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Original Research Article

Anti-inflammatory and analgesic effects of aqueous extracts of *Vitex agnus cactus* L. and *Cymbopogon nardus* L. against carrageenan-induced inflammation in rats

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ABSTRACT

Vitex agnus cactus and *Cymbopogon nardus* are widely used in traditional and conventional medicine as natural anti-inflammatory agents. Within this framework, the current study was undertaken to examine *in vivo* the anti-inflammatory and analgesic effects of aqueous extracts of the leaves and seeds of *V. agnus cactus* and *C. nardus*. In this relation, aqueous extracts were prepared from the leaves and fruits of *V. agnus cactus* and the leaves of *C. nardus*. The inflammatory process was induced using the carrageenan method. The analysis of the obtained results revealed that the aqueous extract of leaves of *V. agnus cactus* exhibited the highest antioxidant content (80.22 ± 11.7 mg GAE/g for TPC, 72.14 ± 9 mg RE/g for TFC, 680 ± 19.6 mg QE/g for flavones and flavonols, 355.33 ± 23.36 mg AAE/g for TAC, 0.33 ± 0.04 mg/mL for IC_{50}-DPPH, and 0.97 ± 0.04 mg/mL for EC50-FRAP) and anti-inflammatory effect with dose-dependent manner. These results suggest that these plants have the potential to alleviate pain and inflammation when used for therapeutic purposes.

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1. Introduction

The use of herbs for medicinal purposes dates back thousands of years, showcasing the enduring importance of herbal remedies in ancient healthcare systems (Crozier et al., 2008; Agrawal and Jain, 2023; Mohammadhosseini and Jeszka-Skowron, 2023). From Traditional Chinese Medicine to Ayurveda in India, and even in ancient Egyptian and Greco-Arabic medicinal practices, herbs played a crucial role in treating various ailments and maintaining health. These herbal traditions were deeply rooted in the knowledge passed down through generations, where women often played a significant role as healers in their communities. In fact, these natural resources have intensively delved into their phytochemical composition for pharmaceutical, cosmetic, nutraceutical, and food purposes (Sayed, 1980; Billowria et al., 2022; Heise et al., 2023). Confirming the potential use of herbal-based products can be challenging, despite their widespread use in treating various human conditions like diabetes, obesity, cancer, microbial infections, and inflammation (Andrade-Cetto, 2009; Singh and Singh, 2009; Saima et al., 2014; Silva et al., 2016; Saleh, 2023).

V. agnus cactus and *C. nardus* are medicinal plants belonging to the Verbenaceae and Poaceae families, respectively (Kaur et al., 2021; Boujbiha et al., 2023). The plants are commonly well-known in Morocco as "Chajarat Mariam'' and "Lwiza Lhamda" for *V. agnus cactus* and *C. nardus*, respectively.

Traditional medicine, such as Ayurveda, Unani, Chinese, Malay, European, Arabic, and ancient Greek medicines, evoked the medicinal utility of medicinal plants such as *Vitex agnus cactus* and *Cymbopogon nardus* (Kamal et al., 2022; Zeqiri et al., 2022). Different ethnopharmacological studies have documented that both plants have been utilized to treat disorders related to the reproductive, digestive, and integumentary systems (Odenthal, 1998;

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Zahid et al., 2016; Niroumand et al., 2018), and also for the treatment of inflammation (Wuttke et al., 2003; Karami et al., 2021). Other pharmacological activities, including analgesic, sedative, and anticonvulsant effects of these plants have been reported in numerous studies (Avoseh et al., 2015; Karami et al., 2021; Tibenda et al., 2022).

The presence of a broad spectrum of biologically active compounds is highly associated with the remarkable therapeutic properties of medicinal plants such as *V. agnus cactus* and *C. nardus* (Adamov et al., 2022; Bayala et al., 2020; Gebashe et al., 2020). New scientific trends are based on the formulation of new drugs using chemically active agents isolated from natural resources (Ousaaid et al., 2022).

Nowadays, phytochemicals present an excellent alternative to chemical agents, which are often associated with moderate to severe side effects (Kamal et al., 2022). Both medicinal plants mentioned above have been identified as natural remedies for various ailments, as documented in different ethnopharmacological studies (Koshta and Sharma, 2023; Lyoussi et al., 2023). The unraveling of the phytochemistry of both plants showed several bioactive compounds with unique beneficial properties against a huge list of chronic diseases (Bayala et al., 2020; Adamov et al., 2022; Gul et al., 2023). In fact, the main phenolic compounds detected in *C. nardus* were *p*-coumaric, ferulic, salicylic, and vanillic acids (Gebashe et al., 2020). In the same context, *V. agnus cactus* contains citronellal, citronellol, and geraniol as the main active components in its essential oil (EO) (Kaur et al., 2021). The EO of *V. agnus cactus* has an excellent antifungal effect against *Candida albicans, C. tropicalis, C. parapsilosis, C. krusei, C. dubliniensis, Aspergillus flavus, A. niger,* and *Penicillium* (Katiraee et al., 2015). Furthermore, it has been found to be effective against numerous cancer cell lines, including breast adenocarcinoma (MCF-7), lung carcinoma (NCI-H292), promyelocytic leukemia (HL-60), and cervical adenocarcinoma (HEP-2) human cell lines (Ricarte et al., 2020). *V. agnus cactus* induces apoptosis by modulating Bcl-2, Bcl-XL, Bax, Bad, FADD, caspase-8, caspase-9, TRAIL R1/DR4, and TRAIL R2/DR5 (Ilhan, 2021). Interestingly, the *V. agnus cactus* extract selectively suppressed cyclooxygenase-2, which is implicated in the inflammatory process (Ibrahim et al., 2021).

