



Medicinal Potential from Plant Biodiversity in a Mediterranean Scrubland, *Thymo Piperellae-Cistetum Crispi* Costa, Peris & Stübing 1986

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Received date: Mar 31, 2014, Accepted date: May 21, 2014, Publication date: May 29, 2014

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Abstract

Objective: The main aim of this study is establishing a research protocol for assessing the medicinal potential from the plant biodiversity in the plant association *Thymo piperellae-Cistetum crispum* Costa, Peris & Stübing 1986, an endemic scrubland.

Methods: The proposed protocol was a multidisciplinary study that it included: a literature review on the traditional use from characteristic plants in the plant community, appropriate pharmacological assays and antimicrobial tests for confirming medicinal properties and phytochemical analysis for studying the active substances. Finally, the percentage of scientifically studied medicinal plants was compared before and after the study for assessing the effect of protocol implementation for establishing the real potential medicinal in plant community.

Results: The review study of ethnobotanical literature showed that two of the characteristic species in plant community, *E. scoparia* and *T. piperella*, had traditional uses. The first species was used as medicinal plant for inflammations of the bladder and kidney and urinary tract disorders. The other one was used as preservative and flavoring of olives, and as an infusion for the tummy pain. After implementation the medicinal study protocol, medicinal properties of two species were demonstrated. *E. scoparia* showed analgesic and antiinflammatory activities and *T. piperella* presented spasmolytic and antimicrobial activities. In addition, the botanical characterization of the plant organs with medicinal activity was performed, and data, indumentum and other anatomical characteristics, allowed differentiating these specific plant drugs from another plant organ and other species, avoiding confusions and increasing the safety of medicinal herbal products. So the real percentage of scientifically studied medicinal plants increased in the studied vegetation after applied the proposed protocol for assessing its medicinal potential.

Conclusion: The proposed protocol for assessing the real medicinal potential in a plant community is valid for this aim.

Keywords: Landscape biodiversity; Natural resources; Medicinal plants; Toxic plants; *Thymo piperellae-Cistetum crispum*.

Introduction

The biodiversity conservation is one of the priorities for many Nations pursuing sustainable development. The plant resource potential use should be a criterion for protecting, controlling and conserving landscapes. The plant resource rational use can only be obtained through the knowledge of the plant properties. In this connection, the quest for medicinal properties in plants can go together with their control and conservation; this will hopefully turn out in a reduction of improper use of the plants, an event that could otherwise lead to hazards for both the environment and the population.

On the one hand, Mediterranean Region is particularly rich in medicinal species; the presence of these plants increases the landscape interest. An interesting plant community is *Thymo piperellae-Cistetum crispum* Costa, Peris & Stübing 1986, an endemic plant association in La Safor region (Valencia, Spain). Moreover, a bigger ethnobotanic tradition exists in Spain; it is confirmed by the wide literature available [1-8].

Given the above, we projected a research protocol designed for showing the real medicinal properties from characteristic species in the plant community, that they had not been scientifically studied, although references on their traditional use could exist. In order to obtain this objective, after an ethnobotanic bibliography review, we carried out different studies (botanical, toxicological, pharmacological (included microbiological tests) and phytochemical) from the characteristic species that were not considered as medicinal plants or

their uses were not scientifically demonstrated, or not exists information on its use. This multidisciplinary research facilitates the rational use of medicinal plants because it provides a possibility for finding new medicinal species and new molecules that may drive, in the future, the design of new synthetic drugs.

The *Thymo piperellae-Cistetum crispum* characteristic species are: *Cistus crispus* L., *Cistus salvifolius* L., *Lavandula stoechas* L., *Pinus pinaster* Aiton, *Erica scoparia* L., *Thymus piperella* L., *Xolantha tuberaria* (L.) Gallego, Muñoz Garm. & C. Navarro, *Pteridium aquilinum* (L.) Kuhn in Kerst. and *Cistus monspeliensis* L. In this study, only the characteristic species were considered because they are constant in the plant community floristic composition that defines the plant association.

Materials and Methods

The developed protocol is reported below:

1. Literature review and selection of plants for the study.
2. Selection of the drug (organ that was used for the assays).
3. Recollection of plant material.
4. Extraction from the leaves of the species and preparation of essential oils.
5. Study on medicinal properties.
 - a. Preparation fresh solutions from extracts.
 - b. Experimental animals.
 - c. Study of acute oral toxicity.
 - d. Pharmacological study.
 - e. Antimicrobial tests.
6. Phytochemical analysis.
7. Characterization of the plant organ (drug) that was used for the assays.
8. Evaluation of the medicinal potential in plant community.

Literature Review and Selection of Plants for Study

A literature review on characteristic species traditional uses in the plant association was performed. Later, other literature review focused on the scientific evidence on the properties of traditionally used species. So, the not used and not scientifically studied medicinal plants came out.

Selection of the Plant Drug

The selection of the plant part for studying the medicinal properties was based on the bibliographic information about the plant organ of each species that has been traditionally used.

Plant Material Recollection

Plant material was collected from the locality Pla de Corral in the Valencia region (Spain) and was authenticated at the Botanical Garden, University of Valencia.

Extraction from the Leaves of the Species and Preparation of Essential Oils

a. Preparation of the extracts

Leaves (192 g) were air-dried, powdered and extracted at room temperature with 80% methanol (2 L, 2 days) and 50% methanol (2.5L, 2 days). Combined extracts were separated into two parts: 2L. were concentrated in vacuo for obtaining 1.78 g, while the remaining 3 L. were concentrated in vacuo with 0.5 L and extracted successively with hexane (3 x 0.5 L) and dichloromethane (3 x 0.5 L). Organic extracts were dried with a vacuum pump for yielding 4.4 g for hexane extraction, 5.20 g for dichloromethane extraction and 2.27 g for butanol extraction (w/w from dry plant). All extracts were stored at -20°C.

b. Preparation of the essential oils

Essential oil was obtained through hydrodistillation with a Clevenger's apparatus during 2 h 30 min. after with no further essential oil was collected. Qualitative analysis was performed with a GC-MS using a non-polar capillary column BPx5 (30 m x 0.22 mm i.d.) 5% phenylpolysiloxane (0.25 µm film thickness) and with the temperature programmed in two stages: T1=60°C; isothermal time, 1.5 min; rate 1, 5°C/min; isothermal temperature 2, 170°C; isothermal time 2, 2 min; rate 2, 10°C/min; isothermal temperature 3, 240°C; isothermal time 3, 60 min; helium T3, 280°C. The carrier gas was helium (flow: 50 kpa). The injector and detector temperatures were 250°C and 200°C respectively. The electron ionization voltage was 70 eV and the quantification of the different components of the essential oil was accomplished with a GC-FID using the same capillary column and temperature programmes as in the case of the GC-MS. Compounds were identified by comparing their retention indices (RI) on the capillary column with those of α -thujene and their mass spectra with the corresponding data of internal authentic samples. Quantification of the components was performed on the basis of their GC-FID peak areas.

