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EVALUATION OF ANTINOCICEPTIVE ACTIVITY, ANTIOXIDANT PROPERTIES AND TOTAL PHENOLIC CONTENT OF THE ETHANOLIC EXTRACTS OF *RHUS PENTAPHYLLA* LEAVES AND FRUITS FROM MOROCCO

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ABSTRACT

The aims of study were to investigate the analgesic, antioxidant activities of *Rhus pentaphylla* ethanolic extracts (leaves and fruits). Three animal models were used to evaluate the possible analgesic effect such as, hot plate, writhing and formalin tests. The antioxidant activity of both extracts were evaluated using DPPH and FRAP tests. Total phenolic compounds, condensed tannins and flavonoids were also estimated. Our findings demonstrates that ethanolic extracts of *Rhus pentaphylla* possess an analgesic remarkable effect of leaves followed by fruits in all experimental models, in hot plate assay time of latency was increased significantly ($p \leq 0.001$) in treated groups at all doses in comparison to the negative control. In writhing test, treated animals by both extracts at all pharmacological doses demonstrates notable reduction ($p \leq 0.001$) of writhing numbers. Concerning formalin test, administration of *Rhus pentaphylla* extracts reduce significantly ($p \leq 0.001$) licking time in both phases indicates a possible central and peripheral mechanisms respectively. Moreover, leaves extract possess an important antioxidant capacity (IC50= 47,20 ± 0,23 µg/ml; IC50= 64,10 ± 0,73 µg/ml for DPPH and FRAP assays respectively) than fruits extract (IC= 55,24 ± 0,26 µg/ml; IC50= 75,30 ± 1,01 µg/ml). This last recorded activity due to the amount of total polyphenols in leaves (13,25 ± 0,01 mg GAE/g DW) as compared to the fruits (12,97 ± 0,26 mg GAE/g DW).

Keywords: *Rhus pentaphylla*; antinociceptive activity; antioxidant capacity; total phenolic compounds.

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INTRODUCTION

Humans to prevent, relieve or treat many of the diseases use various plants found in the environment. The use of medicinal plants has evolved gradually with the changing needs of humans. Subsequently, the accumulated experimental knowledge has enabled humans to take plants as an essential source of medicine (Sofowora *et al*., 2013).

Today modern medicine relies heavily on plants, the therapeutic benefits of which have been confirmed. What parts and forms of plants are used, they are extremely rich in compound chemical complexes with significant antioxidant capacities and health benefits (Naczk and Shahidi, 2004). Plants contain thousands of different constituents, which present to very diverse chemical families, such as phenols, flavonoids, terpenoids and steroids (Zekri, 2017).

Mediterranean regions such as Morocco have a significant plant biodiversity, it is full of plant species of therapeutic, nutritional, cosmetic and industrial interest (Idm'hand *et al*., 2020; Kachmar *et al*., 2021). Unfortunately, several wild plants traditionally known for their interesting biological and therapeutic properties are few or not investigated. In this context, the genus *Rhus* belongs to the family Anacardiaceae is known for these pharmacological activities, including antitumor, hypoglycemic, antiacetylcholinesterase and antidiarrheal effects (Ghouila *et al*., 2014; Sabzghabaee *et al*., 2014 ; Ben Barka, 2016 ; Benamar and Bennaceur, 2021).

In Morocco, *Rhus pentaphylla* (named Tizra), is a sumac shrub or small tree species. It is a Moroccan food and medicinal species used to treat diarrhea and abdominal colic and is used as a digestive (Lahsissene *et al*., 2009 ; El Abbouyi *et al*., 2014), but there is little information available on its bioactive composition and its pharmacological effects.

The aim of this study was to quantify and compare the bioactive compounds and to study the antioxidant and analgesic activities of the extracts obtained from the leaves and fruits of *Rhus pentaphylla* in order to prove its possible use as a potential natural source for human health.

MATERIALS AND METHODS

Animals: The experimental protocols were executed using Swiss male mice (N= 150) weighing 28-33 g, bred in the central animal care facilities of Cadi Ayyad University, Marrakech, Morocco. Animals were reared (six per cage) under controlled temperature (20 ± 2 °C), humidity (60%), and lighting (12/12h light/dark cycle), with free access to water and food. All the experiments were carried out in accordance with the European Council Directive (EEC, 1986/609) and duly approved by the Council Committee of research laboratories of the Faculty of Sciences, Cadi Ayyad University of Marrakech. All efforts were made to minimize the number of animals tested by using only six mice per experimental groups.

Collection of Plant Material: *Rhus pentaphylla* was collected in April 2022 from the region of Sidi Rahal (GPS: 31°35'05.5"N 7°28'11.3"W), Morocco. Representative specimens were identified by Professor A. Ouhammou (Laboratory of Environment and Ecology) and deposited in the Herbarium of the Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakech, Morocco.

Preparation of *Rhus pentaphylla* extracts: In order to extract the compounds contained in the leaves and fruits of *Rhus pentaphylla* we proceeded to the realization of the ethanolic extracts by cold maceration. The plant material was dried at room temperature under the shade, rudely powdered and 70 g of the leaves and the fruits each were subjected to extraction using ethanol, for 24 hours with continuous stirring using a magnetic bar. The macerates were filtered and concentrated with a rotary evaporator at 45 ° C to finally obtain the following yields: 22 % of ethanolic extract of the leaves and 21.5 % of ethanolic extract of the fruits of *Rhus pentaphylla*.

Assessment of Total Phenolic Compounds, Tannins and Flavonoids Content in *Rhus pentaphylla:* Total phenolic compounds were determined spectrophotometrically using the Folin-Ciocalteu reagent. A quantity of 100 µl of the extract was mixed with 3.9 ml of distilled water and 100 µl of the freshly prepared Folin-Ciocalteu reagent, the sodium carbonate ($Na_2 CO_3$) (1 ml of 20 %) was added after 3 minutes. The whole was agitated and incubated at room temperature for 60 minutes in the dark. The absorbance measurement was carried out using a spectrophotometer (VR-2000, Spain) at 725 nm. The results were expressed in milligrams equivalent of gallic acid per g of dry plant material (Wong *et al*., 2006).