The second medicinal plant under study was *C. nardus*, which is extensively utilized in traditional medicine and has been scientifically investigated to validate its biological properties. In fact, from the phytochemical point of view, *C. nardus* has been known to possess various active compounds such as citronellal, geranial, geraniol, citronellol, and neral that were found to be effective against *C. albicans* (CA-ATCC 90028, CA2, CA3, CA4); *C. krusei* (CK-ATCC 6258, CK2, CK3, CK4), *C. glabrata* (CG-ATCC 2001, CG2, CG3, CG4), *C. tropicalis* (CT-ATCC 13803, CT2, CT3, CT4), *parapsilosis complex-C. parapsilosis* (CP-ATCC 22019, CP1), and *C. orthopsilosis* (CO-ATCC 96141, CO1) (De Toledo et al., 2016). *C. nardus* has also excellent antibiofilm and cytotoxic effects against HepG-2 (hepatic) and MRC-5 (fibroblast) (De Toledo et al., 2016). Moreover, the EO of *C. nardus* shows an important anti-inflammatory effect and antiproliferative activity on the prostate cancer cell line LNCaP (Bayala et al., 2020). Each plant possesses a distinctive and complex chemical composition renowned for its pertinent pharmacological activities. The combinations of these components and the development of new formulations could represent a promising approach to create an effective, safe, and stable product with significant effects.

To the best of our knowledge, no comparative study has been conducted to determine the anti-inflammatory and analgesic effects of both studied plants. In this vision, the current work was designed to determine the anti-inflammatory and analgesic capabilities of both plants (*Vitex agnus-castus* and *C. nardus*) using an experimental animal model.

2. Experimental

2.1. Preparation of aqueous extract

The leaves and fruits of *V. agnus cactus* along with the leaves of *C. nardus* were collected from the Rabat region (33°58′09″ N 6°51′26″ W) during 2020. The vegetal matrices were air-dried and powdered using a blender and the obtained powder of each sample was dissolved in water at a 1/10 ratio (10 g/100 mL). The extraction process (maceration) was sustained for 72 h at room temperature. The obtained extracts were then concentrated using a rotary evaporator and subsequently kept at normal conditions until experimentation.

2.2. determination of total phenolic content (TPC)

Quantification was made according to the Folin-Ciocalteu method (Laaroussi et al., 2020), with slight modifications. Briefly, 500 µL of each extract was blended with 500 µL of Folin-Ciocalteu solution (0.2 N) and 400 µL of sodium carbonate (10 *w/w*%) reagent. The mixture was kept for 2 h in the dark. Then, the optical density of the resulting mixture was measured at 760 nm. The obtained results were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g).

2.3. Determination of total flavonoid content (TFC)

Flavonoid content was determined using the aluminum chloride method as previously described (Laaroussi et al., 2020). Briefly, 150 µL of aluminum chloride ($AlCl_3$), 100 µL of sodium nitrite, and 200 µL of NaOH (1.0 %w/w) were added to 100 µL of each extract and then placed in the incubator for one hour. After this period, the optical density was read at 510 nm. The obtained results were expressed as milligrams of rutin equivalent per gram of extract (mg RE/g).

2.4. Determination of flavones and flavonols

Quantification of flavones and flavonols was made using the aluminum trichloride method as previously described by Kosalec et al. (2004). Briefly, 1 mL of

distilled water, 0.1 mL of $AlCl_3$, 1 mL of sodium acetate (50 g/L) were added to 1 mL of each extract. After 90 min of incubation, the optical density was measured at 420 nm. The findings were expressed as milligrams of quercetin equivalent per gram (mg QE/g).

2.5. Antioxidant activity

The antioxidant ability of the different prepared extracts was examined using three complementary assays, namely total antioxidant capacity (TAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric ion reducing antioxidant power (FRAP).

2.5.1. Total antioxidant capacity (TAC)

Total antioxidant capacity was determined by the phosphomolybdenum method (Laaroussi et al. 2020). Briefly, 1 mL of phosphomolybdenum reagent was blended with 100 µL of each extract and then incubated in a bath at 95 °C for 90 min. The optical density was finally read at 695 nm and the obtained results were expressed as milligrams of ascorbic acid equivalent per gram of extract (mg AAE/g).

2.5.2. DPPH assay

The free radical scavenging ability of the extracts under study was determined according to a technique previously described by Laaroussi et al. (2020). Accordingly, the reaction medium consisted of a blend 25 µL of each extract with 875 µL of DPPH solution (63.4 µM). The optical density of the mixture was read at 517 nm after 30 min of incubation in the dark. The percentage of inhibition was determined using the following equation (Eqn. 1):

$$\text{Inhibition}(\%) = \frac{\text{Control absorbance - sample absorbance}}{\text{Control absorbance}} \times 100 \quad \text{(Eqn. 1)}$$

The term IC_{50} for the DPPH assay was deducted from the corresponding percentage inhibition curve.

2.5.3. FRAP assay

Ferric reducing antioxidant power was determined according to the method described previously by Laaroussi et al. (2020). Briefly, the reaction mixture involved combining 1 mL of FRAP solution with 50 µL of each extract, followed by an incubation period of 15 min. After this period, optical density was measured at 593 nm. The obtained results were expressed as the EC_{50} calculated for the expression of findings.