Study on Medicinal Properties

a. Preparation solutions from extracts

Fresh solutions from extract and essential oils were prepared immediately before administration in Tween 20 (<5%) for in vivo studies and in DMSO (<10%) for in vitro assays. The solvents had previously been demonstrated to be pharmacologically inert in all experiments.

b. Experimental animals

Groups of albino mice weighing 25-30 g were employed for the writhing test and hot plate experiments for assessing analgesic activity (in vivo assays). Female and male Wistar rats were employed for assessing spasmolytic (in vitro assay) and anti-inflammatory (in vivo assay) activities. Animals were housed in standard cages at room temperature with food and water ad libitum. Each experimental group consisted of six animals. Treatments of animals during the experiments fulfilled international criteria for the care and use of animals in experimentation. In vivo essays, the solutions from extracts were administered intraperitoneally (i.p.) at a constant volume of 1 mL/kg. The tested doses were usually lower than 1/10 LD50. In vitro essay, essential oils were added to the organ bath.

c. Study of acute oral toxicity

An acute oral toxicity study was performed in accordance with OECD guidelines [9]. The experiment was performed with male albino mice (25-30 g, n=5). Five mice were administered with a single dose of the extract via an intubation cannula. The initial dose was selected on the basis of an observational study, which showed it to produce some signs of toxicity without causing severe toxic effects or mortality. Based on the results of this study, the main study was carried out with a group of five mice administered with a dose of 2 g extract/Kg body weight. The dose volume administered was 0.2 mL in both the observational and the main study. Two control groups with which to compare the treated mice were included in each study and administered 0.2 mL saline (NaCl 0.9%) or 0.2 mL Tween 20 (<5%). Animals were kept under observation for a period of 14 days. This study was carried out with all the selected species and were discarded the toxic ones.

d. Pharmacological study

Analgesic activity

Chemical stimulus: The Koster & Anderson method was employed in male albino mice, using i.p. acetic acid (3 % in a volume of 0.25 ml) as an algogenic substance. Control animals received a similar volume of saline. Thirty minutes after the administration of the different extracts, the acetic acid solution was injected and the number of writhings recorded over the following 20 minutes. Results were evaluated by calculating the average total number of writhings observed (n>6), and this number was represented as % of inhibition of writhing movements compared with the control vehicle group.

Thermal stimulus: the nociceptive threshold of male albino mice was determined by employing a hot plate and the method of Eddy and Leimbach. The device (Ugo Basile) consisted of a metal plate (25 x 25 cm) heated to a constant temperature (55° C), on which a plastic cylinder (20 cm diameter, 16 cm high) was placed. The maximum time allowed for an animal to respond was 90 sec. Both licking and jumping movements (determined in seconds) were recorded. The percentage of analgesic effect was evaluated by calculating the accumulated values (seconds) for each group (n>6 per group) and control.

Antiinflammatory activity

Carrageenan-induced paw edema [10]: carrageenan was injected into the plantar aponeurosis of the right hind paw of Wistar rats (0.1 mL, 2%). Animals (150-175 g, n=10) received extract (200 mg/Kg) 30 minutes prior to the injection of carrageenan, and the paw volume was measured with a plethysmometer (Ugo Basile, Italy) 1, 2, 3 and 24 h later. Results were expressed as a percentage of the inhibition of the edema in each group with respect to controls.

Spasmolytic activity

Assay: Male wistar rats (160-180 g) were used. The animals were killed by a blow on the head, and their tissues were then excised and suspended at 37°C in a 10 mL organ bath with a Krebs Henseleit solution (composition in mmol/L: ClNa 118.4, ClK 4.7, Cl₂Ca 2.5, SO₄Mg 0.6, PO₄H₂K 1.2, CO₃HNa 25 and glucose 11.1) and oxygenated with 95% O₂ and 5% CO₂. 1 g was used as preload for all the preparations. Concentration-response curves to acetylcholine were recorded in the absence and presence of the essential oil (85, 42, 8.5 µg/mL) which was added to the bath 20 min. before the second curve. Modifications of the maximum effect (Emax) and efficient concentration 50 (EC50) were evaluated. A value of 100 was assigned to maximum effects obtained with acetylcholine in the absence of the essential oil. The mechanical activity was measured isometrically using

a transducer (UF-1) connected to a system PowerLab/400 (Cibertec S.A.).

Animal organ (rat ileum): strips of rat ileum were suspended in the same conditions as described above, and concentration-response curves to acetylcholine in the range 10⁻⁹ to 10⁻³ M were recorded. The essential oil (85, 42, 8.5 µg/mL) was assayed.

e. Antimicrobial tests

Tests were performed against the following microorganisms: *Escherichia coli* (CECT 434), *Staphylococcus aureus* (CECT 435), *Streptococcus pyogenes* (CECT 191), *Mycobacterium phlei* (CECT 3009), *Pseudomonas aeruginosa* (CECT 108), *Klebsiella pneumoniae* (CECT 143) and *Candida albicans* (CECT 1472), all of which were obtained from the Colección Española de Cultivos Tipo (Spanish National Collection of Type Cultures).

Characterization of the Plant Drug (plant leaf)

The morphology, epidermal micromorphology and anatomy of the plant leaf were studied. The morphological analysis was performed using a Wild Heerbrugg magnifying glass. The epidermal micromorphology was studied employing the Scanning Electron Microscope (HITACHI S-4100) at SCSIE-University of Valencia. Histologic techniques were applied for studying the foliar blade anatomy. A freezing microtome (LEICA CM 1325 coupled to CU MICRON COOLING SYSTEM K-400) was used for obtaining cross sections, and the safranin-fast green staining was employed for observing different plant tissues. The images were captured using an optical microscope with coupled digital camera (OLYMPUS PROVIS AX-70).

Evaluation of the Medicinal Potential in Plant Community

Medicinal potential was measured considering the characteristic species in the plant association, calculating the percentage of species with proven medicinal use, first taking into account data from literature on species that their medicinal properties were scientifically proven; subsequently, the same percentage was calculated taking into account the obtained results following the proposed protocol. Percentages were compared graphically.

