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Original Article

Anti-Inflammatory, Analgesic Activities, and Phytochemical Study of *Traganum nudatum* Delile

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Abstract

Traganum nudatum Delile is a North African endemic medicinal plant commonly used in traditional medicine to treat wounds, hemorrhoids, rheumatism, and ear infections. Nevertheless, there are no scientific reports on the anti-inflammatory and analgesic effects of this plant. The present study aims to assess the therapeutic effect of the aqueous extract of the aerial part of the plant at three doses (40, 80, and 120 mg/kg) on experimentally pain-induced animals. First, the anti-inflammatory activity was assessed by carrageenan-induced paw edema on Swiss albino mice. Secondly, the analgesic activity was assessed by the acetic acid-induced writhing test. In addition, UPLC-MS-PDA and GC-MS-FID analyses were performed to screen the possible therapeutic compounds. The anti-inflammatory effect of the aqueous extract at 120 mg/kg for 4 h experiment was significantly higher (89.97± 0.17%) than those of the reference drug acetylsalicylic acid (ASA) (0± 0.25%);. In comparison, the analgesic test showed a remarkable reducing pain effect (69.8 ± 1.7%) at the dose of 80 mg/kg, almost similar to those of Acetaminophen (Paracetamol) (72.54 ± 2.26 %). The phytochemical screening revealed the presence of therapeutic biomolecules such as flavonoids, mainly rutin (6440± 3.0 µg/g), flavonols (narcissoside 115.1± 1.4 µg/g) and phenolic acids (chlorogenic acid 1480± 1.6 µg/g). GC-MS-FID showed the presence of saturated acids such as behenic and palmitic acid with percentages of 35.58 ± 0.06 % and 17.54 ± 0.09 %, respectively. These were in a higher percentage than the unsaturated fatty acids. These results validated the use of *T.nudatum* Delile for treating inflammatory and analgesic disorders in folk medicine. The presence of bioactive compounds, including polyphenols and fatty acids, may explain the pharmacological effect of the medicinal plant.

Keywords: Analgesic, Anti-inflammatory, GC-MS-FID, Polyphenols, *Traganum nudatum* Delile, UPLC-MS-PDA.

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1. Introduction

According to Farnsworth and Kass, around 80% of the world's population use medicinal plants to treat various health problems [1]. In North Africa, regional pharmacopoeias are mainly based on traditional Arabic medicine and the experience of herbalists. However, the rules of use of medicinal plants often lack the rigor of modern therapeutic techniques regarding their safety and effectiveness [2]. In North Africa, Algeria is known for the diversity of its vegetation and its privileged ecosystem between the Mediterranean Sea and sub-Saharan Africa. The Saharan region has a specific flora, which is characterized by a large floristic diversity highly adapted to the arid climate of this zone [3].

T.nudatum Delile is an endemic medicinal plant belonging to the family of Amaranthaceae. It is a 15-40 cm tall plant with drifting stems, white branches and alternating fleshy and oval leaves, with a small yellowish curved spine at the top (Figure 1). It is used by the local population as herbal tea, powder or balm to treat various diseases such as wounds, skin diseases, hemorrhoids, rheumatism, exhaustion, and ear infections [4]. It is also a favored pastoral plant for sheep, camels, and fat sand rats *Psammomys obesus* [5]. Despite its therapeutic uses, there is a lack of scientific studies about the anti-inflammatory and analgesic effect of *T.nudatum* Del. Only one report is available on the phytochemical screening of this plant. The study revealed the presence of flavonoids, flavonols, tannins, alkaloids, and high amounts of polyphenols [5]. The present study aimed to assess pharmacological activities of the plant's aqueous extract, and use UPLC-MS-PDA and GC-MS-FID analysis to perform a phytochemical screening to detect and quantify bioactive compounds showing promising activities.

2. Material and Methods

2.1. Plant Material

Traganum nudatum Delile synonym of *Traganum acuminatum Maire & Weiller ; Traganum nudatum var. acuminatum (Maire & Weiller); Traganum nudatum var. microphyllum Maire* was collected in March 2017 in the Souara region (south-west of Algeria), and identified by the higher national agricultural school in Algiers. A voucher specimen of the plant was deposited in the herbarium of the laboratory. For this study, we used the aerial parts of the plant (leaves, stems, and flowers), shade-dried at room temperature, and ground them into a fine powder using an electric blender.

2.2. Animals

Studies were carried out using male and female Swiss albino mice weighing 18–22 g, obtained from the animal house, Institut Pasteur of Algeria, Algiers, under the grant agreement N°778298 signed by the Algerian Association of sciences in animal experimentation. The animals were grouped in polyacrylic cages, with six animals per cage, and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycles (14/10h) and free access to standard dry pellet diet and water.

2.3. Anti-Inflammatory Activity

The anti-inflammatory activity was evaluated using the protocol described by Winter *et al.* [6]. Albinos mice were divided randomly into five groups (n=6). Control mice were treated by oral administration of 0.9% sterile saline water (0.5 mL / 20 g), positive control with 150 mg/kg of acetylsalicylic acid (ASA) as a drug reference, while the remaining groups received the plant's aqueous extract at three different doses (40, 80 and 120 mg/kg). Edema was induced by injecting carrageenan suspension (50 µL, 1 % w/v) into the sub-plantar tissues of the right paw 30 min after oral treatments. The evolution of paw edema was measured hourly by a caliper for 4 h after injection. The edema percentage was calculated using the mean diameter of the right paws after carrageenan injection and the mean diameter of the healthy left paws, according to the following equation:

$$\%\ Edema = \frac{Mean\ diameter\ of\ left\ paw - Mean\ diameter\ of\ right\ paw}{Mean\ diameter\ of\ left\ paw} \times 100$$

The anti-inflammatory activity was calculated as the inhibition percentage of edema between the tested and control groups following the equation:

$$\%\ Inhibition = \frac{\%\ Edema\ of\ control\ group - \%\ Edema\ of\ tested\ group}{\%\ Edema\ of\ control\ group} \times 100$$

2.4. Analgesic Activity

The analgesic effect was evaluated using the method described by Koster *et al.* [7], and Collier *et al*. [8] with a slight modification [9, 10]. Albinos mice were divided randomly into five groups (n=6). The control group received orally 0.9% sterile saline water (0.5 mL /20 g), positive control received Acetaminophen (Paracetamol) as a drug reference (100 mg/kg), while other groups of mice received the plant's aqueous extract at three different doses of 40, 80 and 120 mg/kg. To induce a positive response (stretching of the hind legs and cramps in the abdomen), acetic acid (0.6%; v/v) was injected intraperitoneally into mice (10 mL /kg) 30 min after the oral administration of drugs. The number of cramps of each mouse was counted 5 min after the injection during 15 min. The protection percentage was calculated according to the following equation:

$$\%\ Pain\ inhibition = \frac{Cramps\ of\ control\ group - Cramps\ of\ tested\ group}{Cramps\ of\ control\ group} \times 100$$

2.5. Phytochemical Study

All the analyses of this study were carried out in triplicates and summarized in Fig. 2.

