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Research Article

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Chemical Composition and Antinociceptive Effect of *Pistacia atlantica* desf. Essential Oil from Morocco

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Abstract The chemical composition of crushed fruits and aerial parts essential oil, hydrodistilled from *Pistacia atlantica* DESF., were studied by gas chromatography-mass spectrometry, a significant difference in their chemical compositions were observed. The analgesic activity was carried out with the essential oil for its peripheral and central antinociceptive potentials on acetic acid-induced writhing and tail immersion test in rodents, respectively. The analgesic activity of *Pistacia atlantica* DESF. Essential oil was found to be significant ($P<0.05$) on acetic acid induced model, as well as the tail immersion test. From this study we can conclude that *Pistacia atlantica* DESF. from Morocco possess significantly high analgesic property without any acute toxicity. The results indicate that the analgesic effect of this plant is both centrally and peripherally mediated.

Keywords Analgesic activity, Essential oil, GC/MS, *Pistacia atlantica* DESF, Rodents

Introduction

In Morocco, a wide range of medicinal plants is used in folk medicine for the treatment of different diseases. Ethno-botanical and ethno-pharmacological studies on such plants continue to attract investigators throughout the world. In current scenario, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems [1].

The genus Pistacia, including *P. lentiscus* L., *P. terebinthus* L., *P. atlantica* Desf. and *P. vera* L., is widely present in North Africa however, *Pistacia atlantica*, its the most common [2]. *Atlas pistachio* (*Pistacia atlantica*) is an endemic perennial woody plant in North Africa belongs to the anacardiaceae family in Morocco it is called (butm or butum) [2]. It used to be widely distributed across arid and semi-arid regions; it occurs as isolated scattered trees or in dense populations at a range of altitude from less than 200m up to 1200m [2]. Atlas pistachio is one of the main constructive species of arid and semi-arid Mediterranean forest ecosystems and plays a very important role in retaining ecological stability preventing desertification. It is a drought-resistant plant able to grow in harsh conditions in which few tree species can be grown and established; its large rooting system renders it suitable for reforestation programmes in the semi- arid and arid areas. In modern pharmacology, Pistacia species have been reported to have various biological effects such as antiatherogenic, hypoglycemic, antioxidant, antiprotozoal, analgesic and anti-inflammatory [2].

Different parts of Pistacia species have been investigated for various pharmacological and biological activities with mostly focusing on the resin of *P. atlantica* Desf. that is known as mastic.Pain is one of the problems that occurs in

many diseases [3]. So Identification, production and use of chemical analgesics medicines for pain relief, have been reported as side effects [4].

In Morocco *Pistacia atlantica* Desf. has various traditional uses including astringent and anti-diarrheal as well as improving some of the symptoms of gastrointestinal upsets. It has been also used for sedation and analgesia in traditional medicine.

The main objectives of this study were to evaluate the analgesic activity and the chemical composition of *Pistacia atlantica* DESF. essential oil from Moroccoto record and document the traditional use of this plant to cure and relief pain.

Materials and Methods

Plant Material

Plants were collected from their natural habitat (East of Morocco (Oujda)). They were identified by botanist at the Department of Plant Biology, Ibn Tofail University, Morocco. A voucher specimen (101537) was deposited in the Herbarium of Botany Department of Scientific Institute of Rabat [5].

Extraction of Essential Oil

Gas Chromatography /Mass Spectrometry (GC/MS) Analysis

GC-MS analysis of the EO was performed on a TRACE GC ULTRA equipped with non-polar VB5 (5% phenyl; 95% methylpolisyloxane) capillary column (30m X 0.25mm X 0.25µm film thickness), directly coupled to a mass spectrometer (Polaris Q) (EI 70 eV). The temperature of the injector and detector was set at 220°C and 300°C, respectively. The oven temperature was programmed from 60°C to 200°C at 2°C/min, and then from 200°C at 300°C at 20°C/min. The components of the oil were identified by comparison of their mass spectra with those in the Wiley-NIST 7th edition library of mass spectral data. The percentage composition of the oil sample was calculated from GC-MS peak areas [6].