Results

Literature Review and Selection of Plants for Study

According scientific literature, essential oils from *C. salvifolius* and *C. monspeliensis* presented properties in prevention of neurodegenerative disorders [11]. Also, extracts from leaves of *C. monspeliensis* presented antimicrobial activity [12]. Another characteristic species in the plant community, *L. stoechas*, was a very used medicinal species and their properties had already been demonstrated [13,14]. The essential oils of the maritime pine or cluster pine, *P. pinaster*, presented a moderate antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* [15]. *X. tuberaria* was a species with ethnobotanical and ethnoveterinary uses, and the scientific evidence that explained their traditional medicinal uses was found, highlighting the interest of its decoctions and infusions as a source of bioactive compounds and functional beverages [16, 17]. Tsumbu et al. [18] published a paper about the modulatory activities in extracts from tropical dietary plants with oxidant activity, one of the studied species was *P. aquilinum*, and the authors

concluded that the role of those dietary and medicinal plants in the treatment of ROS-dependent inflammatory diseases could represent a new interesting starting point as a therapeutic approach.

After screening the literature, it resulted that only *C. crispus*, *E. scoparia* and *T. piperella* were not used for their medicinal properties, but our research group researched on *T. piperella* and preliminary tests showed pharmacological activity [19,20], which we proposed to confirm in this work. Thus the studied species were: *C. crispus*, *E. scoparia* and *T. piperella*

According to popular usage, we deduced that *E. scoparia* might have anti-inflammatory and analgesic properties, because this specie and other ones of genus *Erica* were used for urinary problems that produce pain and inflammatory symptoms [4,21]. Regarding *T. piperella*, we thought that it had antimicrobial and spasmolytic properties, taking in account the preliminary studies performed by our research group [19,20]. Finally, we thought that *C. crispus* might present similar medicinal properties to other *Cistus* species.

Selection of the Plant Drug

Selection criteria of the plant organ for studying are, first, the information from literature [4,12,19,20]; subsequently, when information from literature was not existed, the characteristic of easiest collecting and most durable plant organ was taken in account. Also, the most used organ in other similar pharmacological studies was taken in account. So, the leaf was the selected organ.

Recollection of Plant Material

Plant material was collected in spring, when the plants were flowered and fructified and had all necessary plant organs for identifying the species using taxonomic botanical keys. Leaves from each species were collected and stored in a conventional refrigerator. Furthermore, the aerial part with flowers and fruits from each species was collected for the determination of species name.

Study on Medicinal Properties

Study of acute oral toxicity

Oral Acute Toxicity was evaluated and no evidence of toxicity was detected at the assayed dose (2 g/Kg) for *T. piperella* and *E. scoparia* extracts. The animals died at the same doses of assayed *C. crispus* extracts, then lower doses were tested (2, 20, 200 mg/Kg) and toxic signs remained, therefore, pharmacological studies were not performed with this species.

Pharmacological study

Analgesic activity

Chemical stimulus: Pre-treatment with methanol, hexane and dichloromethane extracts (2, 20 and 200 mg/Kg) from *E. scoparia* leaves showed an analgesic effect that reduced the percentage of writhing movements induced by the i.p. administration of 0.25 ml of a solution of 3% acetic acid by 11.0%, 21.4% and 85.0% respectively for the methanol extract, 59.6%, 78.0% and 100% respectively for the dichloromethane extract, and 22.4%, 49.6% and 77% respectively for the hexane extract (Figure 1).

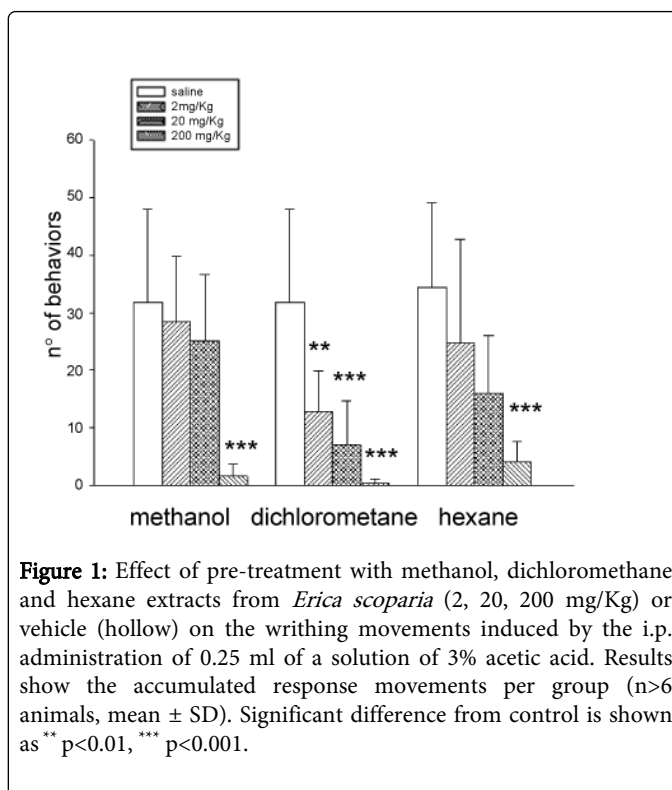


Figure 1: Effect of pre-treatment with methanol, dichloromethane and hexane extracts from *Erica scoparia* (2, 20, 200 mg/Kg) or vehicle (hollow) on the writhing movements induced by the i.p. administration of 0.25 ml of a solution of 3% acetic acid. Results show the accumulated response movements per group (n>6 animals, mean ± SD). Significant difference from control is shown as ** p<0.01, *** p<0.001.

Thermal stimulus: Pre-treatment with methanol, hexane and dichloromethane extracts from *E. scoparia* leaves showed a significantly (p<0.005) increased response time in both the licking and jumping parameters in the hot plate test (Figure 2-4).

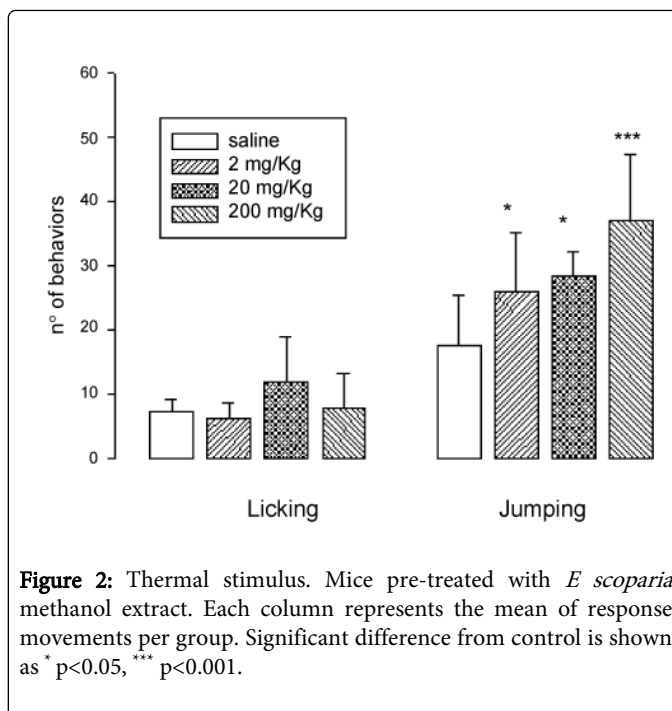


Figure 2: Thermal stimulus. Mice pre-treated with *E. scoparia* methanol extract. Each column represents the mean of response movements per group. Significant difference from control is shown as * p<0.05, *** p<0.001.

