Antioxidant, Analgesic and Anti-Inflammatory Effect of Matricaria Chamomilla L. Aqueous Extract

Karima Loucif [1*], Radia Mebarki [2], Asma Youmbai [3], Mohammed Said Nedjimi [4], Hana Nedjma Belabed [5], Ilyes Zatla [6], Wafaa Lemerini [7], Chawki Bensouici [8], and Smain Amira [1]

[1]Laboratory of Phytotherapy Applied to Chronic Diseases, Department of Animal Biology and Physiology, Faculty of Nature and Life Sciences, University of Setif 1, Setif 19000, Algeria.

[2]Laboratoire de Biologie Animale Appliquée, Département de Biologie Faculté des Sciences, Université Badji Mokhtar Annaba, BP 12, 2300 Annaba, Algérie.

[3]Departement of Cellular and Molecular Biology, Faculty of Natural and Life Sciences, University of El Oued, 39000 El-Oued, Algeria.

[4]Laboratory of Valorization and Promotion of Saharan Resources (VPRS), University of Kasdi Merbah Ouargla, BP 511 Route Ghardaia, Ouargla 30000, Algeria.

[5]Laboratory of Bioactive Molecules and Cellular Physiopathology (LMBPC), University of Batna 2, Algeria.

[6]Laboratory of Microbiology applied to the Food industry, Biomedical and the Environment, Faculty of Natural and Life Sciences, Earth and Universe Sciences, Department of Biology, University of Tlemcen, Algeria.

[7]Laboratory of Organic Chemistry, Natural Substances, and Analysis, Faculty of Natural and Life Sciences, Earth and Universe Sciences, Department of Biology, University of Tlemcen, Algeria.

[8]Biotechnology Research Center (CRBt), UV 03 BP E73, Nouvelle Ville Ali Mendjli, Constantine, Algeria.

Corresponding author: karimaloucif19@gmail.com ; loucif.karima@univ-setif.dz

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Abstract : *Matricaria chamomilla* L. is a famous medicinal plant distributed worldwide. It is widely used in traditional medicine to treat many diseases, including infections, neuropsychiatric disorders, as well as respiratory, gastrointestinal, and liver pathologies**.** The aim of this present work was to assess the *in vitro* antioxidant effects and the *in vivo* analgesic and anti-inflammatory activities of aqueous extract of aerial part from ***Matricaria chamomilla* L.** The antioxidant potency of the extract was evaluated by metal chelating, CUPRAC, and β-carotene bleaching methods. The anti-inflammatory effect of the extract was evaluated using the Croton-oil-induced ear edema and

xylene-induced ear edema in mice. The estimation of polyphenols and flavonoids showed that the MCAE contains a high amount of polyphenols and flavonoids: 108±1.22 mg Gallic acid equivalent and 75.72±1.06 mg Rutin equivalent/g of dried material, respectively. The results exhibited that MCAE had a good cupric reducing antioxidant, **metal chelating and β-carotene bleaching** activities. Oral administration of the extract produced good analgesic activities against acetic acid-induced abdominal constriction. The latter exerted significant dose-dependent anti-inflammatory activity in the xylene-induced ear edema and croton-oil-induced ear edema assays. MCAE had no significant effect against edematous response caused by croton-oil (58.75^{ns} % inhibition ; P ≥0.05) compared to indomethcin as standard drug (60.32% inhibition). The present study confirms the use of ***Matricaria chamomilla* L.** in traditional medicine as an anti-inflammatory agent. Our findings highlight the medicinal use of ***Matricaria chamomilla* L.** as an additional source of natural analgesic and anti-inflammatory agents.

Keywords: *Matricaria chamomilla* L., polyphenols**,** flavonoids, antioxidant, analgesic activity, anti-inflammatory effect.

