Journal of Applied Sciences Research, 8(6): 2876-2880, 2012
ISSN 1819-544X
This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLES

Analgesic properties of secondary metabolites from Algerian *Centaurea pullata* and Greek *C. grisebachii* ssp. *grisebachii*

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ABSTRACT

Twenty five secondary metabolites isolated from *Centaurea pullata* L. and *C. grisebachii* (Nyman) Heldr. ssp. *grisebachii* were evaluated for their *in vivo* analgesic activity using acetic acid-induced abdominal pains. The metabolites mainly belonged to the classes of Sesquiterpene Lactones, Flavonoids and Lignans. Among the compounds tested 5-hydroxy, 6, 7, 3', 4'-tetramethoxyflavone (97%), arctigenin (89%), 8*α*-*O*-(4-hydroxy-2-methylen-butanoyloxy)-11*β*,13-dihydro-sonchucarpolide (88%), 8*α*-O-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)-4-*epi*-sonchucarpolide (86%) and cirsilineol (71%) showed a significant decrease in pain at 30 min post treatments with a dose of 1 mg/kg. The obtained results are of interest since they could be therapeutically useful for mitigating inflammatory pain.

***Key words**:* analgesic activity, *Centaurea pullata* L.; *C. grisebachii* (Nyman) Heldr. ssp. *grisebachii*; Sesquiterpene Lactones, Flavonoids, Lignans

Introduction

Pain was officially defined as an unpleasant sensory that is a consequence of complex neurochemical processes in the central and peripheral nervous systems (Musa *et al.,* 2006). It acts as a warning signal against disturbances of the body and has a proactive function. Typically, it is a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause (e.g. trigeminal neuralgia), or be persistent long after the precipitating injury has healed (e.g. phantom limb pain). It can also occur as a consequence of brain or nerve injury (e.g. following a stroke or herpes infection) (Paschapur *et al*., 2009). Though considerable progress has been achieved in medical science during the last decades, management of pain still remains a challenge for medical community (Raquibul Hasan *et al*., 2009; Basbaum and Field, 1984). Non-steroidal anti-inflammatory drugs and opioids are used in management of mild to moderate and severe pains respectively. However, these drugs have serious limitations due to their side effects. There is therefore, a need to intensify research with the aim of developing efficacious agents with low toxicity profile. Herbal plants are an important source of chemical substances with therapeutic potential that could be useful to treat a large variety of ailments and symptoms. Scientific evaluation of relevant medicinal plants that are used in traditional medicine offer some hope for developing new agents that may be suitable for the treatment of pathological complications such as inflammatory processes and pain. Many bioactive substances are involved in the modulation of pain sensation and eclectic physicians rely upon natural remedies to treat disease (Ebrahimzadeh *et al.,* 2006). Likewise, more and more people are turning to herbal medicines as the alternative treatment of pain.

Polyphenols and Sesquiterpene Lactones are chemical groups, which deserve special attention, since they have raised considerable interest because of their complex pharmacological actions (Heinrich, 2000). The possible medicinal contribution of Polyphenols to human health has long been studied. Among others, they have been reported to possess antioxidant, estrogenic, cytotoxic/antitumor activities, and inhibition of the cyclo-oxygenase and/or the 5-lipoxygenase pathways of arachidonate metabolism (Harborne and Williams, 2000; Pietta *et al*., 2003). Sesquiterpene Lactones as well are known to possess a considerable anti-inflammatory activity against different inflammation models and are reported as the active anti-inflammatory components of a variety of traditional remedies. It is suggested that the *α*-methylene-*γ*-lactone moiety in the molecule might be responsible from their anti-inflammatory effect (Negrete *et al*., 1993).

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J. Appl. Sci. Res., 8(6): 2876-2880, 2012

In the present study an attempt was made to investigate the pharmacological properties of two *Centaurea* species from different geographical origins. The objective was to evaluate the analgesic effect of 25 secondary metabolites isolated from *C. pullata* L. and *C. grisebachii* (Nyman) Heldr. ssp. *grisebachii*.