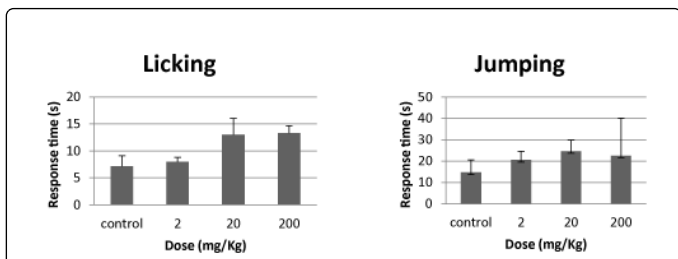


Figure 3: Thermal stimulus. Mice pretreated with *Erica scoparia* dichlorometane extract.

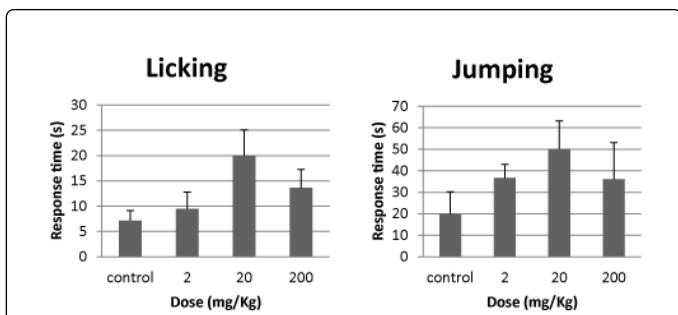


Figure 4: Thermal stimulus. Mice pretreated with *Erica scoparia* hexane extract.

Antiinflammatory activity

Pre-treatment with a methanol extract from *E. scoparia* leaves at doses of 2, 20 and 200 mg/Kg induced a significant antiinflammatory activity after 3 hours and throughout the 24 h experimental period. Dichloromethane and hexane from *E. scoparia* leaves extracts exhibited significant activity 1, 2, and 3 hours after pre-treatment (Figures 5-7).

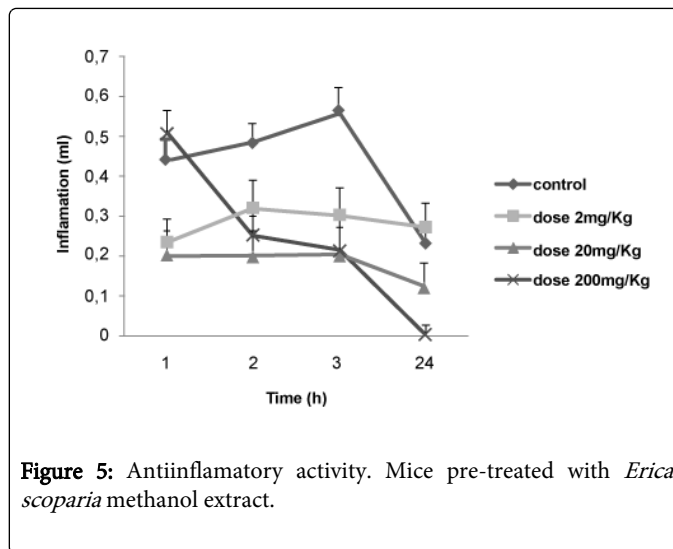


Figure 5: Antiinflammatory activity. Mice pre-treated with *Erica scoparia* methanol extract.