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1. Introduction

Oxidative stress is the lack of balance between the occurrence of reactive oxygen/nitrogen species (ROS/RNS) and the organism's capacity to counteract their action by the antioxidative protection systems (Pisoschi and Pop, 2015). Free radicals can damage cells, and may play a role in cancer and other diseases (Yaseen et al., 2017), and antioxidants that can neutralize these free radicals may be used to protect the human body from illnesses. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which led to the consideration of naturally occurring antioxidants (Chung et al., 2005). For many years, herbal medicines have been, and are still used in developing countries as the primary source of medical treatment. Plant secondary metabolites such as polyphenols, play an important role in the defense against free radicals. Medicinal plants are commonly rich in phenolic compounds, such as flavonoids, tannins and lignans. The antioxidant properties of polyphenols are due to their redox properties, which allow them to act as reducing agents, hydrogen donators, metal chelators and single oxygen quenchers (Piluzza and Bullitta, 2011).

In traditional popular medicine, Chamomile (*Matricaria chamomilla*) is used in the form of tea, for gastric and intestinal inflammatory and painful diseases like diarrhea or the distensions (Franke and Schilcher, 2005; McKay and Blumberg, 2006). Also it is used as an anti-inflammatory drug and as a disinfectant. (Sachin et al., 2012). Currently, chamomile is used to treat all the disorders where the spasm occupies a significant place, such as in the case of painful digestive spasms (Julve, 2004). The aim of this study is to quantify the polyphenolic and flavonoids content of the aqueous extract from *Matricaria chamomilla* L. (MCAE) in addition to evaluate its antioxidant capacity *in vitro* and its analgesic and anti-inflammatory effect *in vivo*.

2. Material and Methods

2.1. Plant material

During the flowering period, the aerial parts of *Matricaria chamomilla* L. were collected from Setif in the North-East part of Algeria.

2.1.1. Preparation of the extracts

100 g of *Matricaria chamomilla* L. powder was decocted in 1L of boiling distilled water at 100 °C for 20 minutes. The mixture was filtered using Wattman filter paper n°1 and then dried at 45 °C to obtain *Matricaria chamomilla* L. aqueous extract which was stored at 20°C until further analysis (Ferreira et al., 2006).

2.2. Animals

Swiss albino mice weighting 20–30 g were used in this study, that were purchased from 'Institut Pasteur d'Algérie', Algiers. They were maintained in polycarbonate cages during 7 days in normal laboratory conditions (12/12 h light/dark cycle, 23 ± 2 °C with free access to food and water) before the beginning of the experimentation. The animal studies were conducted after obtaining clearance from Institutional Ethic Committee, and the experiments were conducted in strict compliance according to ethical principles and provided by Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA).

2.3. Phenolics content determination

2. 3. 1. Determination of total phenolic content (TPC)

TPC of the *Matricaria chamomilla* L. extracts was evaluated spectrophotometrically using the Folin–Ciocalteu reagent (Singleton and Rossi, 1965) 20 µl of a sample were blended with 100 µl of Folin-Ciocalteu reagent (1:10) and 75 µl of sodium carbonate solution (7,5%). The microplate was incubated for 2 h at room temperature in the dark. Absorbance at the wavelength of 765 nm was measured by using the microplate reader. The results were expressed as micrograms of gallic acid equivalents per milligram of extract (µg GAE/mg).

2. 3. 2. Determination of total flavonoids content (TFC)

The total flavonoid content of the *Matricaria chamomilla* L. extracts was determined spectrophotometrically according to the method described by Topçu et al., (2007) with some modifications to adapt it to the microplate. Briefly, 130 µl of methanol were added to 50 µl of samples. Subsequently, 10 µl of 1M potassium acetate (CH_3COOK) and 10 µl of 10% aluminum nitrate ($Al(NO_3)_3$, $9H_2O$) were added and the microplate was incubated at room temperature for 40 min. Absorbance was read at the wavelength of 415 nm. The values were presented as micrograms quercetin equivalents per milligram of extract (µg QE/mg).