2.5. 1. Reagents and Standards Used in Extractions for GC-MS and UPLC Analysis

2, 6-di-t-Butyl-4-methyl-hydroxybenzene (BHT) and heptadecanoic were purchased from Aldrich (Sigma-Aldrich, Madrid, Spain). Chlorotrimethylsilane (CTMS) was obtained from Fluka (Sigma-Aldrich, Madrid, España). Methanol, i-octane, hexane, and pyridine were acquired from J.T. Baker (J.T. Baker, Daventer, Holland). Anhydrous magnesium sulphate was purchased from Panreac (Panreac, Barcelona, Spain). A set of fatty acid methyl esters (FAMEs) reference standards (10 mg/mL in dichloromethane) was obtained from Supelco (Sigma-Aldrich).

A methanol stock solution of the following 57 commercial phenolic standards was prepared and stored at -80°C.

(-)-Chlorogenic acid and gallic acid were purchased from Acros Organics (Fisher Scientific SL, Madrid). Arbutin, caffeic acid, (+)-catechin, p-coumarin acid, cyanidin chloride, 3,5-dicaffeoylquinic acid, daidzein, ellagic acid, epicatechin, epicatechingallate, (+)-eriocitrin, eriodictyol, ferulic acid, galangin, genistein, gallocatechingallate, hesperidin, hydroxytyrosol, hyperoside, isorhamnetin-3-0-beta-D-glucoside, kaempferol, luteoloside, myricetin, narcissoside, naringindihydrochalcone, narirutin, orientin, 1,2,3,4,6-pentagalloyl glucose, phloretin, phloridzin, rutin, procyanidin A2, procyanidin B1, procyanidin B2, procyanidin C1, quercetin, quercitrin, +(-) p-salicylic acid, taxifolin, theaflavin, theaflavindigallate, trangeretin, vanillic acid, vicenin II and vitexin were from Chengdu Biopurify Phytochemicals Ltd. (Cymit Quimica, Barcelona). Cyanidin-3-glucoside chloride, delphinidin-3-O-rutinoside chloride, keracyanin chloride and quercetin-3-o-glucopyranoside were from Extrasynthese (Lyon, France). Fisetin and pelargonidin-3-rutinoside chloride were from Carbosynth Ltd (Berkshire, England).

2.5.2. Metabolite Analysis

UPLC and CG were used as chromatography techniques for the detection of phenolic and other compounds. UV-visible spectrometry was used for the detection of pro-anthacyanidin (condensed tannins). Extraction procedures used were according to the analytical techniques used.

2.5.2.1. Extraction of Free Phenolic Compounds for UPLC Analysis

Plant powder (250 mg) was extracted with 5 mL of acidified (0.1% v/v acetic acid) methanol/water (50:50) solution and then twice with 5 mL of acetone: water (70:30) solution in an ultrasonic bath for 30 min. Extracted solutions were joined and diluted with 1:4 acidified Milli-Q-water (0.1% v/v of acetic acid) and filtered using 0.20 µm PTFE filters (poly-tetrafluoroethylene). Solutions were kept at 6°C until UPLC analysis. All solvents contained ascorbic acid (0.2% w/v) [11].

2.5.2.2. Extraction of Bound Phenolic Compounds for UPLC Analysis

Bound phenolic compounds are covalently conjugated to cellulose, pectin, and polysaccharides by ester bonds and can be difficult to hydrolyze. Basic and acidic hydrolysis methods can be used to release bound phenolic compounds [12].

2.5.2.2.1. Basic Hydrolysis of Bound Phenolic Compounds for UPLC Analysis

The residue of the previous extraction was lyophilized and 50 mg were mixed with 1.5 mL of 2M NaOH containing 10 mM EDTA and 1% ascorbic acid. The mixture was shaken in an Eppendorf® Thermomixer for 30 min at 45°C, acidified at pH 3 with 0.285 mL of 7.2 M HCl and centrifuged at 1400xg for 5 min [13][11]. Sample was extracted three times with ethyl acetate (x4 volumes), centrifuged at 1400 x g for 10 min and the supernatant was collected after each extraction. All the acetate extractions were joined and kept at the freezer until analysis. Prior to the UPLC analysis, samples were dried under nitrogen stream. The residue was dissolved in 0.5 mL MeOH, then diluted with running buffer (1.25% glacial acetic acid, 7% MeOH in water) and the sample analyzed by UPLC [14, 11].

2.5.2.2.2. Acidic Hydrolysis of Bound Pro-Anthocyanidins for Spectrometry Analysis

The lyophilized sample obtained above (10 mg) was incubated with 5 mL of n-butanol/HCl (95:5) solution and 200 µL of ferric reagent (2% ferric ammonium sulphate in 2M HCl) for 60 min [15]. The mixture was cooled in ice water and centrifuged. Pro-anthocyanidins were measured at λ_{max} = 550 nm, using cyanidin chloride as a reference compound [11].