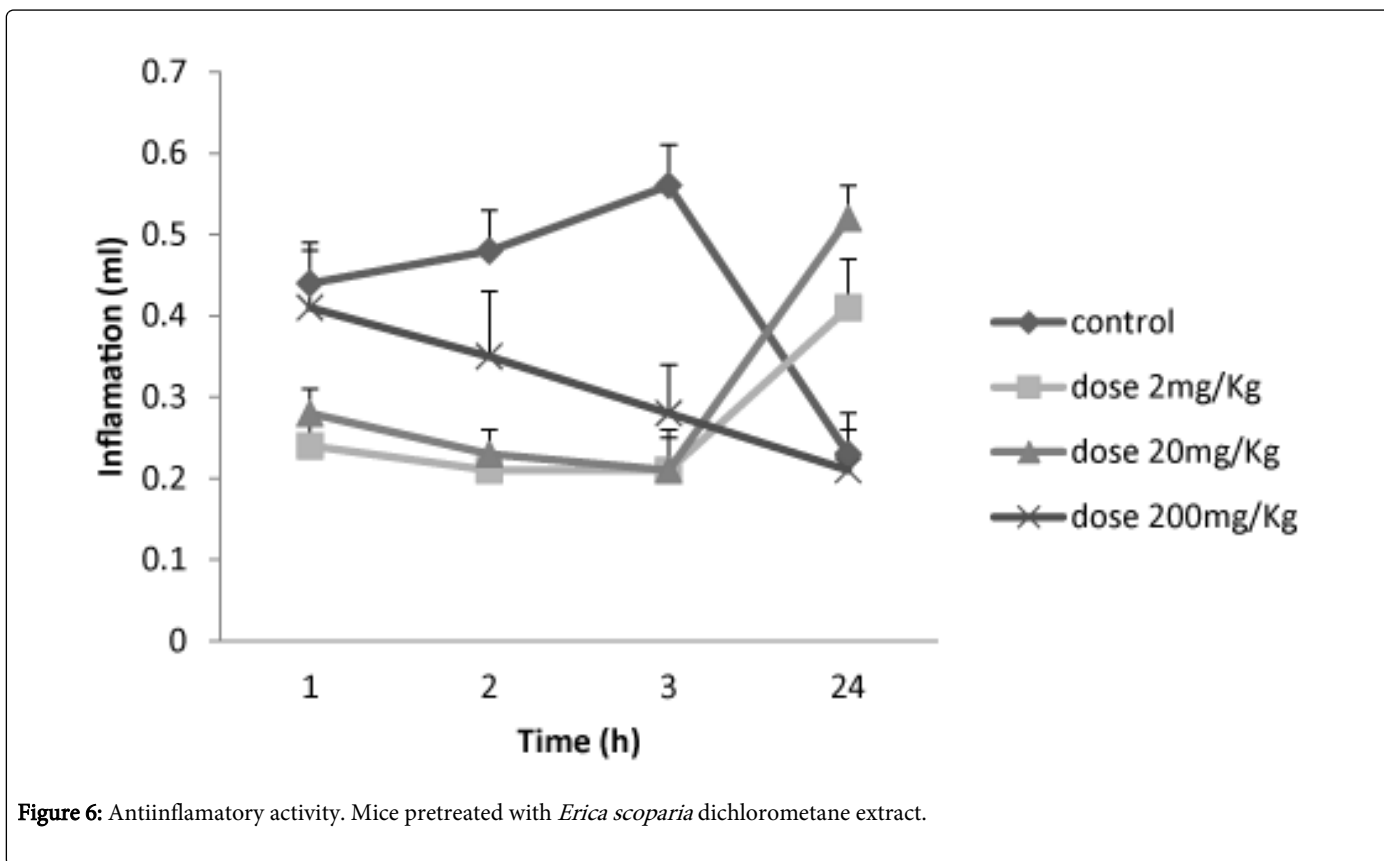


Figure 6: Antiinflammatory activity. Mice pretreated with *Erica scoparia* dichlorometane extract.

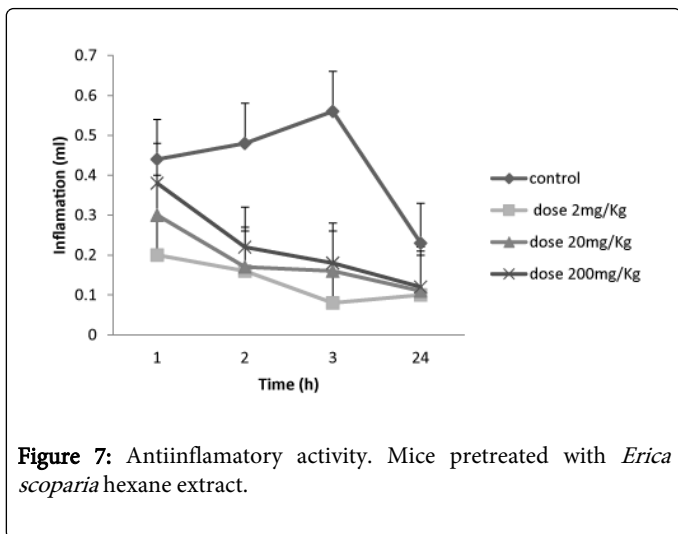


Figure 7: Antiinflammatory activity. Mice pretreated with *Erica scoparia* hexane extract.

Spasmodic activity

The in vitro contractile effect of acetylcholine on isolated rat ileum was reduced significantly in a concentration-dependent manner through preincubation with maximal concentrations of *T. piperella* essential oil. In this case, the E_{max} was reduced by a 21.96% ($p < 0.001$), by a 42.96% ($p < 0.001$) and by a 27.03% ($p < 0.001$) at the concentrations of 8.5, 43 and 85 $\mu\text{g/ml}$ respectively, without a significant modification of the EC_{50} (1.555×10^{-6} M, 1.420×10^{-6} M, 1.641×10^{-6} M and 1.406×10^{-6} M for control, 85, 43 and 8.5 $\mu\text{g/ml}$ of essential oil respectively, as shown in (Figure 8).

Antimicrobial tests

The antimicrobial test demonstrated that essential oil from *T. piperella* leaves showed activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Streptococcus pyogenes* and *Mycobacterium phlei*.