2. 4. Antioxidant activities

2. 4.1. Cupric reducing antioxidant capacity (CUPRAC)assay

The CUPRAC was determined according to the method of Apak et al., (2004). In each of the 96 well, the reaction mixture containing 40 µl of sample and 50 µl of copper (II) chloride solution, 50 µl of neocuproine alcoholic solution, and 60 µl of ammonium acetate aqueous buffer at pH 7 was combined to give a final volume of 200 µl. After 30 minutes, the absorbance was measured at the wavelength of 450 nm. Results were recorded as absorbance ($A_{0.5}$) compared with the absorbance of BHT, which were used as antioxidant standards.

2.4.2. Metal chelating activity assay

The metal chelating activity by the ferene–Fe^{2+} complex assay was measured spectrophotometrically (Decker and Welch, 1990 ; Labed et al., 2016), with slight modifications. 40 µl of the extract were added to 40 µl of 0.2 mM of iron dichloride ($FeCl_2$) solution. The reaction was initiated after the addition of 80 µl of ferene solution (0.5 mM). The obtained mixture was shaken then incubated at room temperature for 10 min, and the ethylenediaminetetraacetic acid (EDTA) was used as standard. The absorbance was read at the wavelength of 593 nm, whilst metal chelating potential was estimated using the following equation. The results were given as IC_{50} values (µg/ml) (50 % inhibition):

$$\text{Metal chelating activity (\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Where $A_{control}$ represents the absorbance of the reaction mixture in the absence of sample, and A_{sample} represents the absorbance of the reaction mixture in the presence of extract or standard.

2.4.3. β-Carotene bleaching assay

This anti-oxidant activity was investigated according to the methods of Marco (Marco, 1968; Loucif et al., 2020a).A volume of 160 µl of solution of β-carotene and 40 µl of a sample were mixed. The absorbance was read at 470 nm after 0 min and 120 min incubation at 50°C. BHT was used as a reference. The bleaching rate (R) was assessed as follows: R = (ln A/B)/t. Where: A= absorbance at time zero, B= absorbance at time 120 min (t) and ln= natural log. β-Carotene bleaching activity (%) = $[(R_{Control} - R_{Sample})/R_{Control}] \times 100$. The results were given as IC_{50} values (µg/mL) corresponding to the concentration of 50% inhibition.

2.5. Analgesic activity

Writhing study was performed by following the method of Ishola et al. (2011) to investigate the analgesic effect of MCAE. In this protocol, female mice were divided into five groups of five animals each (n = 5). Group 1 (positive control): treated orally with aspirin, used as a standard drug (100 mg/kg). Group 2: (negative control), treated orally with distilled water. Groups 3–5:

treated orally with MCAE (100, 250, and 500 mg/kg in 0.5 mL H2O, respectively). After 60 min, writhes were induced in mice through intra-peritoneal injection with 0.1% (v/v) acetic acid. After 5 min of injection, the number of abdominal contractions was counted over a period of 30 min. The percentage inhibition of writhing reflex was calculated using the formula:

Inhibition (%) = 100 x (Cn–Ct)/Cn, where Cn = Mean of contractions' count in animals in the negative control, and Ct = Mean of contractions' count in animals treated with different concentrations of PSSE and aspirin.

2.6. Anti-inflammatory activity models

2.6.1. Xylene-induced ear edema

The anti-inflammatory activity of the extract was investigated in xylene-induced ear edema in mice, following the procedure outlined by Delaporte and coworkers (Delaporte et al., 2004). Mice were divided into five groups (n = 6). Group 1 (positive control): treated orally with indomethacin, used as a standard drug (50 mg/kg), group 2: (Negative control), received orally distilled water, groups 3–5 received orally MCAE (100, 300, and600 mg/kg in 0.5 mL H2O, respectively). Edema was then topically induced in each mouse using 30 µL/ear of xylene after 60 min of treatment. The thickness of the ear was measured with a digital caliper before and 2 h after the xylene application. The inhibition percentage of ear edema was computed as follows: Inhibition percentage (%) = 100 × (Dn–D)/Dn, where D is the difference of ear edema thickness in the treated group and Dn is the difference of ear edema thickness in the negative group.