2.5.2.3. Direct Methylation-Silylation for GC-MS Analysis

A combined derivatization method based on the methylation of carboxylic acids and silylation of hydroxyl groups was used to allow the identification of different metabolites by GC-MS [16]. Derivatization is typically performed to change the analyte properties for a better separation and enhance the method sensitivity, which may improve the capability of identifying compounds. In a 2 mL reaction vial fitted with a poly-tetrafluoroethylene lined cap (PTFE), 20 mg of plant powder, 60 µL of dry MeOH and 250 µL of chlorotrimethylsilane (CTMS) were mixed and vortexed for 2 min, the vial was heated in a digestion stirrer block at 75°C for 15 min. Dry isooctane (600 µL) was added to the cooled vial and vortexed for 1 min. Dry pyridine (180 µL) was added to the reaction vial and vortexed for 2 min. Finally, the vial was centrifuged at 1400 x g for 5 min and the upper phase was recovered and analyzed immediately by GC-MS.

2.5.2.4. Methylation of Fatty Acids for GC-FID Analysis

In order to quantify the free fatty acids as methyl esters, a derivatization method based on acid catalysis was used [16]. To a 15 mL reaction vial fitted with a PTFE-lined cap (poly-tetrafluoroethylene) were added 50 mg of

sample, 0.5 mL of hexane containing 1 mg/mL of internal standard solution (heptadecanoic acid methyl ester), 1 mL of methanol (MeOH) saturated with dry nitrogen and 0.5 mL of chlorotrimethylsilane (CTMS). The vial was filled with nitrogen and placed in a digestion block at 80ºC for 45 min. After cooling, 2 mL of hexane/diethyl ether solution (1:1) were added and the mixture was neutralized carefully by adding 0.2 g of powdered sodium bicarbonate and 2 mL of saturated aqueous sodium bicarbonate solution. The upper phase was recovered, dried over anhydrous magnesium sulphate and analysed by GC-FID.

2.5.3. Chromatography Analysis

To identify and quantify the phenolic compounds in the plant's extracts, UPLC coupled with a photodiode array detector (PDA) in series with a mass spectrometry detector (MS) was used. The screening of bioactive constituents with low mass polar, semi polar and non-polar compounds present in the sample was carried out with GC-MS after derivatization [17]. The GC-FID was used to quantify the fatty acids as methyl esters by calibration with the internal standard method. The response factors of methyl esters of commercially available saturated and unsaturated fatty acids were calculated under the same chromatographic conditions.

2.5.4. Chromatography Parameters

2.5.4.1. UPLC-PDA Parameters

Ultra-performance liquid chromatographic analysis was carried out on a Waters ACQUITY UPLC™ system (Waters, Milford, MA, USA) consisting of an ACQUITY UPLC™ binary solvent manager and ACQUITY UPLC™ sample manager, coupled to a photodiode array detector ACQUITY UPLC™ PDA. The separation of compounds were carried out by an ACQUITY UPLC™ HSS T3 column (1.8 µm; 2.1 mm x 150 mm) (Waters, Manchester, UK) using a mobile phase consisting of solvent A, H_2O: ACN (95:5) with 0.1% v/v acetic acid, and solvent B, ACN 100% (0.1% v/v HAcO). The flow rate was 0.50 mL /min. The linear gradient : 0-1.89 min, 1% B, (isocratic); 1.89 – 17.84 min, 30% B, (linear gradient); 17.84 – 21.39 min, 5% B, (linear gradient); 21.39 – 21.56 min, 1% B (linear gradient); 21.56 - 25 min, 1% B (isocratic). The injection volume was 10 µL in full loop mode; the column was kept at 45°C as the temperature in the sample manager was maintained at 10°C.

2.5.4.2. UPLC-MS Parameters

MS analysis was performed on a Waters ACQUITY XEVO TQS tandem quadrupole mass spectrometer (Waters, UK), using an electro-spray source (ESI) in positive and negative ion mode. The ESI parameters were as following: capillary voltage of 3.0 kV and -2.5 kV in positive and negative mode, respectively; the source at 150°C; desolvation temperature 500°C; cone gas (nitrogen) flow 150 L/h; and desolvation gas flow 800 L/h. Flow injections of each individual standard were used to optimize the cone voltage and Multiple Reaction Monitoring (MRM) parameters. Collision-induced dissociation was achieved

using argon at a flow rate of 0.15 mL /min in the collision cell. MassLynx 4.1 software (Waters, USA) was used for data acquisition. Polyphenols were identified on the basis of the comparison of retention times, the UV and MS spectra of unknown peaks with those of reference standards.

2.5.4.3. GC-MS Parameters

GC-MS analysis was performed on an Agilent 7890A GC with a capillary column: DB 5-MS-UI; 30 m x 0.25 mm x 0.25µm. The carrier gas used was helium with constant flow rate of 1 mL /min. The sample injection volume was 1 µl in splitless mode. The chromatographic conditions were: a) temperature program: initial temperature 80°C (isothermal for 2 min), an increase rate of 6°C/min up to 310°C with no hold; pressure of 6.4 10^4 Pa. The compounds were identified based on GC retention time with those available in the GC-MS computer library's NIST 17 and NIST MS Search 2.3.

2.5.4.4. GC-FID Parameters

GC-FID technique was used to quantify the individual components of the mixture. The temperature program (oven temperature) was the same as mentioned above but instead we used the column: DB-35-MS-UI (25 m x 250 µm x 0.25 µm), with a pressure of 8.1 10^4 Pa and a constant flow rate of 1 mL /min. The compounds were quantified using response factors considering the peak area of internal standard.

2.6. Statistical Analysis

Data were expressed as mean ± standard error of the mean. Statistical differences among the treatment groups were assessed by two-way Anova analysis of variance, followed by Tukey test with GraphPad Prism 5.03 software and differences were considered to be significant when $P < 0.05$.

3. Results and Discussion

3.1. Anti-Inflammatory Activity

The anti-inflammatory effect of *T.nutadum* Del was evaluated during 4 h of experiment. We recorded an increase in inhibition effect in both ASA and aqueous extract until the second hour (57.47± 0.21 %; 56.96± 0.29 %) corresponding to a decrease of edema diameter (4.98± 0.21%; 5.04± 0.29 %) respectively. After the second hour, the inhibitory effect of ASA decreased until fading out (0± 0.25%), corresponding to an increase in edema diameter (10.83± 0.25%), while that of the aqueous extract kept increasing significantly until the end of the experiment (89.97± 0.17%) corresponding to a decrease in edema diameter (1.00± 0.17%). (Figure 3, Figure 4; Table 1, Table 2). Inflammation is a two-phase process; the initial phase is observed around the first hour after the injection of carrageenan, which is attributed to the release of chemical mediators such as histamine and serotonin. The second phase is due to the release of prostaglandins at the end of the second to the third hour [18, 19]. According to Abayomi *et al*. [20], aqueous extract of some medicinal plants of *the Chenopodiaceae family has significant anti-*

inflammatory properties due to flavonoids, polyphenols, and saponins. These compounds oppose the formation of prostaglandin, lysosomes, and proteases at the inflammatory site [21].