2.6. Design of experiment

2.6.1. Animal handling and housing

Male wistar rats weighing 160 ± 7 g were procured from the animal house breeding center at the Faculty of Sciences Dhar El Mahraz, Fez, and housed there in a typical habitat (25 °C, 55% humidity, and a 12-hour of light and 12-hour of dark). The investigation was planned in compliance with the National Academy of Sciences and the National Institutes of Health's respective "Guide for the Care and Use of Laboratory Animals" documents. The current work has received ethical approval according to the SNAMOPEQ, USMBA, 2017-03.

2.6.2. Ointment preparation

Weighted amounts of the extracts were combined with petrolatum (Vaseline) as an excipient to create an ointment based on the extracts under study. After adding each dose of the excipient, the resulting mixtures, which included two distinct amounts (5% and 10%), were homogenized (Kaur et al., 2021; Boujbiha et al., 2023). Different ointments were prepared by mixing 5 g of each extract with 95 g of vaseline for 5% and 10 g of each extract with 90 g of vaseline for 10%.

2.6.3. Carrageenan-induced rat paw inflammation

The anti-inflammatory effects of *V. agnus cactus* and *C. nardus* extracts were examined using two different routes of administration (Per os and dermal administration) according to the protocol previously described by Winter et al. (1962). The animals adopted for this assay were allocated into 17 groups of 6 rats each. Groups 1, 2, and 3 were treated with *C. nardus* leaf extract at doses of 150, 300, and 500 mg/kg bw. Groups 4, 5, 6 were treated with *V. agnus cactus* seed extract at doses of 150, 300, and 500 mg/kg bw. Groups 7, 8, and 9 received *V. agnus cactus* leaf extract at doses of 150, 300, and 500 mg/kg bw. Group 10, used as a standard, and treated with diclofenac at a dose of 10 mg/kg.
Other groups were treated using ointments containing 5% and 10% of different extracts under study. Group 11 served as negative control receiving Indomethacin 1%. Groups 12 and 13 were treated with ointment based on 5% and 10% of *C. nardus* extract. Groups 14 and 15 were treated with ointment based on 5% and 10% of *V. agnus cactus* seed extract. Group 16 and 17 treated with ointment based on 5% and 10% of *V. agnus cactus* leaf extract.
Before the injection of the freshly prepared carrageenan suspension (1.0%), the right hind leg circumference of all animals under study was measured. The measurement was repeated several times at 3, 4, 5, and 6 h later. We used the following equation (Eqn. 2) to determine the percentage of inflammation inhibition:

$$\text{PI}(\%) = \left((\text{Ct-C0})\text{Control} - \frac{(\text{Ct-C0})\text{Treated}}{(\text{Ct-C0})\text{Control}} \right) \times 100 \quad \text{(Eqn. 2)}$$

Where the terms C0 and Ct respectively account for average circumference of the rat's hind paw before injection and average circumference of the rat's hind paw after carrageenan injection at a specific time.

2.6.4. Analgesic activity

The analgesic activity of the plants under study was examined by adopting the protocol previously described by Hernández-Pérez and Rabanal (2002), using acetic acid (0.7%). Animals were allocated into 5 groups with 6

rats of each. The first one, served as the negative control receiving distilled water. The second group, served as a positive control receiving tramadol. Groups 3, 4, and 5 were received a dose of the following extracts, *V. agnus* leaf extract, *C. nardus* leaf extract, and *V. agnus cactus* seed extract. All animals were administered an intraperitoneal injection of acetic acid (0.7 *v/v*% on saline, 70 mL/kg) 90 min after oral administration. Following a 30 min acetic acid injection that lasted for 5 min, abdominal concentration counts were taken. The formula used to determine the percentage inhibition of abdominal concentrations is as follows:

$$PI(\%) = \left(\frac{Mn - Mt}{Mn}\right) \times 100 \quad \text{(Eqn. 3)}$$

Where Mn and Mt respectively represent average number of abdominal contractions in the negative control group and average number of abdominal contractions in each group treated with extracts or standard.

2.7. Statistical analysis

Two-way ANOVA and the Tukey test were used in the statistical analysis, which was performed using Graph Pad Prism 5 (Microsoft software). Differences were deemed significant at $p < 0.05$.