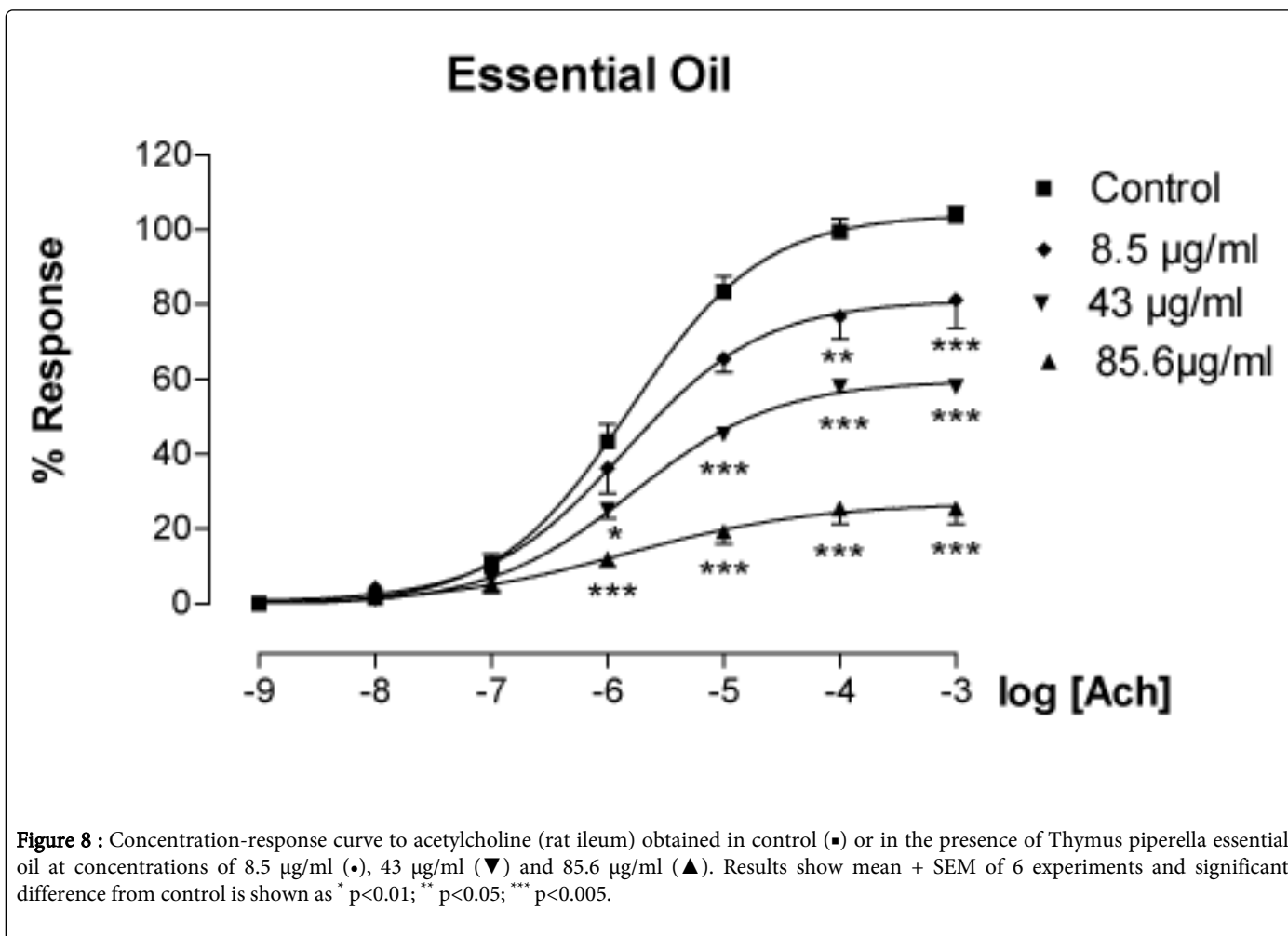


Figure 8 : Concentration-response curve to acetylcholine (rat ileum) obtained in control (■) or in the presence of *Thymus piperella* essential oil at concentrations of 8.5 $\mu\text{g/ml}$ (●), 43 $\mu\text{g/ml}$ (▼) and 85.6 $\mu\text{g/ml}$ (▲). Results show mean + SEM of 6 experiments and significant difference from control is shown as * $p < 0.01$; ** $p < 0.05$; *** $p < 0.005$.

Phytochemical analysis

Phytochemical analysis from *E. scoparia* leaves showed the presence of lupeol and α -amyrin, that they were isolated. Column chromatographic fractionation of the hexane extract (4 g) on silica gel with dichloromethane: methanol (95:5) as eluent, provided lupeol and

α amyrin, in a ratio of 90:10, as major constituents. The residue from the combined hexane extracts (4 g) was fractionated by column chromatography on silica gel 60 (230-400 mesh) with dichloromethane: methanol (95:5) and provided a crude mixture of lupeol and α -amyrin (figure 11). By TLC an approximate ratio of 9:1 was determined between the two triterpenes that represented the

major constituents of the selected fractions. The fractions were combined and the solvent evaporated under reduced pressure. The residue was purified by recrystallization from MeOH and 2.2 g of lupeol and α amyryrin mixture were isolated. It was not possible to further separate the lupeol from the α amyryrin in a reasonable purity and yield due to the close similarity between the two structures. The analytical data observed from the spectra of EI-mass and $^1\text{H-NMR}$ are reported. For lupeol mixed with α amyryrin: EIMS m/z: 426[M⁺] (33), 408 (75), 365 (58), 207 (8), 189 (35); $^1\text{H-NMR}$ (400 MHz, CDCl₃): δ : 4.68 and 4.56 (each 1H, bs, H-29), 3.20 (1H, m, overlapped with H-3 from amyryrin H-3), 2.39 (1H, m, H-29), 1.68 (3H, s, H-30), 1.03 (3H, s, H-26), 0.97 (3H, s, H-23), 0.95 (3H, s, H-27), 0.83, 0.79 and 0.76 (each 3H, s, H-25, 28, 24). For β -amyryrin mixed with lupeol, EIMS m/z: 426 [M⁺] (8), 218 (100), 203 (25), $^1\text{H-NMR}$: δ : 5.13 (1H, t, J 3.5 Hz), 3.22 (1H, m, overlapped with H-3 from lupeol), 1.07, 1.00, 0.99 and 0.95 (each 3H, s, H-27, 26, 23, 25), 0.91 (3H, s, H-30), 0.79 (6H, s, H-28, H-24) and 0.79 (3H, d, J 6Hz, H-29). $^1\text{H-NMR}$ and mass spectral data were in accordance with published data for lupeol and α -amyryrin (Ahmad, 1994).

Essential oil yield from *T. piperella* dry leaves was 2.1v/w. The most abundant compounds obtained with GC-MS were γ -terpinene (11.3%), p-cymene (31.7%) and thymol (37.6%); indeed, they share the same biosynthetic route of which the precursor is γ -terpinene.

Characterization of the Plant Organ (drug) that was used for the Assays

Concerning the characterization of *E scoparia* leaves:

Morphology: Leaves were small (4-7 x 0.5-0.9 mm) and arranged around the stem node in groups (3-4 leaves). They were linear with recurved margins. The young leaf had pilosity and the adult one was glabrous. There were two channels or furrows from the base to the apex of the leaf, next to the midrib, on the underside of the leaf (Figure 9). Hispid and prickly trichomes, subglandular hairs and considerably ornamented indumenta with a small barrel form were in these channels (Figure 10).

Anatomy (cross section of leaf blade): The leaf cross section (Figure 11) had an elliptical-oval form. It showed a monostratified epidermis with a thick cuticle and stratified external wall, characteristic of mucilage presence. On the epidermis, the foliar indumenta were situated on the lower epidermis, but the ornamented indumentum was situated on the leaf blade margins. The palisade parenchyma showed one layer of thin-walled, long, and compactly arranged cylindrical cells. The spongy parenchyma showed isodiametric loose cells and was located underneath the palisade. Both parenchyma's showed druses in idioblasts. There were collateral vascular bundles in the spongy parenchyma. Primary vascular bundle or midrib was surrounded by sclerenchymatous cells in the oldest leaves.

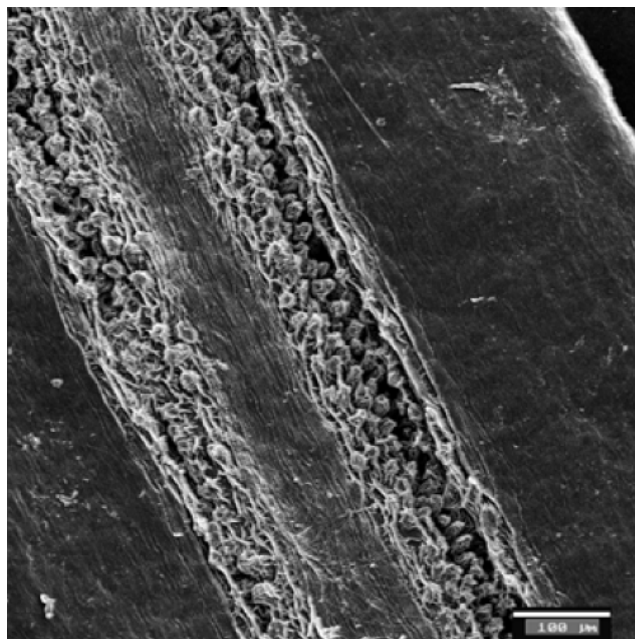


Figure 9: *Erica scoparia*. Two channels on the underside of the leaf blade.