Animals

Young Swiss female mice aged 4-5 weeks, average weight 20-30 gm and healthy Wistar rats of either sex weighing 200-250g were used for the experiment. The rodents were obtained from the the animal experimental centre of Mohammed V University, Medicine and Pharmacy Faculty, Rabat. They were kept in standard environmental condition (at 26.0±0°C temperature & 60-70% relative humidity and 12 hour light/12 hour dark cycle) and received a standard rodent diet and water ad libitum. The set of rules followed for animal experiment were approved by approved by the Institutional Research Committee regarding the care and use of animals for experimental procedure in 2010; CEE509 [7,8].

Acute toxicity test

A total of 9 mice were used in this acute oral toxicity study according to OECD 423 guideline. Each group consisted of three animals (n=3) and allowed to access to food and tap water ad libitium. Six female mice were treated with EOPA (2000mg/kg, p.o.) while the control group was treated with the respective vechile, peanut oil. After oral administration of EO, mice were observed for 24 h and signs of toxicity and mortality were recorded. All the animals were weighed before the treatment and and during 14 days after oral administration EOPA [9].

Analgesic Activity

Acetic Acid-Induced Writhing Test

Analgesic activity of the essential oil of *Pistacia atlantica* was tested using the model of acetic acid induced writhing in mice. The test consists of injecting (3% with 300 mg/kg) acetic acid solution and observing the animal for specific contraction of body referred as 'writhing'. The animals were divided into three groups consisting of 6 mice in each group and were treated with essential oil (100 and 200mg/kg, p.o.) and Aspirin (200 mg/kg, p.o.) 30 min prior to the injection of acetic acid. Five minutes after acetic acid injection, mice were placed in transparent box and number of writhes was counted for a period of twenty minutes [10].

Tail immersion test

Four groups of rats (n=6) were treated orally with EOPA (100 and 200 mg/kg) or vehicle. Morphine (5mg/kg i.m) was used as the standard drug in this test.

The analgesic activity was determined by measuring drug-induced changes in the sensitivity of the pre-screened rats to thermal stimulus (hot water) by placing the tail 5cm in the glass beaker, using tail immesion test at temperatures of 55°C, applied to their tails [10]. The latency in tail withdrawal from the glass beaker was recorded in seconds as response before and after 15, 30, 45, 60 and 120 minutes of drug administration in this method. A cut-off period of 10 sec was taken to prevent the damage to the tail.

Statistical Analysis

All data were expressed as mean±SEM. The statistical analysis was performed using one-way ANOVA followed student test. P values of less than 0.05 were considered significant.

Results

Essential Oil Constituents

Chemical composition of the essential oil of *Pistacia atlantica* DESF. from Morocco was analysed using GC-MS. About 29 compounds were identified in the EO. The most abundant compounds included Isophorone (23.96%), Delta 3-Carene (10.35%), Trans-sabinene hydrate (8.12%) and Sabinène (5.20%) which included about 54% of the volatile oil compounds. Other compounds comprised less than 5% of total volatile oil. The results are listed in table 1 with their retention times, retention indices and percentage shares.