3. Results and Discussion

3.1. Antioxidants

Table 1 summarizes the obtained results of antioxidant determination of extracts of both medicinal studied plants. The findings indicated that the extract of *V. agnus cactus* was the richest extract in phenolic content with a value of 80.22 ± 11.7 mg GAE/g, followed by the seed extract of the same plant with a concentration of 52.14 ± 6.2 mg GAE/g. Extracts prepared from *C. nardus* registered the lowest phenolic content with a value of 20.11 ± 1.14 mg GAE/g (Table 1). In the same context, the quantification of flavonoids, flavones and flavonols contents of both plants under study revealed that the *V. agnus cactus* extract registered the highest amounts of TFC and flavones and flavonols with values of 72.14 ± 9 mg RE/g and 680 ± 19.6 mg QE/g, respectively. *C. nardus* extract showed the lowest values of TFC and flavones and flavonols with concentrations of 17.02 ± 2.34 mg RE/g and 22.31 ± 3.22 mg QE/g, respectively.
With regard to antioxidant activity, *V. agnus cactus* leaf extract showed excellent antioxidant ability evaluated by three complementary assays (TAC, DPPH, and FRAP) with values of 355.33 ± 23.36 mg AAE/g, 0.33 ± 0.04 µg/g, and 0.97 ± 0.04 mg/g for TAC, IC_{50}-DPPH, and EC_{50}-FRAP, respectively. While, the *C. nardus* leaf extract registered the weakest antioxidant activity examined by TAC with a value of 22 ± 0.18 mg AAE/g, the same extract exhibited an important antioxidant ability examined by DPPH and FRAP assays with values of 0.35 ± 0.10 µg/g and 0.54 ± 0.02 mg/g, respectively. The obtained results from the present study agree with those previously reported in several published reports (Allanto et al., 2022; Hoxha and Pashollari, 2022; Kavaz et al., 2022; Solekha et al., 2022; Tewari et al., 2022). According to a research on the antioxidant capacity of various *Vitex s*pecies, *V. agnus cactus* leaves exhibit a higher antioxidant capacity than other species such as *V. negundo* and *V. trifolia* (Tewari et al., 2022). Boujbiha et al. (2023) reported that the *V. agnus cactus* fruit decoction exhibited considerable antioxidant ability as evaluated by six complementary assays with EC_{50} values of 0.64 mg/mL for DPPH, 0.35 mg/mL for FRAP, 1.03 mg/mL for ABTS, 0.16 mg/mL for β-carotene bleaching, 0.44 mg/mL for metal chelating, and 3.108 mg/mL for TBARS. In the same context, the examination of antioxidant potency of *C. nardus* essential oil showed that the EO at a concentration of 146.66 µg/mL exhibited a percentage of inhibition of DPPH of 62.14% and an IC_{50} DPPH of 102.19±4.2 µg/µg of DPPH, while gallic acid exhibited an IC_{50} DPPH of 0.11 ± 0.04 µg/µg of DPPH (Bayala et al., 2020). Sagala et al. (2023) found that the optimized extract of *C. nardus* registered a higher phenolic content with a value of 78.762 mg GAE/g extract. Within this framework, optimization constitutes an excellent tool to maximize phenolic extraction.

3.2. Carrageenan-induced pad edema test

3.2.1. Per os administration

Fig. 1 displays the obtained results of the percentage inhibition of different doses of the aqueous extract of *C. nardus* on carrageenan-induced edema. It is clearly observed a significant reduction of edema formation by the aqueous extract of *C. nardus* at all doses from the 3rd hour of the assay. The percentage inhibition increases with time, and it appears that the effect of the aqueous extract under study is long-lasting and dose-dependent manner. It can be seen that the dose of 500 mg/kg showed the most prominent activity with an inhibition percentage of 89.17 ± 4.12% compared to the standard drug used in the present study, diclofenac (1.0%) with a percentage of 92.13 ± 4.12% after 6 h. These findings reveal that *C. nradus* has anti-inflammatory effects on the carrageenan-induced inflammatory response.
Fig. 2 displays the obtained results of the anti-inflammatory effect of seeds of *Vitex agnus-castus*. An excellent decrease in pain by *V. agnus cactus* seed extract was observed at all doses under study from the 3rd h of the assay. The percentage inhibition increases with time and is positively correlated with the dose. Doses of 300 mg/kg and 500 mg/kg showed similar percentage inhibition after 5 h. It can be said that the most effective dose was 500 mg/kg with an inhibition percentage near to 80% compared with the standard drug used diclofenac (1%) percentage of 92.95% after 6 h.
Fig. 3 presents the obtained results of the anti-inflammatory effect of the aqueous extract of *V. agnus cactus* leaves on the carrageenan-induced inflammatory response. Treatment of the obtained results showed a significant reduction of pain by aqueous extract of *V. agnus cactus* leaves of all doses under study from the first 3 h of the assay. The anti-inflammatory

Table 1
Bioactive compound quantification and antioxidant ability of different extracts under study.

Extract	TPC (mg GAE/g)	TFC (mg QE/g)	Flavones and flavonols (mg QE/g)	TAC (mg AAE/g)	IC_{50} DPPH µg/g	EC_{50} FRAP mg/g
V. agnus seeds extract	52.14±6.2	19.11±0.17	390±17.3	210.33±4.85	0.52±0.02	1.33±0.07
V. agnus leaves extract	80.22±11.7	72.14±9	680±19.6	355.33±23.36	0.33±0.04	0.97±0.04[a]
C. nardus leaves extract	20.11±1.14	17.02±2.34	22.31±3.22	22±0.18	0.35±0.10	0.54±0.02[ab]

Values in the same column followed by the same letter are not significantly different by Tukey's multiple range test ($p < 0.05$).