C. pullata is a biennial plant belonging to the section Melanoloma (Hellwig, 2004), with large terminal pink flowers, very variable in height, distributed from Spain to France and N. Africa. In Algeria, known under the common name "Achbet ennegar" (Trabut, 1935), it is a widely edible herb. It is used with other plants in the preparation of a local traditional dish called "El Hammama", which old women prepare in the beginning of the spring.

C. grisebachii ssp. *grisebachii* Heldr. & Sart. ex Boiss is a biennial shrub with purple florets, growing wild in N. and E. Greece. It belongs to the section Acrolophus (Cass.) DC. (Dostál, 1976). The herb is edible by the ruminants.

Materials and Methods

Plant material:

Aerial parts of *Centaurea pullata* were collected from Chréa Mountain in Blida (North Algeria) in April 2006 and were authenticated by Mr. Beloued Abd El Kader (INA of Algiers). A voucher specimen was deposited in the Herbarium of the Department of Biology, Environmental Laboratory, University of Annaba, under the code: Ann-BV 2006/0010. Aerial parts of *C. grisebachii* subsp. *grisebachii* were collected from mountain Olympus (Thessalia, Central Greece; alt. ca. 650 m), on July 2006. A voucher specimen is kept in the Herbarium of the Institute of Systematic Botany, Agricultural University of Athens (ACA) under the number Skaltsa & Argyropoulou 01.

Phytochemical analysis:

As previously described (Djeddi et al., 2007, 2008a, 2008b).

Analgesic activity investigation:

Animals:

Swiss mice (18-22 g) were obtained from Pasteur Institute (Tunis, Tunisia). They were housed in polypropylene cages and were kept in a room maintained under controlled conditions. All animals were fed with a standard diet *ad libitum* and had free access to drinking water.

Analgesic activity:

The analgesic activity was performed according to the method of (Koster, 1959). Swiss mice were selected one day prior to each test and were divided into three groups of six mice each. One group served as the control and was treated with 10 ml/kg of saline administered through intra-peritoneal injection (i.p.). Aspirin (200 mg/kg) was administered (i.p.) in the second group, while the third group was treated with all compounds (i.p.) at a dose of 1 mg/kg. All animals received 10 ml/kg (i.p.) of 1% acetic acid 30 min after treatment. The number of abdominal writhing was recorded for a period of 30 min.

Statistical analysis:

All data are represented as mean ± SEM and as percentage. Results were statistically evaluated using Student's *t*-test. $P<0.05$ was considered significant.

Results:

Acetic acid-induced writhing in mice was used as the process to evaluate the analgesic activity. This test is widely used for the evaluation of peripheral antinociceptive activity (Shibata et al., 1989). The effects of acetic acid-induced abdominal constriction in mice 30 min post treatment with doses of 1 mg/kg are shown in Table 1. Aspirin was used as positive control. Twenty five natural products were tested. Fifteen of them belonged to Sesquiterpene Lactones, six to Flavonoids, two to Lignans, one to Isoprenoids, and one to Triterpenes. The most active compounds were found to be 5-hydroxy, 6, 7, 3', 4'-tetramethoxyflavone (20) with an inhibition of 97%, followed by arctigenin (22) (89%), 8α-O-(4-hydroxy-2-methylen-butanoyloxy)-11β,13-dihydro-sonchucarpolide (4) (88%), 8α-O-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)-4-epi-sonchucarpolide (14) (86%) and

cirsilineol (18) (71%). As it is noticed compounds belonging to sesquiterpene lactones and polyphenols rendered a decrease in pain.

Table 1: Effect of isolated compounds on the acetic acid induced writhing behaviour in mice.