3.2. Analgesic Activity

The aqueous extract of *T. nudatum* Del at the dose of 80 mg/kg showed the strongest protection against abdominal cramps (69.8± 1.7%) with a significant difference compared to Paracetamol (72.54 ± 2.26 %) (Figure 5, Table 3). Analgesic properties were evaluated using acetic acid, which involves the peripheral mechanisms of pain by inducing the release of chemical mediators such as histamine, prostaglandins, serotonin, and bradykinin [8, 22]. Paracetamol act on pain mechanisms by intervening in the biosynthesis of prostaglandins [23]. The results found in this study corroborate with those of Ibironke and Ajiboye, who demonstrated that some medicinal plants of *Chenopodiaceae* family have analgesic properties, which might be related to the presence of phenolic compounds, flavonoids and tannins [24].

3.3. Free Phenolic Compounds

Phytochemical study revealed high amounts in all families of polyphenols, including flavonoids, flavones, flavonols, flavan-3-ol and phenolic acids, corroborating with the results that *Chenopodiaceae* family plants contain high amounts of polyphenols [5, 25].

Free phenolic compounds were analyzed using UPLC-MS-PDA, identified and quantified by comparison to 57 phenolic standard compounds (Figure 6, Figure 7; Table 4). Narcissoside (ishoramnetin 3-O-rutinoside) known for its therapeutic capacity for the treatment of severe vascular inflammatory diseases, was the main compound of all free polyphenols identified (115.1± 1.4 µg/g). Vanillic acid (21.7 ± 0.5 µg/g), was identified in the medicinal plant *Clerodendrum petasites* and is considered a potential candidate for the anti-inflammatory effects [26, 27].

3.4. Bound Phenolic Compounds

Bound phenolic compounds were obtained by basic hydrolysis of the remaining solid material and identified by UPLC-PDA-MS-tQ (Figure 8, Table 5). Many compounds, such as flavonoids, flavonols, flavones, flavan-3-ols, phenolic acids and anthocyanin were detected. The main compound known for its anti-inflammatory and analgesic effects on Albinos mice was rutin (6440 ± 3.0 µg/g), followed by luteolin-7-O-glucoside (3250 ± 2.7µg/g), which has anti-inflammatory, anti-allergic and antioxidant potential [28, 29]. On the other hand, fisetin known for its anti-inflammatory and anti-cancer effect [30][31] was found at 2660 ± 0.4 µg/g. Delphinidin-3-o-rutinoside chloride found at 2614 ± 2.3µg/g is known for its many health benefits (antioxidant, antiplatelet, ophthalmic, and vasoprotective activities) [32]. Finally quercetin-o-glucopyranoside (1761 ± 0.4 µg/g) and chlorogenic acid (1480 ±1.6 µg/g) were identified as anti-inflammatory, antimicrobial and antioxidant agents [33, 34].

3.5. Bound Pro-anthocyanidins (Condensed Tannins)

Total condensed tannins of *T.nudatum* Del were measured by UV-vis spectrophotometry. We noticed their absence in the plant. These results proved the rare occurrence of the condensed tannins in *Amaranthaceae* family [35].

3.6. Identification of Compounds by GC-MS

The GC-MS allowed the identification of other compounds with potential therapeutic interest not previously detected in the sample (Figure 9, Table 6).

In general, these compounds included short alkyl carboxylic, dicarboxylic and aromatic acids, such as malic acid (Peak 3), 3,4-dimethoxycinnamic acid (Peak 15), citric acid (Peak 8), benzoic acid (Peak 1), ferulic acid (Peak 14) and nonanedioic acid (Peak 9).

Long-chain alcohols, hydroxy fatty acids, long chain fatty acids such as 1-hexacosanol (Peak 36), 1-octacosanol (Peak 39), 1-triacontanol (Peak 41), 10,16-dihydroxyhexadecanoic acid (Peak 24), octacosanoic acid (Peak 40), linolenic acid (Peak 19), myristic acid (Peak 11), palmitic acid (Peak 13), margaric acid (Peak 12), arachidic acid (Peak 22), oleic acid (Peak 17), behenic acid (Peak 26), lignoceric acid (Peak 32) hexacosanoic acid (Peak 38) and finally linolenic acid (Peak 19) were also detected.

3.7. *Fatty Acids Quantification*

GC-FID revealed the presence of more saturated than unsaturated fatty acids in the plant. Three major fatty acids were quantified (Figure 10, Table 7); the most abundant acid was behenic acid (C22:0) , 35.58 ± 0.06 % found in some *Chenopodiaceae* family plants and known for its antimicrobial and antioxidant activities, followed by palmitic acid (C16:0), 17.54 ± 0.09 %, which possesses antioxidant and anti-inflammatory properties in the human body and finally oleic acid, 8.57± 0.03 % presenting a pro-inflammatory effect in wound healing rats [36, 37, 38].

4. Conclusion

The pharmacological tests performed in the present study showed significant analgesic and anti-inflammatory activities of the aqueous extract of *T.nudatum* Del and scientifically validated the use of this plant for treating inflammatory and analgesic disorders in folk medicine. These pharmacological properties could be attributed to the presence of various bioactive compounds including polyphenols (narcissoside, rutin, fisetin, vanillic acid) and fatty acids (behenic, palmitic and oleic acid), which were identified and quantified by UPLC-MS-PDA and GC-MS-FID respectively. These results suggest that the chemical constitution of the plant could serve as a basis for the development of new analgesic and anti-inflammatory treatments; so further studies are necessary in order to identify and evaluate the mechanism of action of these therapeutic compounds.