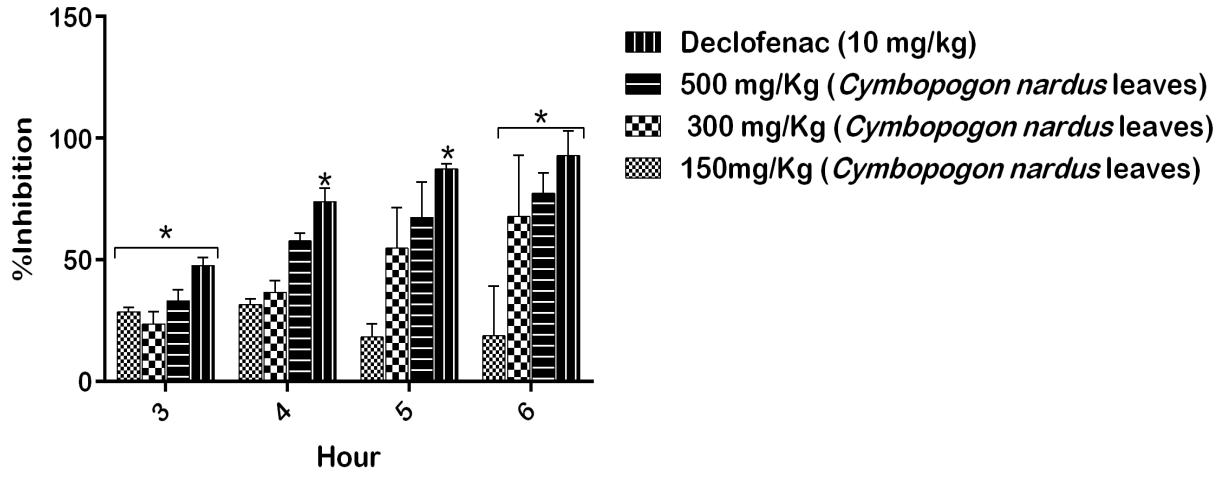

Fig. 1. Inhibition percent of the edema volume after the treatment with the aqueous extract of *Cymbopogon nardus* leaves. The results are expressed as mean±strandard deviation.

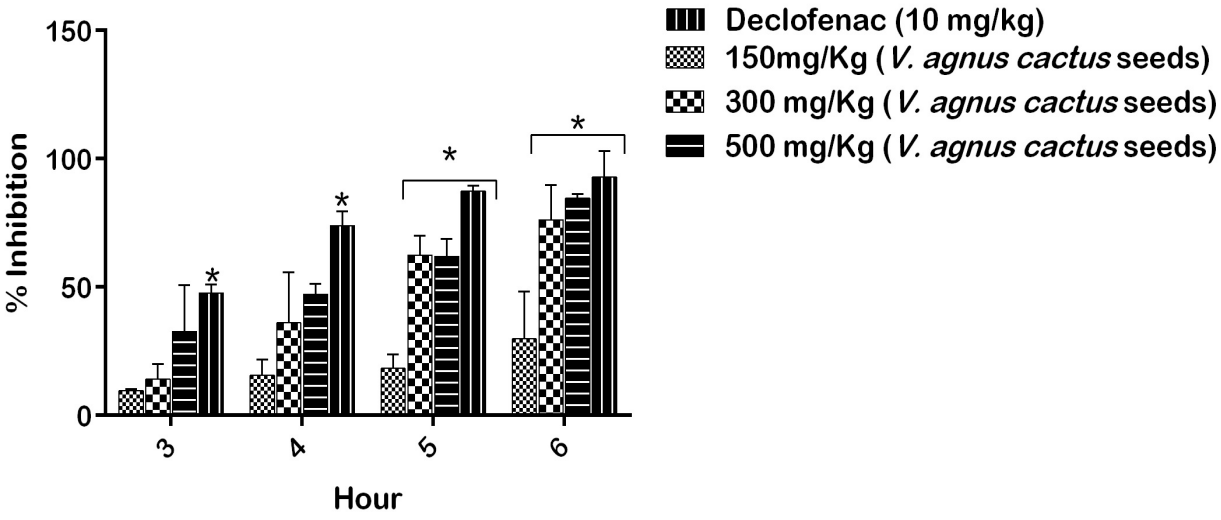

Fig. 2. Inhibition percent of the edema volume after the treatment with the aqueous extract of *Vitex agnus cactus* seeds. The results are expressed as mean±strandard deviation.

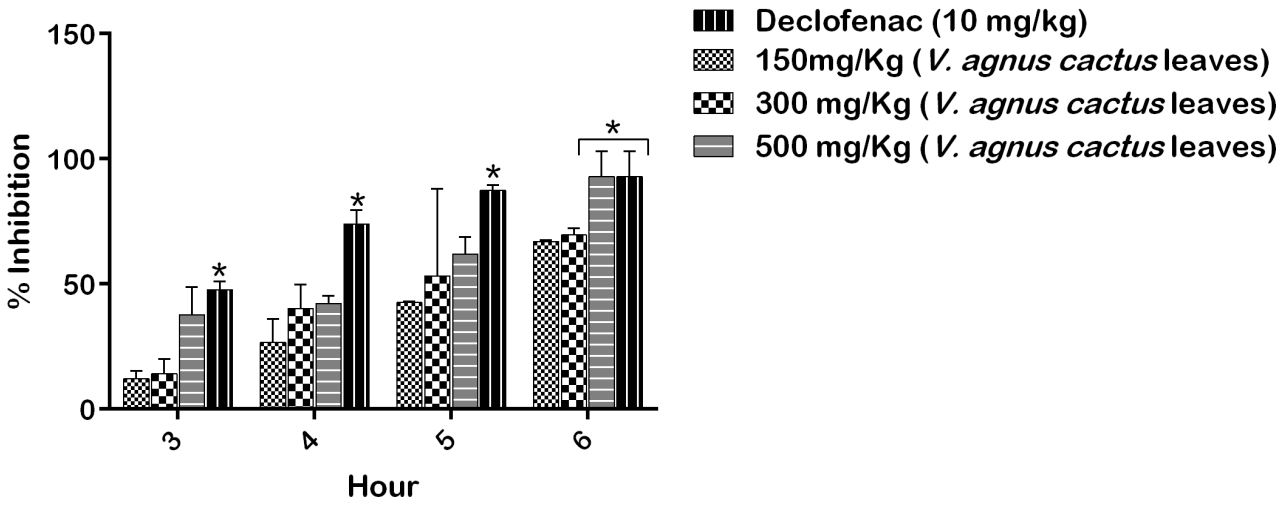

Fig. 3. Inhibition percent of the edema volume after the treatment with the aqueous extract of *Vitex agnus* cactus leaves. The results are expressed as mean±strandard deviation.

effect increases progressively with time, showing a long-lasting. The searching effect is dose-dependent with the dose of 500 mg/kg demonstrating the most potent anti-inflammatory effect and presenting similar inhibition percentage of standard drug used in the present study after 6 h.