The flavonoid compound of the extracts was quantified using the aluminum trichloride colorimetric method. The diluted extract (200 µl) was mixed with 800 µl of distilled water and 60 µl of 5 % sodium nitrite solution ($NaNO_2$). After 5 min, 40 µl of a 10% ($AlCl_3$) solution was added. 400 µl of (Na_2CO_3) solution (1M) and 500 µl of distilled water were added to the mixture after 6 minutes of incubation at room temperature. The mixture was agitated to homogenize the contents and the absorbance was measured at 510 nm. The results were expressed in milligrams equivalent of catechin per g of dry plant material (Zhishen *et al*., 1999).

The method used for the determination of the tannin content is the vanillin method in an acidic medium. A vanillin solution (2 ml, 4% methanol) was added to 200 µl of the sample extract, then the mixture was stirred using a vortex and 1 ml of the HCl was added. The absorbance was measured at 500 nm after 20 min of incubation in the dark at 30 °C. Results were expressed as catechin per gram of extract (Maliki *et al*., 2021).

Antioxidant Activity

DPPH Assay: The antioxidant power of the ethanolic extracts of *Rhus pentaphylla* was evaluated by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging test. 100 µl of each methanolic solution of the extracts at different concentrations were added to 2.9 ml of the methanolic solution of DPPH (0.004 %). The prepared solutions were stirred and incubated for 1 hour at room temperature in the dark. The absorbance reading of each concentration was measured at 517 nm. The negative control was prepared by adding 100 µl of methanol with 2.9 ml of DPPH, and gallic acid was considered as a 50 % positive control. For each concentration, the test was repeated 3 times. The free radical scavenging activity is estimated using the following equation:

(I %) = [Abs control - Abs extract/ Abs control] x 100

Where (I %) is the percentage of anti-free radical activity, Abs control is the absorbance of control and Abs extract is absorbance of the extract. The IC50 values have been determined graphically by linear regression (Bougandoura and Bendimerad, 2013).

Reducing Power Assay: The ferric reducing antioxidant power (FRAP) was tested by preparing a mixture consisting of 1 ml of each concentration of the methanolic solutions of the extracts with the phosphate buffer solution (2.5 ml, 0.2 M, pH 6.6), then a potassium ferricyanide ($K_3Fe (CN)_6$) (2.5 ml, 1 %) was added. The mixture was incubated for twenty minutes at 50 °C. To stop the reaction, 2.5 ml of 10% trichloroacetic acid was added. The mixture was centrifuged for ten minutes at 3000 revolutions per minute. Then, 2.5 ml of the supernatant liquid was added to the same volume of distilled water and 0.5 ml of 0.1% ferric chloride ($FeCl_3$). Finally, the reading of the reaction medium absorbance was taken at 700 nm. BHT and quercetin were used as positive controls whose absorbance has been measured under the same conditions as the extracts. The

concentration of the extract providing 0.5 absorbance (IC50) was determined by measuring the coloration formed by the reduction of Fe^{3+} (Pan *et al*., 2008).

Anti-nociceptive activity

Hot plate test: In this experiment, we used a hot plate apparatus (UgoBasil, Italy; Socrel DS-37) with a temperature maintained at 55 ± 0.2 °C, the mice were placed individually on a plate surface and the reaction time was recorded, it is the latency time before licking the paws or jumping. The test was stopped at a latency period of 20 seconds, which was defined as complete analgesia to avoid damaging the mice tissue. The forty-four animals were divided into eight groups of six mice each. Group I was treated with physiological serum (0.9 % NaCl) as a control, group II, III and IV were treated with *Rhus pentaphylla* leaves extract at 100, 300 and 500 mg / kg, respectively. Group V, VI and VII were treated with *Rhus pentaphylla* fruits extract at 100, 300 and 500 mg/kg, respectively. The treatment was carried out 30 min before the test. Group VIII was treated with morphine (2 mg/kg) as a positive control (Farouk *et al*., 2008; Rios *et al*., 2013).

Writhing test: To induce pain in mice, they were injected intraperitoneally by acetic acid (0.6%), 30 minutes after the treatment by *Rhus pentaphylla* extracts. Forty-eight mice were divided into eight groups of 6 mice each. Group I received physiological serum (0.9% NaCl), group II, III and IV were treated with *Rhus pentaphylla* leaves extract at 100, 300 and 500 mg / kg, respectively; group V, VI and VII were treated with *Rhus pentaphylla* fruits extract at 100, 300 and 500 mg / kg, respectively and acetylsalicylic acid (200 mg/kg) was administered to group VIII. The animals were placed individually in a transparent Plexiglas enclosure to count the number of contortions during a period of 25min (the number of contortions was noted 5min after the injection of acetic acid) (Farouk *et al*., 2008).

Formalin Test: In the current study, we used the method previously reported by De Miranda *et al*., (2001) with modifications. Briefly, 20 µl of 2 % formalin was injected subcutaneously into the right hind paw of mice. Allow each mouse to adjust the test box for 5 min prior to formalin injection. Nociceptive behavior was quantified as the total time spent licking and/or biting the injected paw. Quantification of nociceptive behavior occurs in two phases (Hunskaar and Hole, 1987), early nociceptive responses typically peaked 5 minutes after formalin injection (neurogenic phase), and 15-30 min after formalin injection peaked the late nociceptive response representing inflammatory phase. To this end, fifty-four mice were divided into nine groups of 6 mice each. Group I received physiological serum (0.9% NaCl), group II, III and IV were treated with *Rhus pentaphylla* leaves extract at 100, 300 and 500 mg / kg, respectively. Group V, VI and VII were treated with *Rhus pentaphylla* fruits extract at 100, 300 and 500 mg / kg, respectively. Acetylsalicylic acid (200 mg/kg) and morphine (2 mg/kg) were administered to group VIII and group IX respectively.

Statistical Analysis: Results were shown as mean ± SEM and comparisons between experimental groups were made using t-test and one Way analysis of variance ANOVA followed by Tukey's post hoc test. The value was considered statistically significant at ($p \leq 0.05$), the data were analysed using Sigma Plot 12.5 for Windows.

RESULTS

Assessment of Total Phenolics, Flavonoids and Tannins Content in *Rhus pentaphylla:* Ethanol extracts from *Rhus pentaphylla* leaves and fruits have a higher content of polyphenols, flavonoids and tannins. We noticed that the leaves are the richest in phenolic compounds, flavonoids, and tannins compared to the fruits (Table 1).