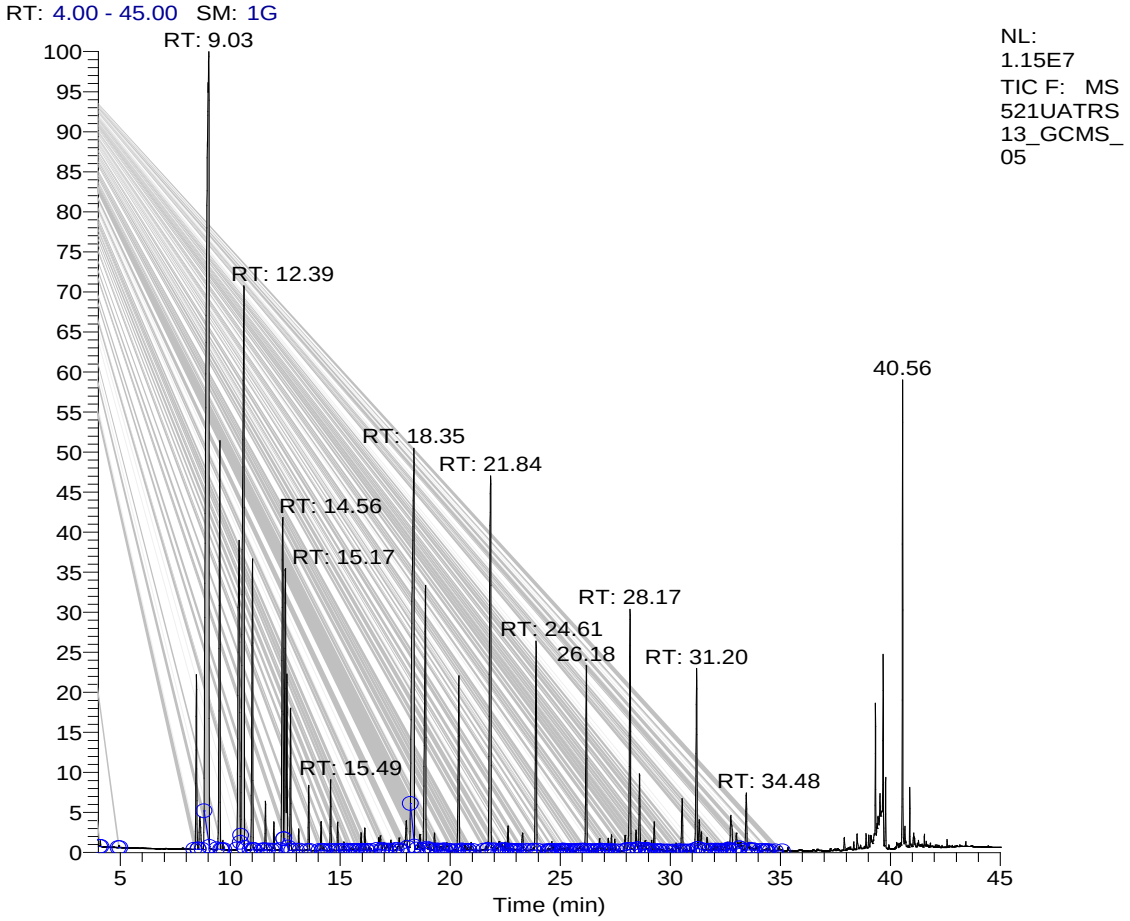

Figure 1: GC–MS chromatogram of essential oil of Pistacia atlantica DESF.

Table 1: Volatile organic compounds identified in the essential oil of *Pistacia atlantica* DESF.

Retention time (min)	Components	Relative area (%)
8.46	α-thujene	1.70
8.62	α-Phellandrene	0.78
9.03	Isophorone	23.96
9.53	Camphene	4.74
9.67	Trans-Verbenol	0.07
10.40	Sabinène	5.20
10.62	Delta 3-Carene	10.35
11.01	α-pinène	3.05
11.60	Santolina triene	0.40
11.98	γ-terpinène	0.83
12.39	p-cymène	4.06
12.51	Limonene	4.34
12.74	Cis-Ocimene	1.33
18.01	Alcool de santoline	0.48
18.35	Trans-sabinene hydrate	8.12
18.88	Ocimenyl Acetate	3.52
20.39	Pulégone	1.90
21.84	Bornyle acetate	5.65
22.63	Verbenol	0.26
23.90	α-terpinène	2.44
26.18	Trans-caryophyllène	1.91
28.17	Germacrène D	2.71
28.45	Valencene	0.97
28.60	α-Gurjunene	0.77
29.27	Cadinène	0.28
30.54	Aromadendrène	0.57
31.20	Spathulénol	1.99
31.31	Naphtalène	0.49
32.75	Patchulane	0.57

Acute Toxicity Testing

Oral administration of PAEO (2 g/kg) did not induce any lethal effect and no significant changes in the body weight between the control and treated group indicating an oral LD_{50} was higher than 2000 mg/kg.