3.2.2 Dermal administration

Fig. 4 displays the obtained results of anti-inflammatory effect of the cream prepared using the aqueous extract of *C. nardus*. The analysis of results revealed a significant reduction of edema at both the doses from the 3rd hour of the experiment. It is worth noting that the anti-inflammatory effect of the cream is long-lasting and sustained after 6 h of the assay. When comparing the potency of both doses, it can be shown that the activity is dose-dependent. The most effective effect was shown with a dose of 10% *C. nardus* with an inhibition percentage near 100% compared with the standard drug used (Indomethacin).

The findings shown in Fig. 5 were obtained after using an ointment made from the leaf extract of *V. agnus cactus*. With a percentage time increase to obtain comparable percentage inhibition of the standard drug employed in the current investigation, both dosages produced a notable anti-inflammatory effect.

Application of ointment prepared on the basis of leaf extract showed the results displayed in Fig. 5. As can be seen in this figure, both doses exerted a remarkable anti-inflammatory effect with a percentage time-raising to achieve a comparable percentage inhibition of the standard drug used in this study.

The results shown in Fig. 6 were obtained after applying an ointment produced from seed extract of *V. agnus cactus*. The percentages recorded at various intervals for the two doses used fall short of those recorded for the often prescribed medication.

The analgesic effect of different extracts under study was evaluated using acetic acid at a dose of 50 mg/kg. Fig. 7 displays the obtained results of the Koster assay. Accordingly, it is clearly seen that the administration of different extracts significantly reduced contractions compared with the control. There is no significant difference between all extracts and the standard drug used in this study.

The ability of plants to fight inflammation was closely tied to their antioxidant effect. The highest concentration of antioxidants was found in *V. agnus cactus,* which also had a significant anti-inflammatory effect with a percentage of inhibition comparable to that of the standard drug employed after 6 h of administration (Fig. 3). These findings are in line with those reported by Boujbiha et al. (2023) found that the analgesic and anti-inflammatory effects of *V. agnus cactus* fruit decoction were dose-dependent. *V. agnus cactus* fruit decoction at a dose of 200 mg/kg exhibited a stronger anti-inflammatory effect than the standard drug (lysine acetylsalicylic acid) (Boujbiha et al., 2023). The anti-inflammatory properties of *Haplophyllum tuberculatum* were surpassed by those of *V. agnus cactus* and *C. nardus* (Agour et al., 2022). According to Csikós et al. (2020), using *C. nardus* essential oil reduces inflammatory airway hyperresponsiveness and some cellular inflammatory indicators. Citral, one of the most prevalent bioactive chemicals found in *C. nardus*, has been shown to reduce edema development, histopathological changes, neutrophil activation and adhesion, and the generation of pro-inflammatory indicators (Abe et al., 2003; Shen et al., 2015; Csikós et al., 2020). At a dose of 2.2 mg/mL, *C. nardus* essential oil exhibited a percentage inhibition of lipoxygenase of 25 ± 3% (Bayala et al., 2020).

Carrageenan caused the development of peripheral inflammation in a time-dependent manner, which in turn caused a marked rise in the levels of the inflammatory proteins tumor necrosis factor (TNF), interleukin A (IL-1), nitric oxide (NO), and prostaglandin E2 (PGE2), as well as iNOS and cyclooxygenase-2 protein production in the affected paw (Mansouri et al., 2015). However, administration of the studied plants markedly reduced carrageenan-induced edema. The antioxidants by their