Table 1: Total phenolic compounds, flavonoids and tannins content of ethanol extract of *Rhus pentaphylla* leaves and fruits.

	Leaves extract	Fruits extract
Total phenolics (mg GAE/g DW)	13.25 ± 0.01	12.97±0.26
Flavonoids (mg /g DW)	16.10 ± 0.04	14.99±0.05***
Tannins (mg CE/g DW)	14.55 ± 0.32	11.69±0.19*

Values expressed as mean ± SEM. ***$p \leq 0.001$; *$p \leq 0.05$ Vs Leaves extract.

Antioxidant Activity: The antioxidant activity of the leaves and fruits of the ethanolic extract of *Rhus pentaphylla* was tested in vitro using two complementary assays: DPPH and assay of the reducing power.

The inhibitory concentration (IC50), is represented in Table 2. The antioxidant properties were compared to those of quercetin and butylated hydroxytoluene (BHT). Stronger antioxidant activity was indicated by lower IC50 values.

Both *Rhus pentaphylla* extracts showed significant antioxidant activity, with the DPPH test the lowest IC50 was obtained for the leaf extract (IC50 = 47.20 ± 0.23 µg/mL). This effect was less potent than that of the reference antioxidants butylhydroxytoluene and quercetin (IC50 values of 2.39 ± 0.01 µg/ml to 1.91 ± 0.02 µg/ml) (Table 2).

Table 2: IC 50 (µg/ml) values of *Rhus pentaphylla* of ethanol extract leaves and fruits compared to synthetic antioxidants (BHT and Quercetin).

	DPPH	Reducing power
BHT	2.39 ± 0.01	2.31±0.01
Quercetin	1.91 ± 0.02	2.66±0.05
Fruits extract	55.24 ± 0.26$^{***, c, ###}$	75.30±1.01$^{***, c, ###}$
Leaves extract	47.20 ± 0.23$^{***, c}$	64.10 ± 0.73$^{***, c}$

Data expressed as mean ± SEM. $^{***}p \leq 0.001$ Vs BHT; $^{C}p \leq 0.001$ Vs Quercetin; $^{###}p \leq 0.001$ Vs Leaves extract.

Anti-nociceptive activity

Heat induced pain model: Hot plate test: The analgesic effect of ethanolic extracts of *Rhus pentaphylla* leaves and fruits on the experimental mice in the hot plate is shown in the table (3). The results revealed that the morphine-treated group (2 mg/kg), leaf and fruit extract showed a significant increase ($p \leq 0.001$) in reaction time compared with the control.

The extract of *Rhus pentaphylla* leaves at 100 and 300 mg/kg, prolonged significantly ($p \leq 0.001$) the latency period in mice compared to the Morphine group at 15 minutes. The fruit extract showed a significant increase ($p \leq 0.001$) in reaction time at 0 min using (100 and 500 mg/kg) and a similar effect was also noted at 100 mg/kg after 15 min of administration compared to the Morphine group. The leaf extract present a lower analgesic effect ($p \leq 0.001$) compared to the fruit extract (500 mg/kg) from 30 minutes (Table 3).

Acetic acid induced pain model: Writhing test: The acetic acid-induced writhing test confirmed the analgesic effect of ethanolic extracts of *Rhus pentaphylla* leaves and fruits at different doses (100, 300 and 500 mg/kg). According to the results represented in figure (1). The treatment with both extracts caused a significant ($p \leq 0.001$) reduction in the number of spasms in mice compared to the control, the leaf extract at 500 mg/kg also showed a significant ($p \leq 0.001$) decrease in number of spasms compared to the Aspirin (200 mg/kg) and the fruit extract group at 500 mg/kg (Figure 1).

Formaldehyde induced pain model: Formalin test: The results of formalin test are reported in figure (2), after treatment with ethanolic extracts of *Rhus pentaphylla* leaves and fruits at 100, 300 and 500 mg/kg in mice model with the paw injected by formalin, a remarkable antinociceptive activity ($p \leq 0.001$) was proved in the early phase (0-5min) compared to control. A marked reduction was recorded from the 300 and 500 mg/kg doses of fruits extract in the late phase. The leaf extract at 500 mg/kg was significantly ($p \leq 0.01$) reduced paw licking during the early phase compared to the fruit extract at 500 mg/kg. Aspirin and Morphine were significantly ($p \leq 0.001$), ($p \leq 0.01$) inhibited the pain response induced by formaldehyde in the neurogenic phase and the inflammatory phase, respectively compared to the control (Figure 2).

Table 3: Effect of *Rhus pentaphylla* leaves and fruits (100, 300 and 500 mg/kg) on latency time of mice exposed to hotplate test.

Groups		T 0	T 15	T 30	T 45	T 60	T 90	T 120
Control		4,38± 0,25	4,87± 0,22	4,52 ± 0,29	4,72 ± 0,39	4,90 ± 0,18	4,90 ± 0,51	4,98± 0,40
Morphine		6,96± 0,31^{a}	15,00± 0,90^{a}	12,70± 0,62^{a,c}	12,96± 0,47^{a,c}	13,20± 0,69^{a}	13,70± 0,77^{a}	11,56± 0,97^{a}
Leaves extract	**100**	5,24± 0,45	8,90± 0,33^{a,b}	11,46± 0,98^{a}	10,52± 2,36^{a}	8,32± 0,97^{a}	9,04± 0,66^{a}	8,64 ± 0,45^{a}
	300	8,10± 0,40^{a}	9,92± 0,52^{a,b}	10,82± 1,21^{a}	11,02± 1,82^{a}	10,10± 0,98^{a}	10,45± 1,11^{a}	10,58± 0,37^{a}
	500	8,97± 0,45^{a}	14,22± 0,35^{a}	12,48± 0,70^{a,c}	12,17± 0,60^{a,c}	11,46± 0,34^{a,c}	11,87± 0,17^{a}	11,32± 0,55^{a,c}
Fruits extract	**100**	10,36± 0,44^{a,b}	10,80± 0,34^{a,b}	12,06± 0,58^{a}	13,30± 0,36^{a}	10,36± 0,89^{a}	10,26± 1,02^{a}	9,23 ± 1,09^{a}
	300	9,40± 0,60^{a}	12,43± 0,56^{a}	14,16± 0,49^{a}	13,40± 0,58^{a}	12,30± 0,55^{a}	11,83± 0,60^{a}	10,76± 0,52^{a}
	500	9,70± 0,80^{a,b}	13,80± 0,70^{a}	14,76± 0,72^{a}	15,03± 2,51^{a}	13,26± 0,65^{a}	12,93± 0,67^{a}	13,10± 0,45^{a}