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Tables:

Table 1. Edema diameter percentage of paw injected by carrageneen 1%, treated with aqueous extract of *T.nudatum* Del. P<0.05 significant (**), P<0.05 highly significant (***).

Groups	Doses (mg/kg)	Edema diameter of the right paw (%)			
		1st hour	2nd hour	3rd hour	4th hour
Control (Saline water)	-	7.78±0.31	11.71±0.18	15.11±0.17	9.97±0.10
Acetylsalicylic acid (ASA)	150	7.15± 0.32	4.98± 0.21	7.58± 0.18	10.83± 0.25
Aqueous extract	120	6.92± 0.09	5.04± 0.29	4.77± 0.20 **	1.00± 0.17 ***

Table 2. Edema inhibition percentage of aqueous extract of *T.nudatum* Del. P<0.05 significant (**), P<0.05 highly significant (***).

Groups	Doses (mg/kg)	Edema inhibition (%)			
		1st hour	2nd hour	3rd hour	4th hour
Acetylsalicylic acid (ASA)	150	8.10± 0.32	57.47± 0.21	49.83± 0.18	0± 0.25
Aqueous extract	120	11.05± 0.09	56.96± 0.29	68.43± 0.20**	89.97± 0.17***

Table 3. Number of abdominal cramps of mice injected by acetic acid 0.6%, treated with aqueous extract of *T.nudatum* Del. P<0.05 significant (**), P<0.05 highly significant (***).

Groups	Doses (mg/kg)	Pain inhibition (%)
Control (saline water)	-	0
Acetaminophen (Paracetamol)	100	72.54±2.26
Aqueous extract	40	45.3±1.6
	80	69.8±1.7 ***
	120	66±2

Table 4. Free phenolic compounds content in *T.nudatum* Del as determined by UPLC-PDA-MS-tQ. ND: Not determined.

Nº Peak	**Retention time (min)**	**Compound**	**Amount (µg/g; n=3)**
2	5.06	Vanillic acid	21.7 ± 0.5

4	5.19	Salicylic acid	6.2 ± 0.3
5	5.31	Syringic acid	5.1 ± 0.2
6	5.57	Procyanidin B2	ND
7	5.69	Kaempferol	ND
11	6.72	Ferulic acid	7.2 ± 0.9
13	6.96	Narcissoside	115.1 ± 1.4
14	7.24	Isorhamnetin glucoside	3.8 ± 0.4
16	8.50	Theaflavindigallate	4.4 ± 0.4
18	8.74	Hydroxytyrosol	4.0 ± 0.1
19	8.95	Quercetin	3.7 ± 0.16

Table 5. Bound phenolic compound content in *T.nudatum* Del as determined by UPLC-PDA-MS-tQ. ND: Not determined.

Nº Peak	**Retention time (min)**	**Compound**	**Amount (µg/g; n=3)**
1	4.25	Chlorogenic acid	1480±1.6
3	5.18	Catechin	940 ± 12.1
8	6.24	Delphinidin-3-o-rutinoside chloride	2614± 2.3
9	6.47	Hyperoside	26 ±0.3
10	6.56	Quercetin-o-glucopyranoside	1761 ± 0.4
12	6.81	Rutin	6440± 3.0
15	7.77	Luteolin-7-o-glucoside	3250± 2.7
17	8.63	Gallic acid	540 ± 1.1
20	9.16	Fisetin	2660 ±0.4

Table 6. Compounds identified in *T.nudatum* Del by GC-MS. The compounds with the hydroxyl groups were identified as trimethylsilylether derivatives and the compounds with carboxylic groups were identified as methyl esters previous derivatization.

Nº Peak	**Retention time (min)**	**Compound name**
1	**8.15**	**Benzoic acid**
2	8.43	Glycerol
3	**10.92**	**Malic acid**

4	**15.02**	**-**
5	**15.76**	**Monosaccharides in furanosic form (coelutions)**
6	15.91	Lauric acid
7	**16.01-22.60**	**Monosaccharides in pyranoside form (coelutions)**
8	**17.32**	**Citric acid**
9	**17.91**	**Nonanedioic acid**
10	**19.55**	**-**
11	**19.73**	**Myristic acid**
12	**24.87**	**Margaric acid**
13	**25.05**	**Palmitic acid**
14	**25.40**	**Ferulic acid**
15	**25.73**	**Cinnamic acid 3,4-dimethoxy**
16	25.80	2-Hydroxyhexadecanoic acid
17	**26.30**	**Oleic acid**
18	26.45	Linoleic and stearic acids (coelution)
19	**26.77**	**Linolenic acid**
20	28.42	1-Eicosanol
21	28.57	16-Hydroxyhexadecanoic acid
22	**29.37**	**Arachidic acid**
23	29.50	1,16-Hexadecanedioic acid
24	30.58	10,16-Dihydroxyhexadecanoic acid
25	31.12	Docohexaenoic acid
26	**32.10**	**Behenic acid**
27	32.25	1,18-Octadecanedioic acid
28	33.39	Tricosanoic acid
29	33.60	1-Tetracosanol
30	33.96	2-Hydroxydocosanoic acid
31	**34.01**	**Docosadienoic acid**
32	**34.64**	**Lignoceric acid**
33	34.81	Eicosanedioic acid
34	34.91	Triacontanoic acid
35	35.01	-
36	35.99	1-Hexacosanol
37	36.20	2-Hydroxytetracosanoic acid and 22-hydroxydocosanoic acid (coelution)
38	**37.01**	**Hexacosanoic acid**
39	38.21	1-Octacosanol
40	39.23	Octacosanoic acid
41	40.29	1-Triacontanol
42	41.30	Stigmasterol
43	42.00	Beta-Sitosterol
44	42.12	Stigmastanol

Table 7. Fatty acids quantification in *T.nudatum* Del as determined by GC-FID. To quantify the fatty acids in the plant by calibration method; Heptadecanoic acid methyl ester was used as an internal standard (IS).