Effect of EOPA on Acetic Acid-Induced Writhing Test

In this test the EO of this plant showed significant analgesic activity at doses of 100 and 200 mg/kg (Table 2). The standard drug exhibited a writhing inhibition percentage of 61.56%, EOPA at dose of 100 mg/kg (50.98) and EOPA at dose of 200 mg/kg (80.88) as comparison to control group (fig.1).

Table 2: Effect of essential oil of *Pistacia atlantic*a DESF. on acetic acid induced writhing in mice

Treatment groups	Dose mg/kg p.o.	No. of writhing
Control	0.5 ml/mouse	45±2.58
Aspirin	200	19.6±2.88*
EOPA	100	25±5.31*
EOPA	200	9.75±1.20*

Values are means ± S.E.M. * P< 0.05, significantly different from control; Student's t-test (n= 6).

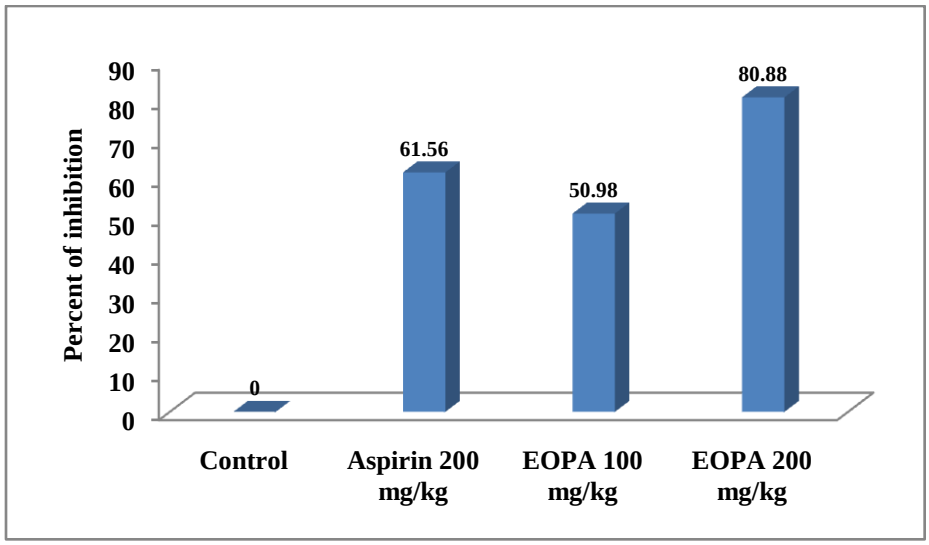

Figure 1: Effect of essential oil of Pistacia atlantica DESF. and aspirin on acetic acid induced writhing response in mice

Effect of EOPA on Tail immersion Test

In Tail immersion method all the test and standard drugs significantly (P<0.05) reduce the pain as compare to the control group. (Table 2) By applying Student test, it was shown that there is significant (P<0.05) effect of EOPA (100 and 200 mg/kg) as compare to the standard at 15, 30 and 45 minutes. The maximum analgesic effect was observed at 30 min and 45 min post administration of the EOPA at dose 100mg/kg which was comparable to that of the standard drug morphine.