The data are expressed as mean ± SEM. $^{a}p \leq 0.001$ Vs Control; $^{b}p \leq 0.001$ Vs Morphine; $^{c}p \leq 0.001$ Vs Fruit extract (500 mg/kg)

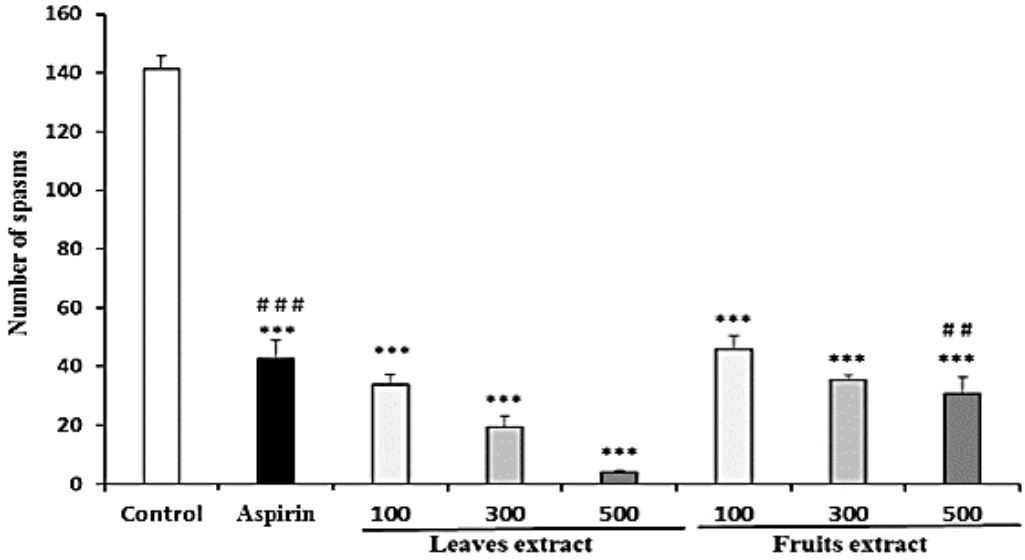

Figure 1: Effect of *Rhus pentaphylla* leaves and fruits (100, 300 and 500 mg/kg) on the acetic acid-induced writhing behaviour in mice. The data are shown as mean ± SEM. ***p≤0,001 Vs Control; $^{\#\#\#}$p≤0,001 Vs Leaf extract (500 mg/kg); $^{\#\#}$p≤0,01 Vs Leaf extract (500 mg/kg).

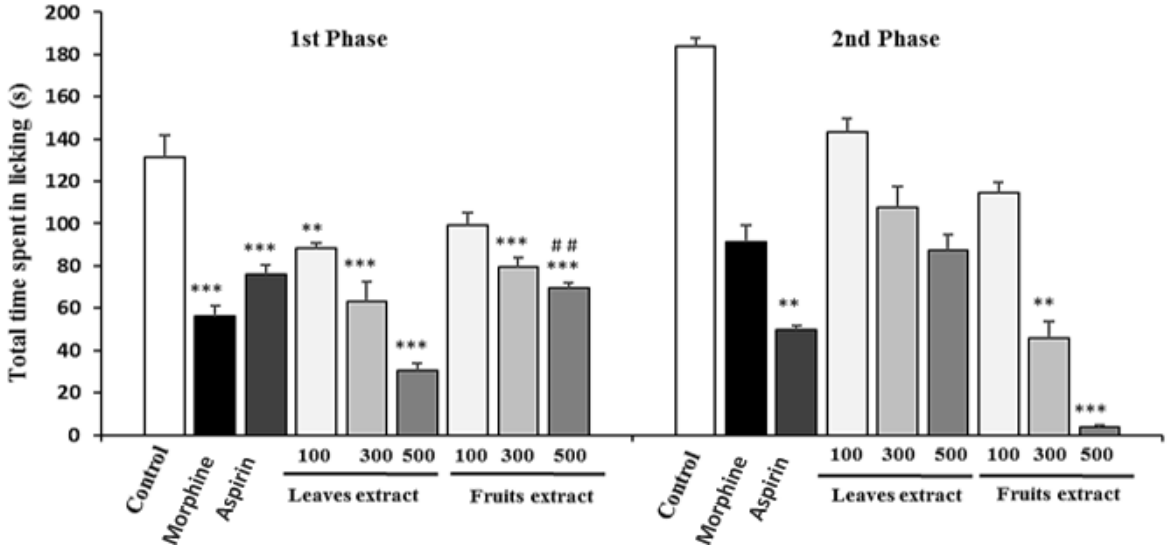

Figure 2: Anti-nociceptive effect of *Rhus pentaphylla* leaves and fruits (100, 300 and 500 mg/kg) in formalin induced nociception assay. The results are expressed as means ± SEM. ***p≤0,001, **p≤0,01 Vs Control; # #p≤0,01 Vs Leaf extract (500 mg/kg).

DISCUSSION

The research of medicinal plants is strongly required for the safe and appropriate use of herbal medicines. Despite the fact that *Rhus* is one of the most widely used genera of medicinal plants (Tewari *et al*., 2017), and even though it has benefited from numerous studies on its various pharmacological effects (Ahmed *et al*., 2001; McCutcheon *et al*., 1992 ; Lee *et al*., 2004 ; Choi *et al*., 2012 ; Lee *et al*., 2002 ; Rima *et al*., 2011). The valuation of *Rhus pentaphylla* species by experimental scientific studies especially in Morocco has not been carried out, for this reason, we have focused on a quantitative study of phenolic compounds with an evaluation of antioxidant and antinociceptive activities by comparing both the extracts of leaves and fruits of *Rhus pentaphylla.*

Both ethanolic extracts of *Rhus pentaphylla* leaves and fruits showed potent antioxidant activity in vitro. Nevertheless, the leaves possess a higher antioxidant power than fruits. However, this effect was less significant than the reference antioxidant agents used in the two DPPH and reducing power-scavenging assays.

