
Standard (paracetamol)	1	91.05
Butanolic fraction (200mg/kg)	7.2	35.9
Butanolic fraction (300mg/kg)	0.5	95.5

The results had shown that *Globularia alypum* L.was found to exhibit higher analgesic activity against acetic acid induced pain in mice and reduces the number of writhing induced by acetic acid with a percentage analgesic activity was 95.5% compared to the effect of the standard paracetamol (91%).The constrictions induced in mice by acetic acid results from an acute inflammatory reaction with production of PEG2 and prostaglandin F2 in the peritoneal fluid as well as lipoxygenase products [20].

4. Conclusion

In this study, flavonic extract (butanolic fraction) of *Globularia alypum* L. revealed strong analgesic and antioxidant activities.The analgesic activity of butanol fraction reduces the number of writhings induced by acetic acid compared to controls with a percentage of protection of 95.5%. We conclude that the extract of plant provides the traditional uses of *Globularia alypum* L. as analgesic.

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