2.6.2. Croton oil induced ear-edema

Anti-inflammatory activity was carried out according to the method of Dulcetti Junior et al. (2004) with slight modifications. Mice were randomly divided into five groups of 6 mice each. Group 1 (positive control), orally received indomethacin, used as a standard drug (50 mg/kg), group 2 (negative control), orally received distilled water, whereas groups 3–5 orally received MCAE (100, 300, and 600 mg/kg in 0.5 mL H2O, respectively). After 1 h, inflammation was induced with 15 µL of croton oil solution (80 µg in 50% water-acetone v/v) locally applied in the inner surface of the right ear of mice. The thickness of the ear was measured by means of a digital caliper before treatment and 6 h after the induction of inflammation. The inhibition percentage of ear edema was computed as in the equation of xylene-induced ear edema activity.

2.7. Statistical analysis

Statistical tests were carried using Graph Pad Prism (version 5.01 for Windows). All *in vitro* results were presented as mean ± standard deviation and *in vivo* results were expressed as mean ± standard error of mean (SEM). Each assay was analyzed by using a one-way analysis of variance

(ANOVA) followed by Dunnett's test. The p-values less than 0.05 were considered statistically significant.

3. Results

3.1. Total polyphenols, and flavonoids contents

Total phenolics and flavonoids content in MCAE (108±1.22 µg GAE/mg and 75.72±1.06 µg QE/mg, respectively), as shown in Table 1. This suggests a potential source of phenolic compounds from *Matricaria chamomilla* L. extract.

Table 1. Total polyphenols and flavonoids content of *Matricaria chamomilla* L. extract.

Extract	Total phenolic content[(a)]	Total flavonoid content[(b)]
MCAE	108±1.22	75.72±1.06

[(a)]: µg GAE/mg and [(b)]: µg QE/mg

3.2. Antioxidant activities

3.2.1. Cupric reducing antioxidant capacity

The cupric ion reducing antioxidant capacity was observed in *Matricaria chamomilla* L. extract as shown in Table 2. This antioxidant activity of MCAE ($A_{0.5}$ = 15.45±0.25 µg/mL) was stronger than BHT ($A_{0.5}$ = 9.62±0.87 µg/mL) with a significant difference (* ; P≤0.05)

3.2.2. Metal chelating activity

Table 2 demonstrates that the metal chelating activity of the aqueous extract had a similar effect (IC_{50}= 11.34±1.12 µg/mL) compared to BHT (IC_{50}= 12.11±0.32µg/mL) as standard (P ≥0.05).

3.2.3. β-Carotene bleaching activity

The results (Table 2) exhibited that MCAE had a β-carotene bleaching activity (IC_{50}= 17.56±1.75 µg/mL).

Table 2. Anti-oxidant activities of MCAE extract

	IC_{50} (µg/mL)		$A_{0.5}$ (µg/mL)
Extract/ standard	Metal chelating activity	β-Carotene bleaching activity	Cupric reducing antioxidant capacity
MCAE	11.34±1.12 [ns]	17.56±1.75 [***]	15.45±0.25 [*]
BHT	12.11±0.32	1.05+0.01	9.62±0.87

ns; no significant difference in antioxidant effect (P ≥0.05), * ; P≤0.05, *** ; P≤0.001 *vs* BHT as standard.

3.3. Analgesic activity

Our findings showed that MCAE exhibits analgesic activity against acetic acid-induced abdominal constriction. Results in Table 3 reveal that this extract exhibits a considerable antinociceptive effect against acetic acid-induced writhing in mice in a dose-dependent fashion.