The DPPH test was performed with a spectrophotometer by following reduction of the free radical that is accompanied by its change from purple to yellow color measured at 517nm. The decrease in absorbance induced by anti-radical substances determined this reduction capacity. The presence of flavonoids and tannins is probably responsible for the free radical scavenging effects observed. These phenolic compounds are a major group of metabolites that act as primary antioxidants or free radical scavengers (Bougandoura and Bendimerad, 2013). The potent anti-radical power of *Rhus pentaphylla* leaves may be due to their richness in flavonoids and tannins compared to fruits.

The presence of reducers in ethanolic extracts of *Rhus pentaphylla* was confirmed by the iron reduction technique. The reduction of the ferricyanide complex to the ferrous form results in the increase of the blue color density in the reaction medium at 700 nm (Benziane *et al*., 2019). The reducing power of the ethanolic extract of *Rhus pentaphylla* leaves is much higher than that of the fruit extract, but lower than that of Butylated hydroxytoluene and Quercetin. This potency of *Rhus pentaphylla* species is presumably due to the presence of hydroxyl group in the phenolic compounds, which can serve as electron donor. Accordingly, antioxidants are considered as inactivators and reducers of oxidants (Siddhuraju and Becker, 2007).

Our results are in agreement with those of Mansour *et al*., (2011), Itidel *et al*., (2013), and Benamar and Bennaceur, (2021) who confirm the antioxidant activity of *Rhus pentaphylla* extracts.

The species of the genus *Rhus* are well known for their antioxidant power. The crude ethanolic extract of *Rhus verniciflua* wood has shown strong antioxidant activity on neuronal cells in culture (Lee *et al*., 2001). Other species can also produce antioxidant potential such as the extract of *Rhus copallina* wood (Young, 1976), *Rhus tripartita* (Itidel, 2013), *Rhus glabra* (Young, 1976), *Rhus typhina* (Kossah *et al*., 2011), *Rhus hirta* (Wu *et al*., 2012), *Rhus chinensis* (Djakpo and Yao, 2010) and *Rhus coriaria* (Mohammadi *et al*., 2010). The chemical structures of synthetic antioxidants that can be toxic, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are related to those of natural antioxidants. However, the development of new naturally occurring antioxidants for food and health reasons is a major goal in the field of sustainable bioproducts (Rayne and Mazza, 2007).

Before evaluating the antinociceptive activity, we demonstrated that *Rhus pentaphylla* does not induce mortality or toxicity in mice, for either acute or sub-acute toxicity. The 50% lethal dose being greater than 5000 mg/kg.

The analgesic effect was demonstrated by different model of pain: The hot plate test, writhing test and formalin test. Our results suggest that the extracts of *Rhus pentaphylla* leaves and fruits at 100, 300 and 500 mg/kg possesses antinociceptive activity concurrently on both central and peripheral pain pathways.

The central analgesic activity was evaluated by the hot plate test. Which demonstrated that the administration of the ethanolic extracts of *Rhus pentaphylla* leaves and fruits at different concentrations exhibit anti-nociceptive effects which may be due to the active substances such as flavonoids and tannins that interfere with the activation of opioid receptor stimulation (Muhammad *et al*., 2012).

The writhing test is a chemical pain model, it detects peripheral analgesic activity. Following the injection of acetic acid, pain mediators such as arachidonic acid, histamine, serotonin, substance P, prostaglandins and lipoxygenases are secreted causing local inflammation, *Rhus pentaphylla* extracts were significantly reduces spasms by the inhibition of peritoneal receptors or peripheral pain mediators (Muhammad *et al*., 2012). The oral administration of Hydroalcoholic leaf extract of *Rhus coriaria* revealed a significant reduction in writhing in Wistar rats (Mohammadi *et al*., 2016). *Rhus typhina* is used for treating abdominal pain (Opiyo *et al*., 2021), *Rhus verniciflua* extract and the flavonoidal mixture of *Rhus retinorrhaea* significantly inhibited the cramps in mice pretreated with these sumac extracts (Alqasoumi *et al*., 2009; Choi *et al*., 2003). Another investigation revealed that anacardic acids isolated from *Rhus semialata* possess an analgesic property due to their similarity to acetylsalicylic acid (Bagchi *et al*., 1985).

Injection of formalin in the paw induces biphasic spontaneous nociceptive behavior (Rezaee-Asl *et al*., 2014). The early phase (0-5min) is a neurogenic phase; it is induced by the direct stimulation of sensory C fibers, then release of substance P, or by the involvement of pain mediators glutamate. The late phase (15-30 minutes) is an inflammatory phase, it is characterized by the release of various inflammatory mediators like prostaglandins, excitatory amino acids, histamine and bradykinin, the latter also affects the first phase (Rezaee- Asl *et al*., 2014; Tewari *et al*., 2021). Our results indicate that both *Rhus pentaphylla* extracts at different doses demonstrated a potential effect in reducing pain sensation in both phases. The richness of these extracts in flavonoids explains their effects since they can inhibit the production of hyperalgesia mediators such as those mentioned above and others as nitric oxide and calcium, which interfere with release of neurotransmitters that stimulate pain and inflammation (Zare *et al*., 2018). Our results are in accordance with those found by Vargas-Ruiz *et al*., (2022) who confirms that the administration of aqueous, methanolic and hexanolic extracts of *Rhus virens* prior to the injection of formalin resulted in a decrease of the licking time in both phases of the formalin test.

Other previous studies on different species of *Rhus* confirm their analgesic and anti-inflammatory effects. The hydroalcoholic leaf extract of *Rhus coriaria,* and the root extract of *Rhus natalensis* have a marked analgesic effect (Kariuki *et al*., 2012 ; Mohammadi *et al*., 2015). Moreover, *Rhus retinorrhaea* and *Rhus chirindensis* present a significant analgesic and anti-inflammatory activities in rodents (Mossa *et al*., 1995; Ojewole, 2007). Rani *et al*., (2016) were demonstrated the anti-inflammatory activity of 5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one and quercetin from *Rhus mysorensis* leave, *Rhus tripartitum* and *Rhus toxicodendron* possesses also a strong power to prevent inflammation (Santos *et al*., 2007 ; Mahjoub *et al*., 2010).