Table 2: Central Analgesic activity of essential oil of *Pistacia atlantic*a DESF. by Tail immersion test

	Reaction time in seconds					
Treatment/Dose	**0 min**	**15 min**	**30 min**	**45 min**	**60 min**	**120 min**
Morphine 5mg/kg	1.78±0.19	3±0.15	5.5±0.19	7.49±0.15	5.05±0.14	3±0.13
EOPA 100 mg/kg	1.85±0.12	6.66±0.25*	7±0.4*	6.57±0.3*	5.65±0.26	2.63±0.18
EOPA 200 mg/kg	1.20±0.23	6±0.55*	6.33±0.44*	6.04±0.17*	5.13±0.24	2.25±0.17

Values are means ± S.E.M. * P< 0.05, significantly different from control; Student's t-test (n= 6).

Discussion

Medicinal plants as potential source of therapeutic aids has attained a significant role in health system all over the world for both humans and animals not only in diseased condition but also as potential material for maintaining proper health. However there is need to know which constituents in the medicinal herb are responsible for therapeutic uses. Therefore the need arises to extract, isolate and identified the phytoconstituent responsible for its therapeutic use [11].

These studies were undertaken to investigate the antinociceptive effect and chemical composition of essential oil of *Pistacia atlantic*a DESF. from Morocco.

Essential oil was analysed by Gas Chromatography/Mass Spectrometry (GC/MS) and different nociceptive test models were employed: the tail-immersion Test (thermoreceptors) in rats for assessing central analgesic effect and the writhing test (visceral chemoreceptors) in mice for assessing peripheral analgesic effect. In the acute toxicity study the LD_{50} of the EO was higher than 2mg/kg. The behavior of mice was observed upon administration of essential oil. So, *Pistacia atlantic*a DESF. essential oil had no impact on the mouse behavior.

In the peripheral antinociceptive effect, Acetylsalicylic acid was used as a standard or reference drug since it is known to have both analgesic and anti-inflammatory action and it is the prototype used for comparison against other analgesic, anti-inflammatory andanti-pyretic drugs. Acetylsalicylic acid continues to be widely regarded as the drug of choice among the available non-steroidal analgesic anti-inflammatory drugs [12]. EOPA significantly increased

the reaction time and decreased the writhing movements in mice in acetic acid-induced writhing test. The EO of *Pistacia atlantic*a DESF. caused a dose-dependent inhibition of pain in this acute pain model used. The analgesic action of this plant can be attributed to its phyto-constituents such as flavonoid and terpenoids, which are known to act through inhibition of PAF and prostaglandin biosynthesis besides of the presence of phenolic compounds in the essential oilwhich was confirmed using CPG/MS.

Previous study showed that polyphenols were found to be more abundant in *P. atlantica* than in the other species studied and that the composition of essential oil was dependent on the geographic site and the most abundant compounds were α-pinene and α-phellandrene and limonene [13].

The EOPA also had a significant effect in the tail immersion test. Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure. The effect of EOPA on this pain model indicates that PA is centrally acting. Characteristic of morphine and other opioid substances, probably because it acts at the level of the μ--opioid receptor regulated by ganglioside GM1 linked to Gs protein [14-15], thus showing an opioid activity without the classic effect of opioid drugs. This antinociceptive effect of EOPA may be related to the reduction in Ca^{2+} influx at the axon terminal of the afferent nerve inducing a decrease in adenylyl cyclase activity, which results in decreased levels of cyclic AMP and efflux of K^+ ions. The latter lead to hyperpolarization of the nerve and finally an apparent antinociceptive effect [16-18].

The results obtained in this study indicate that EOPA possesses analgesic properties, which are mediated via peripheral and central inhibitory mechanisms. This could provide a rationale for the use of this plant in pain and inflammatory disorders in folk medicine in Morocco.

Conclusion

In conclusion, the data obtained in this study suggest that EOPA possesses antinociceptive activity. However, other studies are necessary to elucidate its mechanism of action.

Conflicts of Interest

All contributing authors declare no conflicts of interest.

Acknowledgments

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