Retention time (min)	Fatty acid name	Amount (% ww)
21.50	Myristic	4.25± 0.06
23.25	Pentadecanoic	0.33± 0.01
25.05	Palmitic	17.54± 0.09
27.98	Oleic	8.57± 0.03
28.08	Stearic	7.63± 0.09
28.13	Linolenic	1.26± 0.01
30.88	Arachidonic	6.29± 0.01
30.98	Arachidic	5.19± 0.02
31.12	Eicosapentaenoic (EPA)	1.03± 0.04
32.34	Henicosanoic	4.98± 0.02
32.10	Behenic	35.58± 0.06
33.82	Docosahexaenoic (DHA)	4.23± 0.04
34.93	Tricosanoic	1.20± 0.01
36.00	Nervonic	0.1± 0.26
36.16	Lignoceric	1.82± 0.02

Figures:

Figure 1. *Traganum nudatum* Delile. Morphological characteristics of the plant**.**

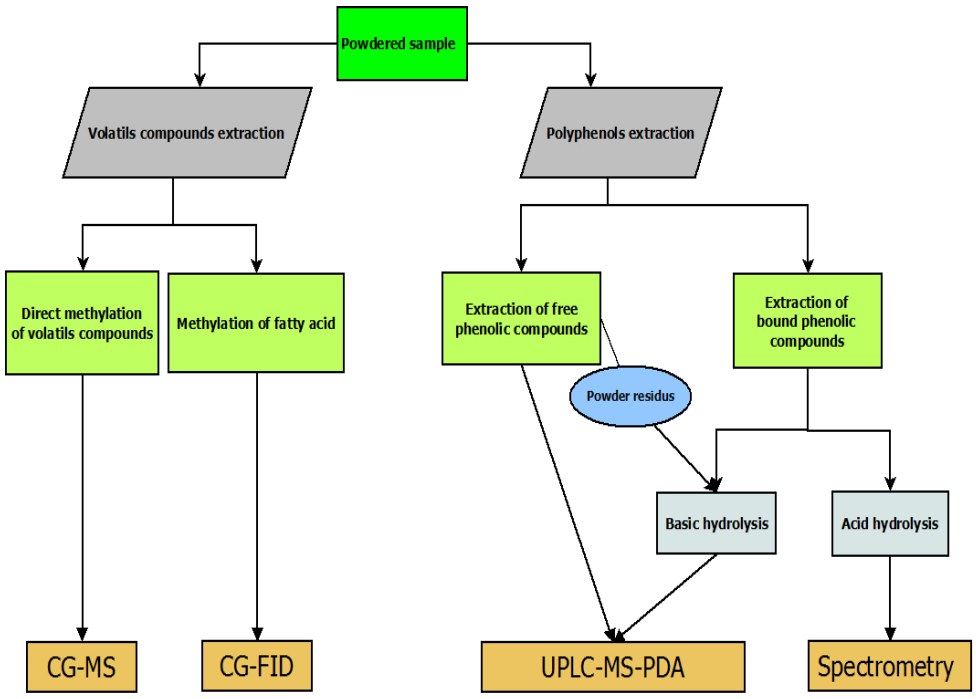

Figure 2. Methodological scheme of the phytochemistry study**.**

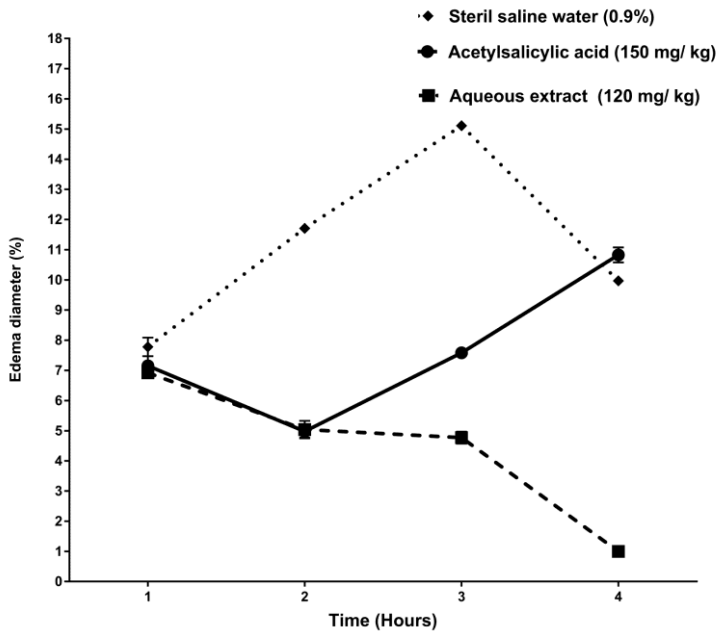

Figure 3. Edema diameter of paw injected by carrageneen 1% of mice treated with aqueous extract of *T.nudatum* Del and acetylsalicylic acid. P<0.05 significant (**), P<0.05 highly significant (***), P> 0.05 NS: No significant.

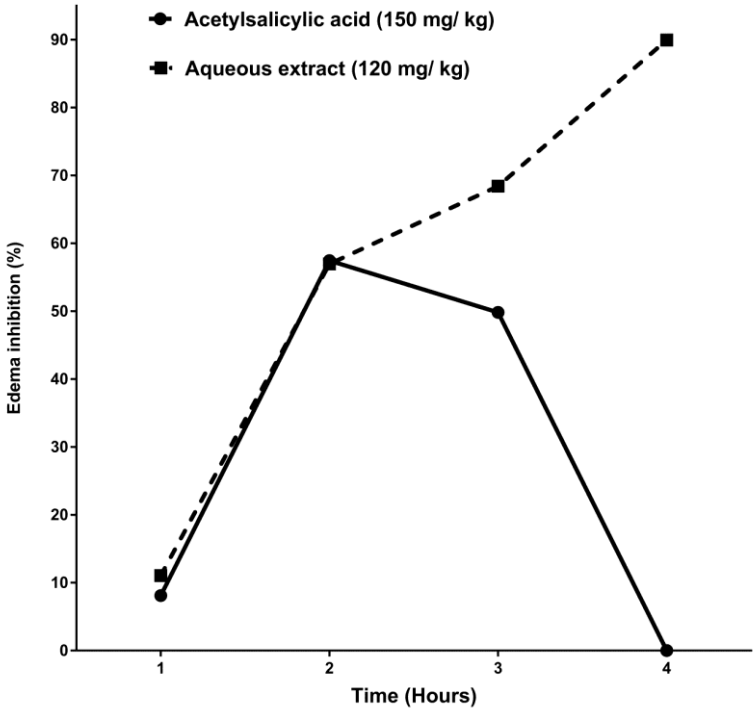

Figure 4. Edema inhibition of Albino mice treated with aqueous extract of *T.nudatum* Del and acetylsalicylic acid. P<0.05 significant (**), P<0.05 highly significant (***), P> 0.05 NS: No significant.