This study suggests that extracts of *Rhus pentaphylla* leaves and fruits from the Sidi Rahal region in Morocco are rich in polyphenols, specifically flavonoids and tannins. The latter are responsible for a significant antioxidant activity, which could be useful in delaying the progression of various pain and inflammation.

Authors' contribution: Category 1, Conception and design of study: Agouram, Fatimazahra ; Chait, Abderrahman acquisition of data: Agouram, Fatimazahra; Chait, Abderrahman analysis and/or interpretation of data: Agouram, Fatimazahra; Sokar, Zahra; Chait, Abderrahman Category 2 Drafting the manuscript: Agouram, Fatimazahra ; Chait, Abderrahman revising the manuscript critically for important intellectual content: Agouram, Fatimazahra; Sokar, Zahra ; Chait, Abderrahman Category 3 Approval of the version of the manuscript to be published (the names of all authors must be listed): Agouram, Fatimazahra; Sokar, Zahra ; Chait, Abderrahman

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REFERENCES

Ahmed, M. S., A. M. Galal, S. A. Ross, D. Ferreira, M. A. El Sohly, A. R. S. Ibrahim, J. S. Mossa and F. S. El-Feraly (2001). A weakly antimalarial biflavanone from *Rhus retinorrhoea*. Phytochemistry. 58 (4): 599-602. https://doi.org/10.1016/S0031-9422(01)00244-8

Alqasoumi, S., A. Galal, A. Gamal, M. Al-Yahya and S. Rafatullah (2009). Antinociceptive, anti-inflammatory and antipyretic effects of a flavonoidal mixture leaf surface of *Rhus Retinorrhaea*. Farmacia. 57(3): 346-354. https://www.academia.edu/download/32163483/issue32009art11.pdf

Bagchi, A., M. Sahai, and A.B. Ray (1985). Phenolic constituents of *Rhus semialata* leaves. Planta medica. 51(5): 467-468. DOI: 10.1055/s-2007-969561

Ben Barka, Z., C. Aouadhi, M. Tlili, H. Alimi, H. Ben Miled, K. Ben Rhouma, M. Sakly, R. Ksouri, Y.J. Schneider, M. Maaroufi and O. Tebourbi (2016). Evaluation of the anti-diarrheal activity of the hydromethanolic root extract of *Rhus tripartita* (Ucria) (Anacardiacae). Biomed. Pharmacother. 83: 827-834. https://doi.org/10.1016/j.biopha.2016.07.055

Benamar, H., and M. Bennaceur (2021). Bioactive Compound Contents and Biological Activities of the Algerian Medicinal Plant *Rhus pentaphylla* (Jacq.) Desf. (Anacardiaceae). Biol. Life Sci. Forum. 6(1): 68. https://doi.org/10.3390/Foods2021-10915

Benziane, M.N.A., K. Acem, H. Aggad and M. Abdali (2019). Phytochemistry, HPLC profile and antioxidant activity of aqueous extracts of fenugreek (*Trigonella foenum graecum* L.) seeds grown in arid zones of Algeria. Acta. Scientifica Naturalis. 6(2): 71-87. https://doi.org/10.2478/asn-2019-0020

Bougandoura, N., and N. Bendimerad (2013). Evaluation de l'activité antioxydante des extraits aqueux et méthanolique de *Satureja calamintha* ssp. *Nepeta* (L.) Briq. Nature & Technologie. 9: 14. https://www.researchgate.net/profile/Nassima-Bendimerad2/publication/273832215_Evaluation_de_l'activite_antioxydante_des_extraits_aqueux_et_methanolique_de_Satureja_calamintha_ssp_Nepeta_L_Briq/links/63f0055d51d7af054032ab3c.pdf.

Choi, J.W., B.J. Yoon, Y.N. Han, S.K. Lee, K.T. Lee and H.J. Park (2003). Sulfuretin, an Antinociceptive and Antiinflammatory Flavonoid from *Rhus verniciflua*. Nat. Prod. Sci. 9 (2): 97-101. https://khu.elsevierpure.com/en/publications/sulfuretin-an-antinociceptive-and-antiinflammatory-flavonoid-from-2.

Choi, W., H. Jung, K. Kim, S. Lee, S. Yoon, J. Park, S. Cheon, W. Eo and S. Lee (2012). *Rhus verniciflora* stokes against advanced cancer: a perspective from the Korean integrative cancer center. J. Biomed. Biotechnol. 181-191. https://doi.org/10.1155/2012/874276

De Miranda, F.G.G., J.C. Vilar, I.A.N. Alves, S.C.H. Cavalcanti and A.R. Antoniolli (2001). Antinociceptive and antiedematogenic properties and acute toxicity of *Tabebuia avellanedae* Lor. ex Griseb. inner bark aqueous extract. BMC Pharmacol. 1(1): 1-5. https://doi.org/10.1186/1471-2210-1-6

Djakpo, O., and W. Yao (2010). *Rhus chinensis* and *Galla chinensis* evidence: folklore to modern

review. Phytother. Res. 24(12): 1739-1749. https://doi.org/10.1002/ptr.3215

El Abbouyi, P.A., N.F. Ansari, P.S.E. Khyari and H. Loukili (2014). Inventory of medicinal plants prescribed by traditional healers in El Jadida city and suburbs (Morocco). Int. J. Green Pharm. 8(4). https://doi.org/10.22377/ijgp.v8i4.420

Farouk, L., A. Laroubi, R. Aboufatima, A. Benharref and A. Chait (2008). Evaluation of the analgesic effect of alkaloid extract of *Peganum harmala* L.: possible mechanisms involved. J. ethnopharmacol. 115(3): 449-454. https://doi.org/10.1016/j.jep.2007.10.014

Ghouila, H., W. Haddar, M.B. Ticha, N. Baaka, N. Meksi, M.F. Mhenni and H.B. Jannet (2014). *Rhus pentaphylla* bark as a new source of natural colorant for wool and silk fibers. J. Soc. Chim. Tunisie. 16: 95-102. http://www.sctunisie.org/pdf/JSCT_v16-14.pdf