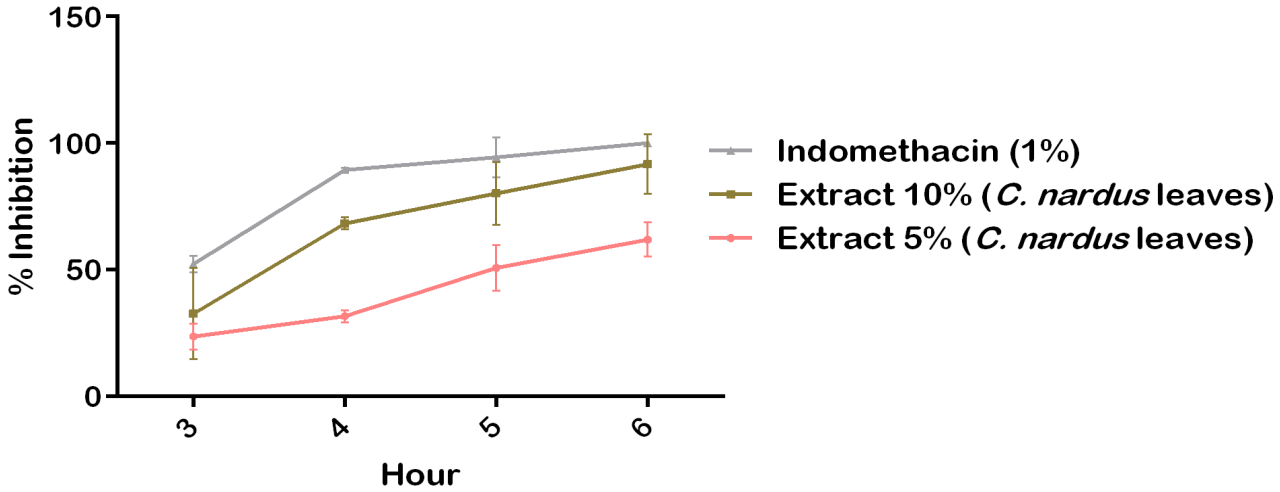

Fig. 4. Inhibition percent of the edema volume after the treatment with the aqueous extract of *Cymbopogon nardus* leaf extract and the standard compound. The results are expressed as mean±strandard deviation.

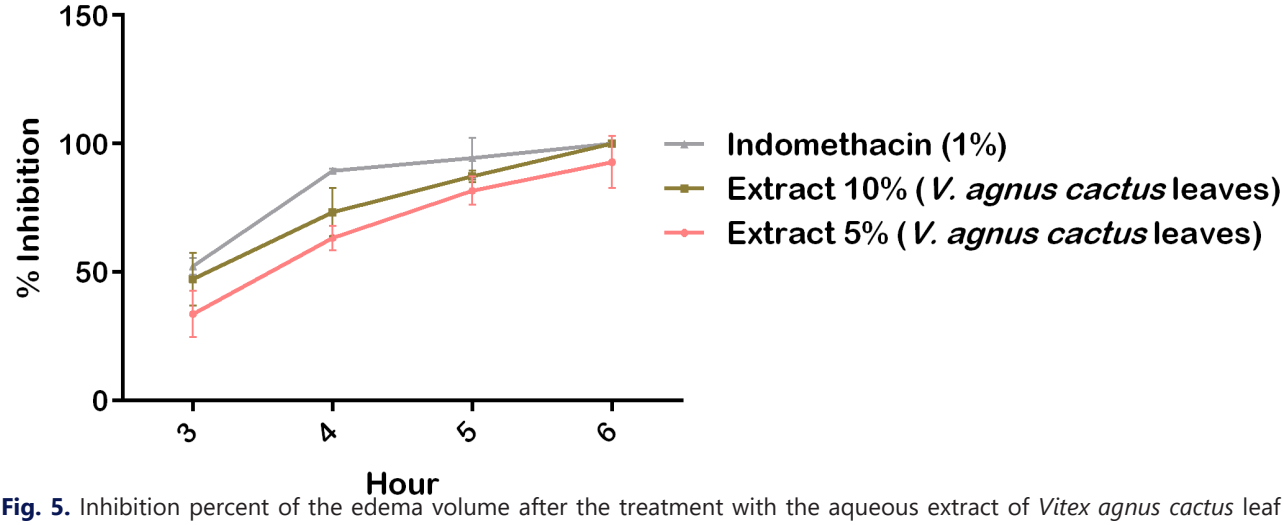

Fig. 5. Inhibition percent of the edema volume after the treatment with the aqueous extract of *Vitex agnus cactus* leaf extract and the standard compound. The results are expressed as mean±strandard deviation.

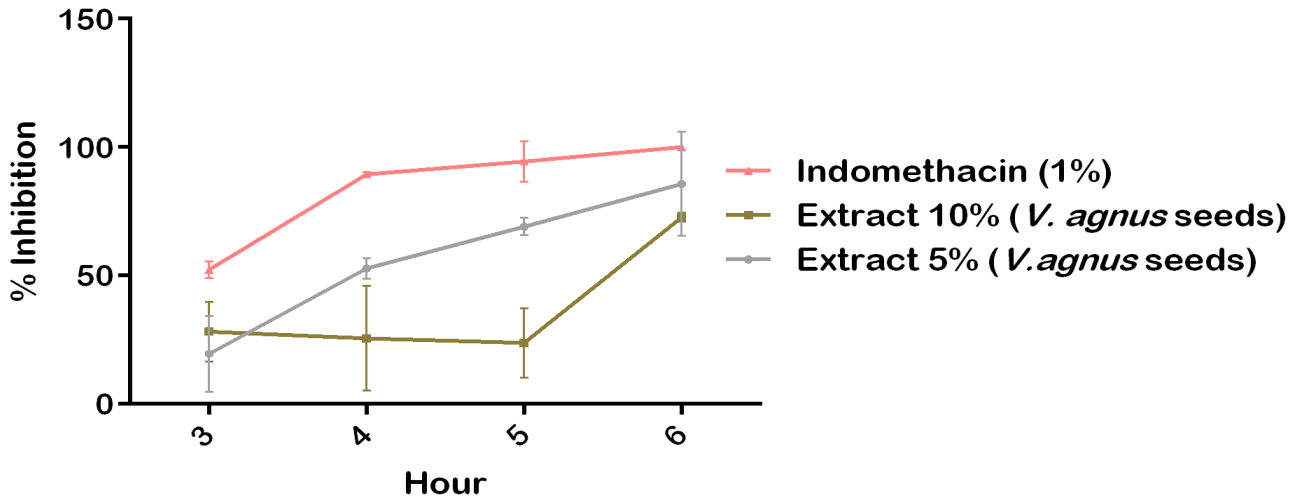

Fig. 6. Inhibition percent of the edema volume after the treatment with the aqueous extract of *Vitex agnus cactus* seed extract and the standard compound. The results are expressed as mean±strandard deviation.