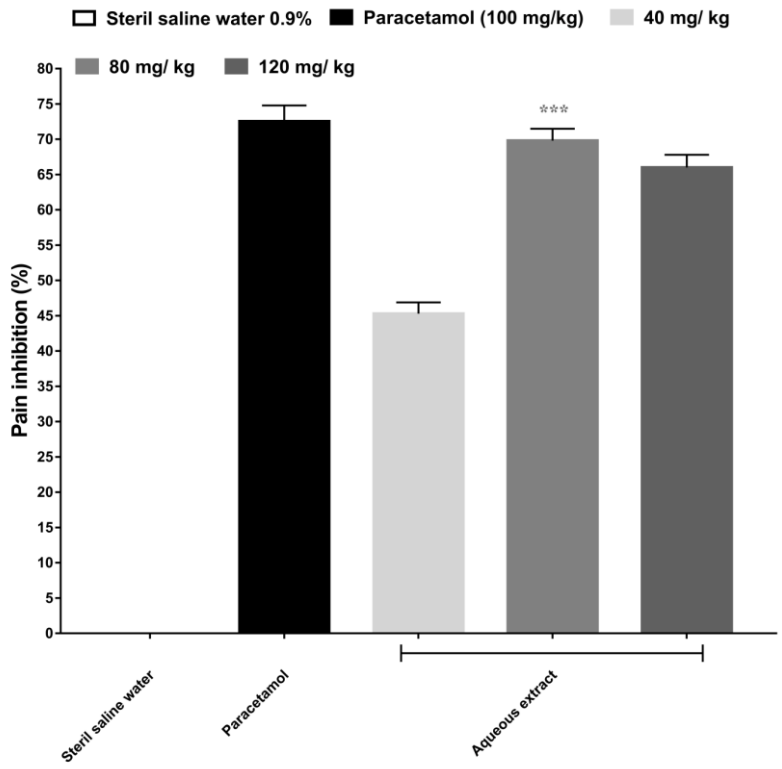

Figure 5. Pain inhibition of Albino mice treated with aqueous extract of *T.nudatum* Del and Paracetamol. P<0.05 significant (**), P<0.05 highly significant (***), P> 0.05 NS: No significant.

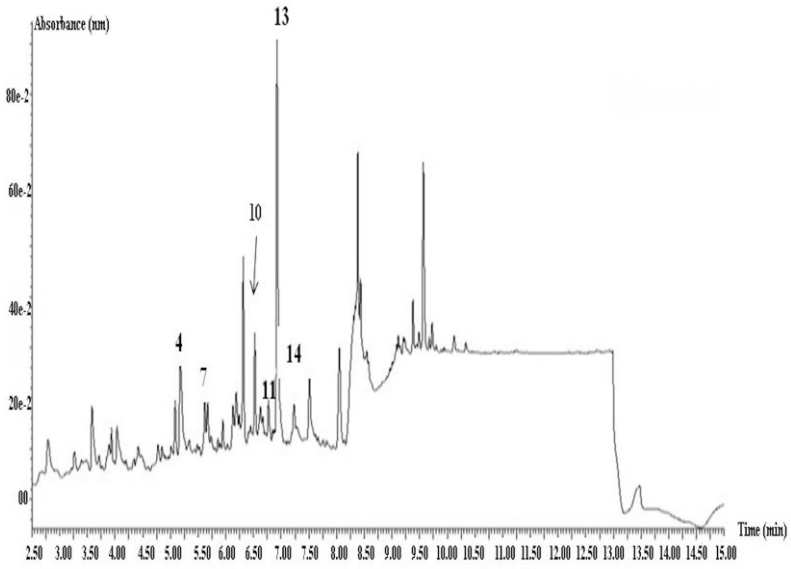

Figure 6. UPLC-PDA chromatograms of extract with MeOH/H_2O and Acetone/H_2O, UV at 240 nm. Numbered peaks correspond to the identified compounds: (4) Salicylic acid, (7) Kaempferol, (10) Quercetin-o-glucopyranoside, (11) Ferulic acid, (13) Narcissoside, (14) Isorhamnetin glucoside.

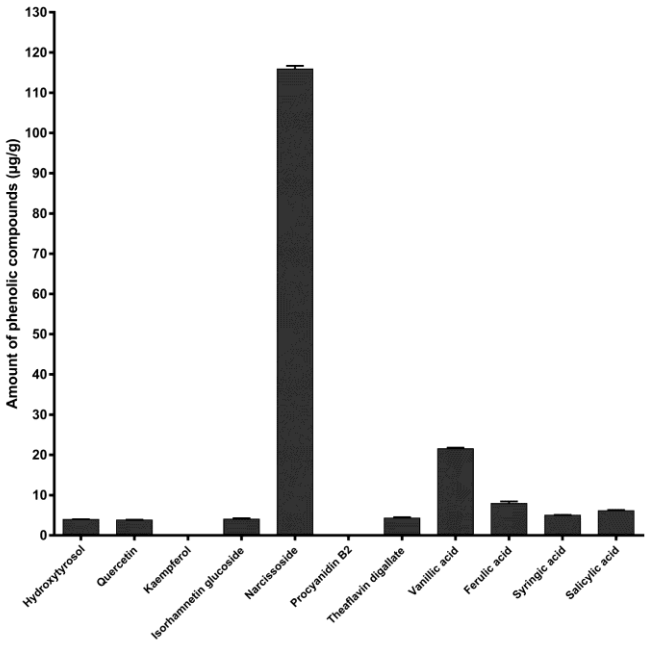

Figure 7. Free phenolic compounds content in the plant as determined by UPLC-PDA MS-tQ. Compounds were identified and quantified by comparison to 57 phenolic standard compounds available. All analyses were carried out in triplicates (n=3).