Hunskaar, S. and K. Hole (1987). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30(1): 103-114. https://doi.org/10.1016/0304-3959(87)90088-1

Idm'hand, E., F. Msanda and K. Cherifi (2020). Ethnobotanical study and biodiversity of medicinal plants used in the Tarfaya Province, Morocco. Acta. Ecol Sin. 40(2): 134-144. https://doi.org/10.1016/j.chnaes.2020.01.002

Itidel, C., M. Chokri, B. Mohamed and Z. Yosr (2013). Antioxidant activity, total phenolic and flavonoid content variation among Tunisian natural populations of *Rhus tripartita* (Ucria) Grande and *Rhus pentaphylla* Desf. Ind. Crops Prod. 51: 171-177. https://doi.org/10.1016/j.indcrop.2013.09.002

Kachmar, M.R., H. Naceiri Mrabti, M. Bellahmar, A. Ouahbi, Z. Haloui, K. El Badaoui and S. Chakir (2021). Traditional knowledge of medicinal plants used in the Northeastern part of Morocco. Evid.-Based Complementary Altern. Med. https://doi.org/10.1155/2021/6002949

Kariuki, H.N., T.I. Kanui, A. Yenesew, P.M. Mbugua and N.B. Patel (2012). Antinocieptive activity of the root extracts of *Rhus natalensis* Kraus and *Senna singueana*. Phytopharmacology. 2(2): 312-317. http://erepository.uonbi.ac.ke:8080/xmlui/handle/123456789/33009

Kossah, R., H. Zhang and W. Chen (2011). Chinese Antimicrobial and antioxidant activities of sumac (*Rhus typhina* L.) Fruit extract. Food Control. 22(1): 128-132. https://doi.org/10.1016/j.foodcont.2010.06.002

Lahsissene, H., A. Kahouadji, M. Tijane and S. Hseini (2009). Catalogue of medicinal plants used in the region of Zaër (Western Morocco). Lejeunia, J. Botany. 186. https://popups.uliege.be/0457-4184/index.php?id=701.

Lee, J.C., J. Kim, K.T. Lim, M.S. Yang and Y.S. Jang (2001). Ethanol eluted extract of *Rhus verniciflua* Stokes showed both antioxidant and cytotoxic effects on mouse thymocytes depending on the dose and time of the treatment. BMB Reports. 34(3): 250-258. https://papersearch.net/thesis/article.asp?key=1726357.

Lee, J.C., K.T. Lim and Y.S. Jang (2002). Identification of *Rhus verniciflua* Stokes compounds that exhibit free radical scavenging and antiapoptotic properties. Biochim Bio- phys Acta. 1570(3): 181-191. https://doi.org/10.1016/S0304-4165(02)00196-4

Lee, J.C., K.Y. Lee and J.N. Kim (2004). Extract from *Rhus verniciflua* Stokes is capable of inhibiting the growth of human lymphoma cells. Food Chem. Toxicol. 42(9): 1383-1388. https://doi.org/10.1016/j.fct.2004.03.012

Mahjoub, M.A., S. Ammar, H. Edziri, N. Mighri, A. Bouraoui and Z. Mighri (2010). Anti-inflammatory and antioxidant activities of some extracts and pure natural products isolated from *Rhus tripartitum* (Ucria). Med. Chem. Res. 19: 271-282. https://doi.org/10.1007/s00044-009-9190-z

Maliki, I., A. El moussaoui, H. Elmsellem, M. Ramdani and K. Elbadaoui (2021). Phytochemical screening and the antioxidant, antibacterial and antifungal activities of aqueous extracts from the leaves of *Salvia officinalis* planted in Morocco. Mor. J. Chem. 9(2): 354-368. https://doi.org/10.48317/IMIST.PRSM/morjchem-v9i2.24840

Mansour, H.B., S. Yatouji, S. Mbarek, I. Houas, A. Delai and D. Dridi (2011). Correlation between antibutyrylcholinesterasic and antioxidant activities of three aqueous extracts from Tunisian *Rhus pentaphyllum*. Ann. Clin. Microbiol. Antimicrob. 10(1): 1-9. https://doi.org/10.1186/1476-0711-10-32

McCutcheon, A.R., S.M. Ellis, R.E. Hancock and G.H. Towers (1992). Antibiotic screening of medicinal plants of the British Columbian native peoples. J. Ethnopharmacol. 37(3): 213-223. https://doi.org/10.1016/0378-8741(92)90036-Q

Mohammadi, S., M. Zarei and M.M. Zarei (2015). Antinociceptive effects of *Rhus coriaria* L. extract in male rats. J. Physiol. Sci. 65(2): S23-S28. https://doi.org/10.1007/BF03405852

Mohammadi, S., M. Zarei, M.M. Zarei and I. Salehi (2016). Effect of Hydroalcoholic Leaves Extract of *Rhus coriaria* on Pain in Male Rats. Anesth. Pain Med. 6(1). doi: 10.5812/aapm.32128

Mohammadi, S., S. Montasser Konhsari and A. Monavar Feshani (2010). Antidiabetic of the ethanolic extract ceut.Sci. of *Rhus coriaria* fruits in rats. Darut. Pharma. 18(4): 270-275. PMCID: PMC3304353

Mossa, J.S., S. Rafatullah, A.M. Gala1 and M.A. Al-Yahya (1995). Pharmacological studies of *Rhus retinorrhaea*. Inter. J. Pharmacogn. 33(3): 242-246. https://doi.org/10.3109/13880209509065371

Muhammad, N., M. Saeed and H. Khan (2012). Antipyretic, analgesic and anti-inflammatory activity of *Viola betonicifolia* whole plant. BMC Complement Altern. Med. 12(59): 1-8. https://doi.org/10.1186/1472-6882-12-59

Naczk, M., and F. Shahidi (2004). Extraction and analysis of phenolics in food. J. chromatogr A. 1054 (1-2): 95-111. https://doi.org/10.1016/j.chroma.2004.08.059