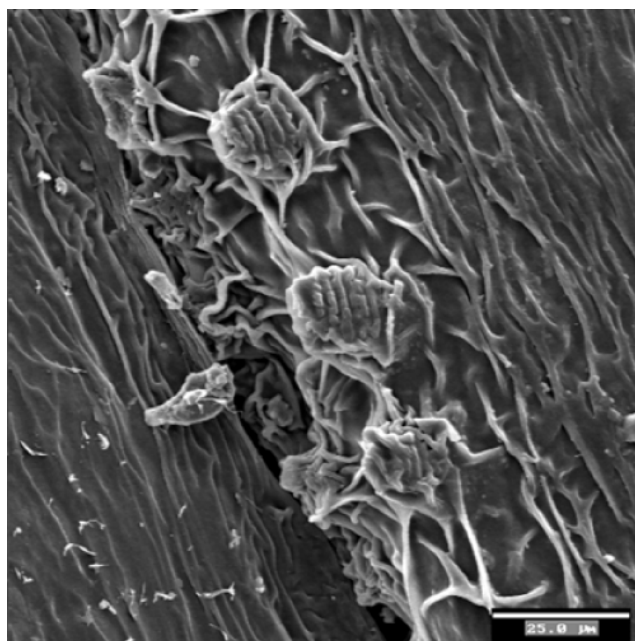


Figure 10: *Erica scoparia* Characteristic foliar indumentum on the leaf margin.

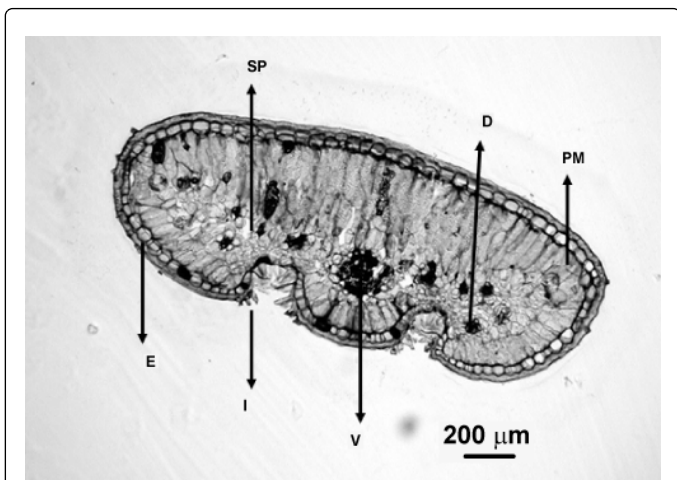


Figure 11: *Erica scoparia*. Leaf blade cross section. D: druse, E: epidermis, I: characteristic indumentum; SP: spongy parenchyma, PM: palisade mesophyll, V: midrib.

Concerning the characterization of *T. piperella* leaves:

Morphology: leaves are broad and oval with small glands located in deep fovea and small glandular trichomes, both essential oil reservoirs, and distributed on the upper and lower side of the leaf blade (Figure 12).

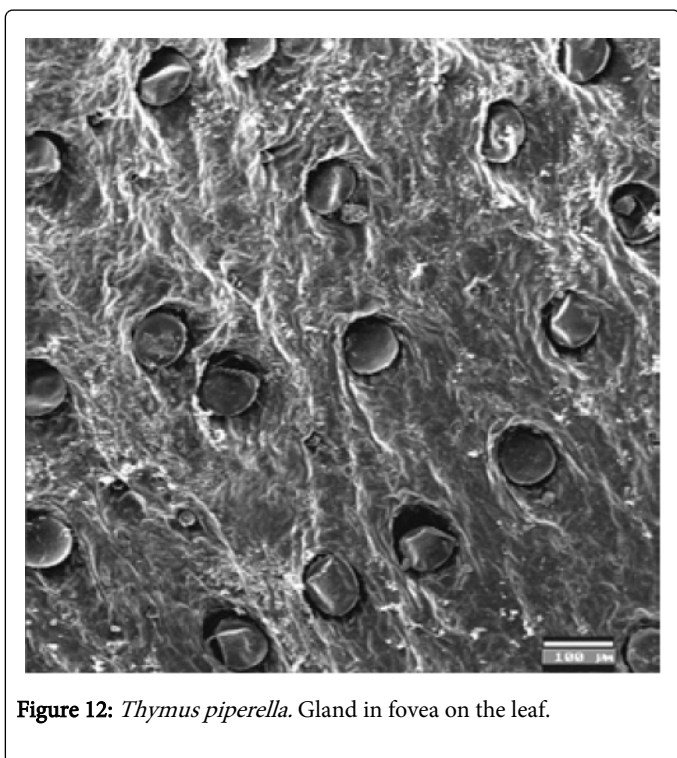


Figure 12: *Thymus piperella*. Gland in fovea on the leaf.

Anatomy: An anatomical characteristic is the presence of continuous sclerenchyma coupled to all the vascular bundles; this structure allowed differentiating other *Thymus* species (Figure 13).

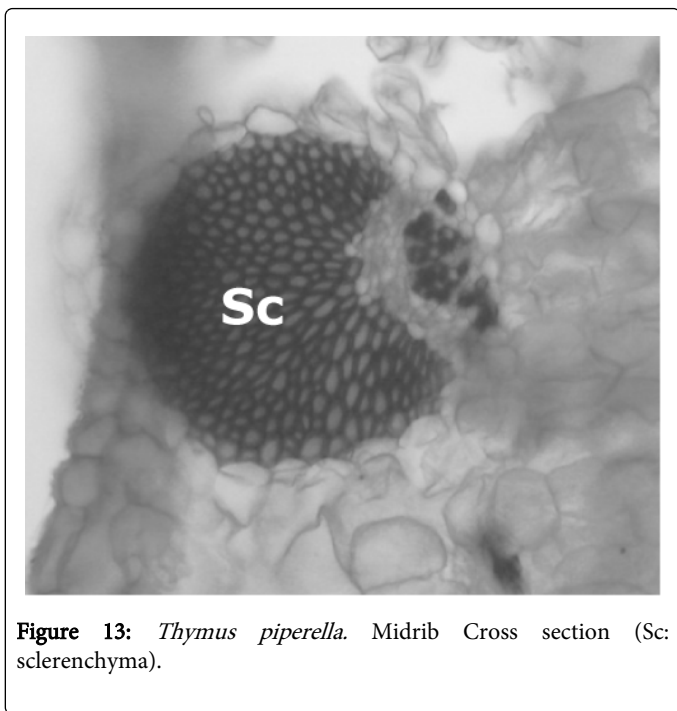


Figure 13: *Thymus piperella*. Midrib Cross section (Sc: sclerenchyma).