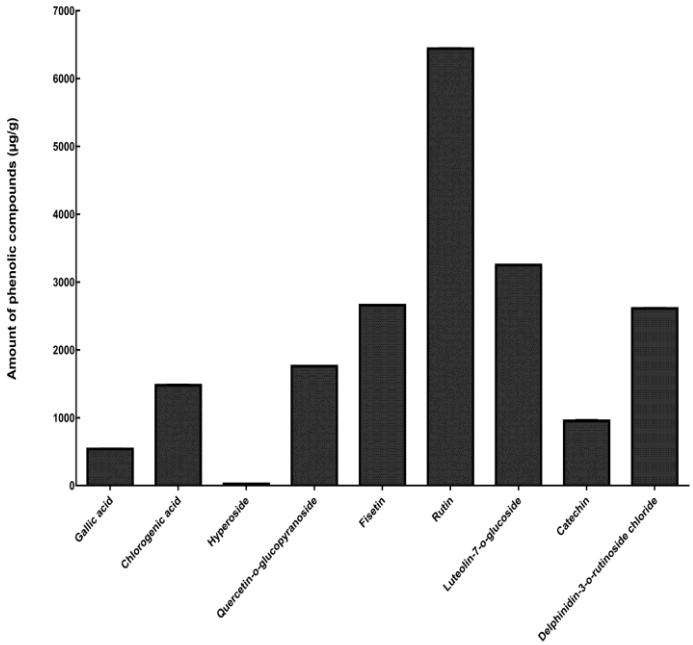

Figure 8. Bound phenolic acid content in the plant as determined by UPLC-PDA-MS-tQ. Compounds were identified and quantified by comparison to 57 phenolic standard compounds available. All analyses were carried out in triplicates (n=3).

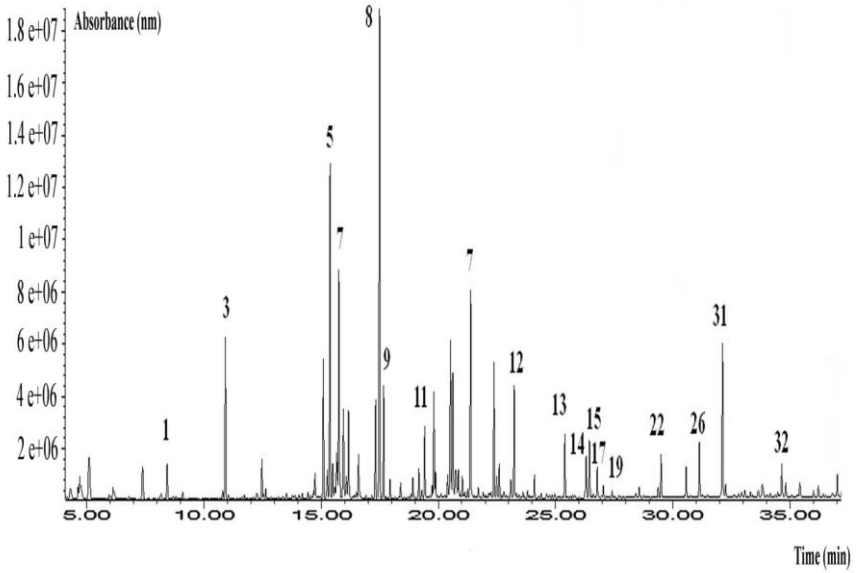

Figure 9. GC-MS chromatograms of volatile compounds of the plant. Numbered peaks correspond to the respective identified compounds: (1) Benzoic acid, (3) Malic acid, (5)-(7) Monosaccharides, (8) Citric acid, (9) Nonanedioic acid, (11) Myristic acid, (12) Margaric acid, (13) Palmitic acid, (14) Ferulic acid, (15) Cinnamic acid, (17) Oleic acid, (19) Linoleic acid, (22) Arachidic acid, (26) Behenic acid, (31) Docosadienoic acid and (32) Lignoceric acid.

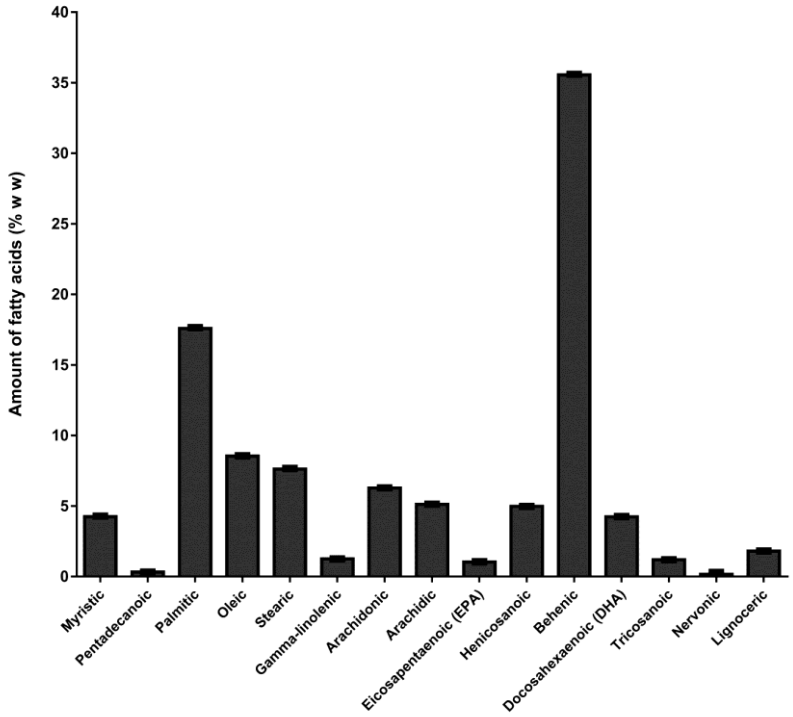

Figure 10. Fatty acids quantification in the plant as determined by GC-FID. Fatty acids were quantified (% ww) as methyl esters by calibration method using Heptadecanoic acid methyl ester as an internal standard. All analyses were carried out in triplicates (n=3).

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