Table 3. Analgesic activities of MCAE extract

Sample	MCE (100mg/kg)	MCE (250mg/kg)	MCE (500mg/kg)	Aspirin
Inhibition (%)	24.45^{****}	43.78^{***}	$73.15.19^{*}$	79.48

* ; P≤0.05, ** ; P≤0.01, *** ; P≤0.001 , **** ; P≤0.0001 *vs* aspirin as standard.

3.4. Anti-inflammatory activity

Results from this study revealed that MCAE exerted significant dose-dependent anti-inflammatory activity in the xylene-induced ear edema and croton-oil-induced ear edema assays as depicted in Table 4. MCAE had no significant effect against edematous response caused by croton-oil (58.75^{ns} % inhibition ; P ≥0.05) compared to indomethacin as standard drug (60.32% inhibition).

Table 4. Anti-inflammatory activity of MCAE extract

Sample	Inhibition (%)			
	MCE (100mg/kg)	MCE (300mg/kg)	MCE (600mg/kg)	Indomethcin
Xylene-induced ear edema	40.78^{***}	50.75^{***}	75.12^{*}	80.75
Croton-oil-induced ear edema	45.42^{**}	52.98^{*}	58.75^{ns}	60.32

ns; no significant difference in antioxidant effect (P ≥0.05), * ; P≤0.05, ** ; P≤0.01, *** ; P≤0.001 , **** ; P≤0.0001 *vs* indomethacin as standard.

4. Discussion

Oxidative stress is a crucial element of the pathogenic process, and anti-oxidants could help treat the disease (Senguttuvan et al., 2014). Anti-oxidants are a class of chemicals that either directly or indirectly suppress oxidation and reduce free radicals. Exogenous anti-oxidant treatment can

ameliorate oxidative damage *in vivo*. Synthetic antioxidants have potentially harmful effects on the body. Thus, researchers focus their efforts on discovering safe, effective, and natural anti-oxidants to combat oxidative stress (Benteldjoune et al., 2019). There is a growing interest in natural antioxidants like polyphenols found in medicinal plants to help prevent oxidative complications (Loucif et al., 2022b). This work showed that *Matricaria chamomilla* L. extract had a strong phenolics content. Its extract was assessed for their possible antioxidative activities by employing three complementary tests, metal chelating, and CUPRAC, β-carotene bleaching methods. CUPRAC method is based on the reaction of an electron transfer, thus the oxidant is reduced, which is monitored by a color change (Lekouaghet et al., 2020 ; Loucif et al., 2020b). In this assay, the *Matricaria chamomilla* L. extract demonstrated a strong antioxidant effect. This cupric reducing antioxidant capacity could be due to TPC and TFC contents in *Matricaria chamomilla* L. extracts. Several authors have reported that the antioxidant capacity depends on the content of phenolic compounds of plant extracts (Angelov et al., 2008; Bozan et al., 2008). The phenolic compounds act as hydrogen donors, free radical acceptors, chain oxidation reaction interrupters, or metal chelators (Loucif et al., 2022c ; Viuda-Martos et al., 2010). The antioxidant activities are partially attributed to the presence of phenolic compounds (Loucif, 2022 ; Loucif et al., 2020a). Metal ion chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent oxidative damage (Loucif et al., 2020b) Metal ion chelating capacity plays a significant role in antioxidant mechanisms since it reduces the concentration of the catalyzed transition metal in lipid peroxidation (Viuda-Martos et al., 2010) In the presence of chelating agents, the ferrozine-Fe_2 complexes are disrupted, resulting in a decrease in the red color of the complex (Loucif et al., 2020b). *Matricaria chamomilla* L. showed a good metal-chelating potential. This activity could be due to the richness of these extracts in polyphenols and flavonoids, and phenolic compounds have been reported to be chelators of free metal ions (Brown et al., 1998 ; Loucif et al., 2022c). Previous study exhibited the correlation between polyphenols and the antioxidant effect of plant extracts (Loucif et al., 2021). β-Carotene bleaching test can be utilized to evaluate the anti-oxidant effect from plant extracts (Loucif et al., 2020a; Loucif et al., 2020b). The presence of anti-oxidant compounds prevents the oxidative degradation process of linoleic acid and inhibits lipid peroxidation (Farahmandfar et al., 2018). *Matricaria chamomilla* L. had an activity *via* lipid peroxidation inhibition, most likely due to the existence of anti-oxidants in extracts (Loucif et al., 2022a).