Treatments	Doses (mg/kg)	Number of writhings	% of inhibition
Control	-	140 ± 6.5	-
1	1	80.2 ± 6.2	50
2	1	65.8 ± 3.5	60
3	1	99.5 ± 4.1	32
4	1	20.4 ± 3.2	88
5	1	60.5 ± 4.5	63
6	1	106.6 ± 6.3	34
7	1	64.8 ± 3.2	63
8	1	130.5 ± 5.2	20
9	1	59.3 ± 5.2	63
10	1	58.3 ± 2.6	64
11	1	76.5 ± 2.3	53
12	1	169.4 ± 6.3	10
13	1	66.5 ± 5.3	59
14	1	22.6 ± 3.6	86
15	1	114.6 ± 5.3	30
16	1	77.2 ± 5.6	52
17	1	66.5 ± 6.3	59
18	1	47.2 ± 3.6	71
19	1	107.8 ± 4.3	34
20	1	5.8 ± 2.3	97
21	1	85.4 ± 5.2	47.5
22	1	18.5 ± 3.6	89
23	1	103.5 ± 6.3	36
24	1	62.5 ± 3.6	62
25	1	129.5 ± 6.3	20
Aspirin	200	12.89 ± 3.24	92.57

Discussion:

The method is very sensitive and able to detect antinociceptive effects of compounds at dose levels that may appear inactive in other methods like the tail flick test (Bentley *et al*., 1981). Acetic acid causes a painful reaction and acute inflammation emerges in the peritoneal area which is accompanied with an increase in peritoneal fluid levels of prostaglandins (PGE2 and PGF2), involving in part peritoneal receptors and inflammatory pain (Deraedt *et al*. 1980). Therefore, a possible mechanism of action of the active compounds could be the inhibition of inflammatory mediators, such as prostaglandins, suggesting that the molecules are antagonists of cyclo-oxygenase or other enzymes involved in the arachidonic acid cascade (Heinrich, 2000). They could also block pain sensation or might exert other specific mechanism to counteract the pain induced by acetic acid (Asongalem *et al*., 2004; Simmons *et al.,* 2004).

Herbs containing Sesquiterpene Lactones are frequently used by Mexican Indians for treatment of stomach-ache, infection of the skin and other organs (Heinrich *et al*., 1998, 2000). Some researches show that several *Centaurea* species, which are very rich in sesquiterpene lactones, are used to alleviate pain and inflammatory symptoms in rheumatoid arthritis, high fever and headache in folklore medicine. As an example, *C. chilensis* is used as antipyretic and anti-rheumatic (Kobayashi *et al*., 2000; Sepulveda *et al*., 1994) while some of its components have been reported to possess anti-inflammatory activity (Negrete *et al*., 1993; Phillipson, 2001). Negrete *et al*. (1993) have shown that a mixture of two elemanolide esters from the aerial parts of *C. chilensis* are the active components of the traditional remedy which is prescribed against gout and rheumatism. Also, the aqueous extract of *C. ainetensis* has anti-inflammatory effect (Talhouk *et al*., 2008). Sesquiterpene lactones are not the only active anti-inflammatory components of *Centaurea* sp. Karamenderes et al. (2007) studied the effect of *n*-hexane, chloroform and methanol extracts from eight *Centaurea* sp. and found that three of them (*C. cadmea*, *C. calolepsis* and *C. hierapolitana*) possess potent *in vitro* inhibitory activity on NF-kB. They postulated that flavonoids might be the active components of these plants.

Conclusion:

In conclusion, and according to the results obtained in this research, the writhing test in mice revealed that several secondary metabolites isolated from both species tend to elicit significant analgesic activity. The results of the present study support the traditional assertion of *C. pullata* in Algerian folk medicine (Beniston and Beniston, 1984). These findings suggest a potential benefit of metabolites isolated from *Centaurea* species in treating conditions associated with inflammation pain. Further studies are on the way to elucidate the underlying mechanisms of action.

Acknowledgements

The authors wish to thank Prof. H. Skaltsa for her supervision during the isolation and structural elucidation of the secondary metabolites from both plants, as well as Dr. A. Karioti for her assistance at the analytical techniques and spectroscopic measurements.

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