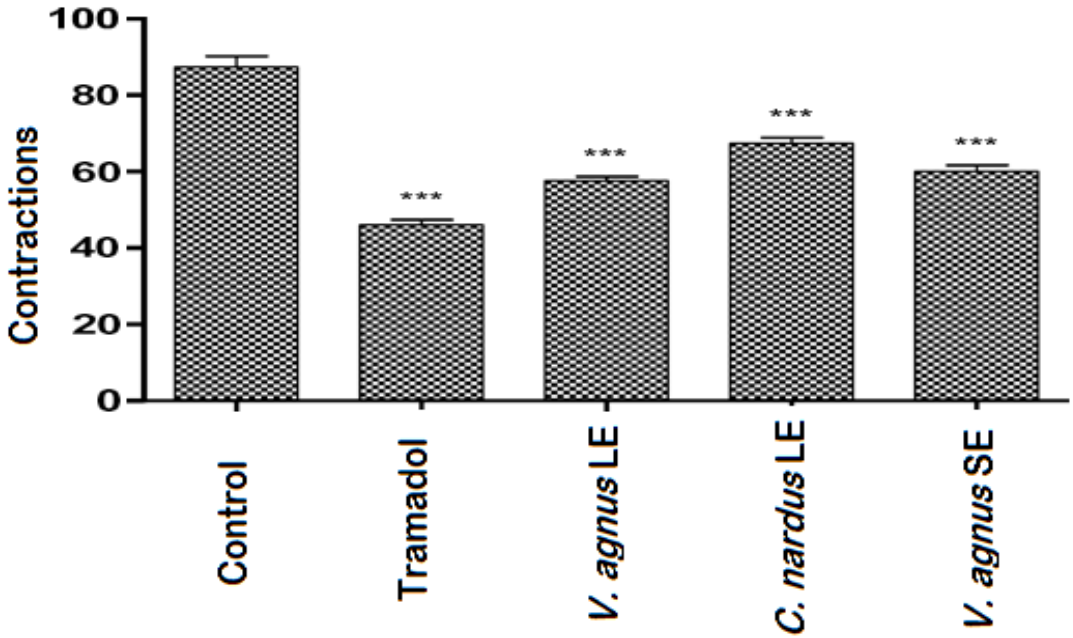

Fig. 7. Contraction inhibition of different extracts under study and the standard compound. The results are expressed as mean±strandard deviation.

pleiotropic effects attenuate the deleterious effects of reactive oxygen species by arresting free radical production and metal chelating, which can achieve anti-inflammation (Chu, 2022). Additionally, they boost the endogenous antioxidant enzymes that are implicated in the ROS elimination process (Chu, 2022). Antioxidants target diverse inflammatory pathways because of their high antioxidant potentials, such as AMPK activation, PI3K/AkT, mTORC1, IKK/JNK, and JAK/STAT inhibition (Yahfoufi et al., 2018). A delve into the phytochemistry of *V. agnus cactus* revealed that 1,8-cineole comprised the highest proportion (30.3%) of the bioactive compounds detected (Boujbiha et al., 2023).

This active component exhibited an interesting anti-inflammatory effect by increasing nuclear factor erythroid 2-related factor 2 (Nrf2) and peroxisome proliferator-activated receptor-γ (PPARγ), whereas, it decreased pro-inflammatory chemokine synthesis (Venkataraman et al., 2023). *In vitro* and *in vivo*, 1,8-cineole inhibits macrophage M1 polarization and prevents HSP90 from suppressing the NLRP3 inflammasome in macrophages (Ma et al., 2023).

Few studies have evoked the anti-inflammatory effect of *C. nardus*. The essential oil of this plant exhibited modest antioxidant and anti-inflammatory activity with a percentage inhibition of lipoxygenase of 0.5 ± 0.9% at a concentration of 0.083 mg/mL compared with gallic acid as the standard active compound used (59.64 ± 2.12%) (Bayala et al., 2020).

The metabolomics profile of *C. nardus* showed the presence of different chemically active compounds like *p*-coumaric, ferulic, salicylic, and vanillic acids, which are well known for their anti-inflammatory effects (Gebashe et al., 2020; Gastelum-Hernández et al., 2023; Song et al., 2023). A plant combination may be a useful product to combat pathogenesis and its complications. To fully exploit the therapeutic potential of medicinal plants and their combinations, additional experimental research is necessary.

4. Concluding remarks

The current comparative study was conducted to investigate the antioxidant, anti-inflammatory, and analgesic effects of two Moroccan medicinal herbs. Our data show that both plant extracts have significant antioxidant, anti-inflammatory, and analgesic efficacy in a dose- and time-dependent manner. *V. Agnus* cactus outperformed *C. Nardus* in terms of antioxidant and anti-inflammatory activity because of its diverse bioactive content. Combining the two plants under consideration can stop or lessen inflammation.

Author contribution statement

Conceptualization and literature search were performed by Fatima El Kamari, Driss Ousaaid, and Badiaa Lyoussi. The first draft of the manuscript was prepared by Fatima El Kamari and Driss Ousaaid. Laila Lahrizi, Abdelfattah El Moussaoui, and Badiaa Lyoussi critically analyzed and gave suggestions to finalize the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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