In a similar fashion, we evaluated both the anti-inflammatory and analgesic activities of MCAE. In this context, various animal models have been used to probe mediators of inflammation and screening of anti-inflammatory agents (Chauhan et al., 2018). For example, xylene-induced ear edema in mice is a commonly used acute inflammation model (Zhao et al., 2018), it is a reproducible experimental model and provides good predictive values for screening anti-inflammatory agents (Lu et al., 2006). Xylene can stimulate vasodilation and raise blood vessel permeability and then induce edema. The molecular and cellular mechanism by which xylene

induces inflammation involves sensory neurons sensitive to capsaicin which, following stimulation, releases a number of mediators that can initiate the inflammatory response; this phenomenon is known as neurogenic inflammation (Richardson and Vasko, 2002). Substance P and peptide linked to the calcitonin gene are the main initiators of neurogenic inflammation. They induce vasodilation and plasma oxidation by acting on the smooth muscles of blood vessels and endothelial cells (Rotelli et al., 2003) as they can directly activate mast and other immune cells. It is also recognized that sensory neurons have cyclooxygenase which can synthesize pro-inflammatory prostaglandins (Richardson and Vasko, 2002). Along this line, the effect of indomethacin on inflammation can be explained by the inhibition of pro-inflammatory prostaglandin synthesis. Similarly, pretreatment of mice with MCAE caused significant inhibition of the development of edema. This could suggest that the extract reduces the release of substance P or antagonizes its action (Zhou et al., 2008), which may be due to suppression of phospholipase A2 that is associated with the pathophysiology of inflammation triggered by xylene (Rahman et al., 2019). In this regard, Kim et al. (2006) showed that gallic acid and its derivatives are responsible for the inhibition of Kappa-B nuclear factor (NF-κB) binding which is necessary for pro-inflammatory cytokines expression. Flavonoids can also affect the expression of pro-inflammatory cytokines by inhibition of NF-κB transcription (González-Gallego et al., 2007). The anti-inflammatory activity of MCAE was further evaluated by the inhibition of croton oil-induced ear edema in mouse model. Croton oil is a highly irritating agent that contains tetradecanoyl phorbol acetate (TPA) which can stimulate an inflammatory response and then induces edema (Da Silva et al., 2015). The molecular and cellular mechanism by which croton oil induces inflammation may be linked to the activation of numerous protein kinases C (Passos et al., 2013) by the secretion of high levels of intracellular factors such as calcium and diacylglycerol. Furthermore, activation of the receptors coupled to G protein results in the production of these factors. Diverse intracellular signal transduction pathways mediated by protein kinases C (PKC) such as phospholipase A2 associated with the release of arachidonic acid and eicosanoid production, and influences the pathogenesis of inflammation. The mechanism of action of indomethacin on inflammation is based on the inhibition of pro-inflammatory prostaglandin synthesis. Similarly, the topical treatment of mice with MCAE causes inhibition of the development of edema. This inhibition may be due to reduction of the release of PKC or its inhibition, and to the suppression of the exudative phase in acute inflammation.

Conclusion

Our study demonstrated that *Matricaria chamomilla* L. aqueous extract had high phenolics contents. The results obtained proved that *Matricaria chamomilla* L. can be an excellent source of antioxidant compounds and anti-inflammatory agents, probably due to its rich phenolic contents. Further research (LCMS, GCMS) is needed to identify and isolate the active principles

present in the extract in addition to investigate the mechanisms of action of the constituents of this extract.

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Declaration of Competing Interest

Authors declare no conflict of interest

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