Ojewole, J.A.O. (2007). Analgesic, anti-inflammatory and hypoglycaemic effects of *Rhus chirindensis* (Baker F.) [Anacardiaceae] stem-bark aqueous extract in mice and rats. J. Ethnopharmacol. 113(2): 338-345. https://doi.org/10.1016/j.jep.2007.06.025

Opiyo, S.A., P.W. Njoroge, E.G. Ndirangu and K.M. Kuria (2021). A review of biological activities and phytochemistry of *Rhus* Species. Am. J. Chem. 11(2): 28-36. DOI: 10.5923/j.chemistry.20211102.02

Pan, Y., K. Wang, S. Huang, H. Wang, X. Mu, C. He, X. Ji, J. Zhang and F. Huang (2008). Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus Longan* Lour.) peel. Food Chem. 106(3): 1264-1270. https://doi.org/10.1016/j.foodchem.2007.07.033

Rani, G.R., C.M.A. Singara, M. Viswanadham, M.K. Thupurani, S. Gorripati, K. Rajashekar (2016). Phytochemical analysis and antimicrobial activities of *Rhus mysorensis*. Int J. Adv Res. 4: 503-515. https://www.journalijar.com/article/7680/phytochemical-analysis-and-antimicrobial-activities-of-rhus mysorensis/

Rayne, S., and G. Mazza (2007). Biological Activities of Extracts from Sumac (*Rhus* spp.): A Review. Nat. Preced. 631: 1. https://doi.org/10.1038/npre.2007.631.1

Rezaee-Asl, M., M. Sabour, V. Nikoui, S. Ostadhadi and A. Bakhtiarian (2014). The Study of Analgesic Effects of *Leonurus cardiaca* L. in Mice by Formalin, Tail Flick and Hot Plate Tests. Int. Sch. Res. Notices. 2014: 687697. https://doi.org/10.1155/2014/687697

Rima, K., Z. Hao and C. Wei (2011). Antimicrobial and antioxidant activities of Chinese sumac (*Rhus typhina* L.) fruit extract. Food Control. 22(1): 128-132. https://doi.org/10.1016/j.foodcont.2010.06.002

Rios, E.R.V., N.F.M. Rocha, A.M.R. Carvalho, L.F. Vasconcelos, M.L. Dias, D.P. De Sousa and M.M. De Franca Fonteles (2013). TRP and ASIC channels mediate the antinociceptive effect of citronellyl acetate. Chem Biol Interact. 203(3): 573-579. https://doi.org/10.1016/j.cbi.2013.03.014

Sabzghabaee, A.M., R. Kelishadi, K. Golshiri, A. Ghannadi and S. Badri (2014). Clinical effects of *Rhus coriaria* fruits on dyslipidemia in adolescents: a triple-blinded randomized placebo-controlled trial. Arch. Med. Res. 68(5): 308. doi: 10.5455/medarh.2014.68.308-312

Santos, A.D., F.F. Perazzo, L.G.V. Cardoso and J.C.T. Carvalho (2007). In vivo study of the anti-inflammatory effect of *Rhus toxicodendron*. Homeopathy. 96(2): 95-101. DOI: 10.1016/j.homp.2007.03.001

Siddhuraju, P., and K. Becker (2007). The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. Food Chem. 101(1): 10-19. https://doi.org/10.1016/j.foodchem.2006.01.004

Sofowora, A., E. Ogunbodede and A. Onayade (2013). The Role and Place of Medicinal Plants in the Strategies for Disease Prevention. Afr. J. Tradit. Complement Altern. Med. 10(5): 210-229. DOI: 10.4314/ajtcam.v10i5.2

Tewari, D., A. Mocan, E.D. Parvanov, A.N. Sah, S.M. Nabavi, L. Huminiecki, Z.F. Ma, Y.Y. Lee, J.O. Horbanczuk and A.G. Atanasov (2017). Ethnopharmacological approaches for therapy of jaundice: Part I. Front. Pharmacol. 8: 518. https://doi.org/10.3389/fphar.2017.00518

Tewari, D., M. Khayatkashani, P. Gupta, S. Bawari, A.N. Sah, D. Barreca, H.R. Khayat Kashani (2021). Himalayan *Ficus palmata* L. Fruit Extract Showed In Vivo Central and Peripheral Analgesic Activity Involving COX-2 and Mu Opioid Receptors. Plants 10(8): 1685. https://doi.org/10.3390/plants10081685

Vargas-Ruiz, R., R. M. Montiel-Ruiz, A. Zamilpa, M. Gonzalez-Cortazar, M. L. Herrera-Ruiz, J. Molina-Cabrera and F. J. Flores-Murrieta (2022). Bio-guided study of the antinociceptive, anti-inflammatory, and free-radical scavenging capacity of the leaves of *Rhus virens* Lindh. ex A. Gray and its possible mechanism of antinociception. J. Ethnopharmacol. 300: 115756. https://doi.org/10.1016/j.jep.2022.115756

Wong, S.P., L.P. Leong and J.H.W. Koh (2006). Antioxidant activities of aqueous extracts of

selected plants. Food Chem. 99(4): 775-783. https://doi.org/10.1016/j.foodchem.2005.07.058

Wu, T., J.L. Mc Callum, R. Liu, H. Zhu and R. Tsao (2012). Evaluation of antioxidant activities and chemical characterization of staghorn sumac fruit (*Rhus hirta*). Food Chem. 138(2-3): 1333-1340. https://doi.org/10.1016/j.foodchem.2012.10.086

Young, D.A. (1976). Flavonoid chemistry and the phylogenetic relationships of the Julianiaceae. Syst. Bot. 1: 149-162. https://doi.org/10.2307/2418766

Zare, A., Z. Khaksar, Z. Sobhani and M. Amini (2018). Analgesic Effect of Valerian Root and Turnip Extracts. World J. Plast. Surg. 7(3): 345-350. doi: 10.29252/wjps.7.3.345

Zekri, N. (2017) : Phytochemical study and biological activities of essential oils and M. extracts. *pulegium* (L.), M. *suaveolens* (Ehrh.) and M.*spicata* (L.) from the Moroccan Middle Atlas. Faculty of Science, Rabat. https://docplayer.fr/111228685-These-de-doctorat-presentee-par-nadia-zekri.html

Zhishen, J., T. Mengcheng and W. Jianming (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chem. 64(4): 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2.