Evaluation of the Medicinal Potential in Plant Community

It is observed that the medicinal potential from plant community, measured as the percentage of medicinal species, increased after this study, from 62% to 66%; it also increased the number of toxic species (Figure 14).

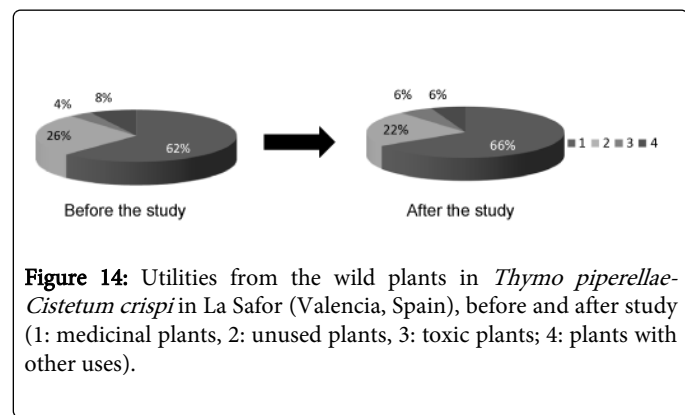


Figure 14: Utilities from the wild plants in *Thymo piperellae-Cistetum crispum* in La Safor (Valencia, Spain), before and after study (1: medicinal plants, 2: unused plants, 3: toxic plants; 4: plants with other uses).

Discussion

The conservation of biodiversity is a fact of current interest. It is not a passing trend; it is a necessity connected to the industrial development of the world, by the migration of people and food, by the cultural interchanges and the effects of globalization. But do we ask ourselves, why actually we conserve the vegetation, what must we protect? And why do we do it? This work arose when we tried to answer these questions. We concluded that we must know the species and the ecosystems, but we must also see how useful they are for humans. The use of the natural resources must be an important subject within the plans of a sustainable development. It is not conserve just for the sake of conservation but also because of its possible medicinal use. We know that wild flora cannot always be used

directly; in these cases, the plants will only be used to research, and it is possible to find some use for them to justify their growing and conservation *ex situ*.

This work is an example about which we have discussed previously. We have observed that the medicinal potential in a plant community increases after doing the pharmacological test from characteristic species initially not considered as medicinal plants or no scientifically proven information that justifies their use.

The oral acute toxicity did not showed evidence of toxicity at the assayed dose for *T. piperella* and *E. scoparia*, but animals died at the same doses of assayed *C. crispus* extracts. So, the toxicity test showed that *T. piperella* and *E. scoparia* could be used as medicinal plants, as a tea that it is the most usual form of consumption, because they were not toxic plants. However, *C. crispus* was a toxic plant at low doses and should not be consumed; Mulet [7] already warned about the toxicity of this species.

With respect to the medicinal properties, on the one hand and in order to determine the type of analgesia induced by the *E scoparia* extract, the activity produced by two different pain stimuli was evaluated: heat and chemical agents, in this case acetic acid. Although both cerebral and peripheral analgesics respond by inhibiting pain stimuli of chemical and mechanical origin, only central analgesics increase the time of response in the hot plate test [22]. In this sense, methanol, hexane and dichloromethane extracts from *E scoparia* leaves showed an analgesic effect by significantly reducing the percentage of writhing movements induced by the acetic acid, and an analgesic effect arising from CNS action, observed in the increase in response time to licking and jumping in the hot plate test. Besides, anti-inflammatory activity was evaluated by the most commonly employed method according to the literature [23]. Oedema induction with carrageenan is preferable to that induced by other phlogistic agents because it is less influenced by non-specific factors such as vasodilatation and diuresis. The activity of anti-inflammatory drugs in this test exhibited a good correlation with their anti-inflammatory activity in humans [24,25]. In the present study, the evaluated *E. scoparia* extracts showed different profiles of anti-inflammatory activity in the rat when they were tested 1, 2, 3 and 24 h after the administration of the inflammatory agent. The extracts from the species showed a significant anti-inflammatory activity by inhibiting the oedema induced by carrageenin up to 24 h after the administration of this phlogistic agent.

On the other hand, the *T. piperella* spasmolytic activity was tested against acetylcholine induced contractions in isolated rat ileum. The different extracts inhibited significantly in a concentration-dependent manner. Maximum effect was obtained with the hexane extract. And the essential oil from leaves was active against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Streptococcus pyogenes* and *Mycobacterium phlei*

The phytochemical study showed the presence of certain substances that could explain the medicinal properties of the studied species. In the *T. piperella* essential oil, p-cimeno, timol and terpineno monoterpenes were the main components; and several triterpenes, amirine and lupeol, in the hexane and diclorometane extracts from *E. scoparia* were isolated.

Finally, the study of plant organs used for evaluating the pharmacological activity (drugs) provided the needed differential characters for distinguishing these parts from other plant organ and other species, especially when the drug is adequately pulverized. The

most interesting characteristics were related to the leaf indumentum. *T. piperella* leaf is well differentiated from other *Thymus* species more abundant, like *Thymus vulgaris L.*, where the indumenta is formed by glands and glandular trichomes of different types such as star-shaped trichomes or branched ones but no glands in fovea [26]. *E. scoparia* presented simple eglandular trichomes located along the channel part of the abaxial surface of leaves. These trichomes are longer and thinner than the upper surface ones; this specie also presented characteristic trichomes on the margin of the leaf.

Given explained above, the medicinal and toxic properties of the *Thymo piperellae-Cistetum crispum* characteristic species are now disclosed and the results of previous works [27,28] are confirmed. It is deduced from this study that *E. scoparia* and *T. piperella* are medicinal plants and their popular uses are now scientifically proven. In addition, the drugs (leaves) are easily recognizable by their leaf morphology and anatomy, this is very important if the use of these species goes become popular. Finally, it is derived from this work that this multidisciplinary research type increases the percentage of medicinal plants from the studied community, thus increasing the value of this vegetation and offers reasons for protecting and preserving it for use.

Conclusion

The proposed protocol for assessing the medicinal potential from a plant community is valid and provides scientific data that justifies popular or new uses. This evaluation can increase the number of medicinal plant in a plant community.

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