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Antinociceptive and Analgesic Activities of *Asparagus Flagellaris* (Kunth) Baker, *Tephrosia Uniflora* Pers., and *Acacia Gerrardii* Benth

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Abstract

Pain is a frequent symptom of various illnesses, and pain management is important for those who suffer from it. The purpose of this work is to estimate the analgesic and antinociceptive potentials of *Acacia gerrardii* Benth, *Asparagus flagellaris* (Kunth) Baker, and *Tephrosia uniflora* Pers in albino rats. Ethanolic extracts of the *A. gerrardii*, *A. flagellaris,* and *T. uniflora* (doses 100, 200, and 400 mg) were used. Normal saline was used as control and standard drugs morphine and diclofenac sodium were used in acetic acid writhing, hot plate, tail-flick, and formalin tests. Acetic acid and formalin were injected after one hour of administering the extracts and the standard drugs in acetic acid and formalin tests. *A. flagellaris* leaves and roots extracts (400 mg/kg) through oral administration caused the highest significant reduction in the writhing's number ($P < 0.001$) as comparing to the control normal saline (87.92% and 70.70%, respectively). Also, the highest activity in formalin test phases I & II; 86.51% and 79.89%, respectively was for *A. flagellaris* leaves (400 mg/kg) extract. For the hot plate test at 120 minutes, *A. flagellaris* leaves (400 mg/kg) extract increased latency period to 27.31 seconds and *A. flagellaris* roots (400 mg/kg) extract to 25.52 seconds ($P < 0.05$); the effect was inhibited by naloxone. In the tail flick test also *A. flagellaris* leaves 400 mg/kg produced retention of 14.80 seconds in 120 minutes ($P < 0.05$). *A. flagellaris* roots 400 mg/kg produced the highest retention of all extracts at 14.97 seconds in 120 minutes ($P < 0.05$). The current study suggests the ethanolic extracts of *A. flagellaris* leaves and roots had analgesic and antinociceptive activities in a dose-dependent manner.

Keywords: Analgesic, antinociceptive, *Acacia gerrardii*, *Asparagus flagellaris*, *Tephrosia uniflora*; health and wellbeing; Life on land

1. Introduction

Numerous clinically useful medicines have been derived from natural products and their derivatives [1]. As a result, natural products continue to be potential lead compounds and precursors for the development of new medicines [2-5]. Traditional

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medicine, according to WHO, is either the backbone of health care delivery or serves as a supplement to it. Sudanese medicinal plants are traditionally utilized for treating a range of diseases and are prepared in a variety of ways, including infusions, macerates, and decoctions, or as described by herbalists [6-8].

Pain is a universal public health issue with a high influence on life quality and economic, and it has emerged as one of modern medicine's most impressive adversaries [9]. The frequent usage of analgesics has considerably grown over the last decades in both developing and developed countries, increasing the risk of overdose and side effects [10]. Many researchers are looking into the use and side effects of analgesics. According to Palos et al, 68% of participants had prior experience with two or more side effects [11]. The worst side effects were nausea, confusion, and vomiting. Analgesics have also been linked to an increase in the risk of cardiovascular disease in osteoarthritis patients [12].

Pain is, at its core, a perceptual phenomenon. It is constructed in the brain from information collected by specialized pain receptors in tissue, altered by supraspinal and spinal mechanisms, and combined with a discrete sensory experience with an emotional valence [13]. Injured tissues liberate chemicals such as K^+, which directly depolarizes nerve terminals and increases the nociceptors responsiveness, in addition to substance P and bradykinin that sensitizes nociceptive terminals further; bradykinin invigorates Aδ and C nerve endings and boosts synthesis and releasing of prostaglandin [14]. During this process, the released histamine, prostaglandins, and serotonin sensitize or activate nociceptors and contribute to the inflammation process [14]. Based on mounting evidence that glutamate plays a critical role in pain sensation and transmission, glutamate receptors have emerged as promising potential targets for pain-relieving drug development [15]. Inflammatory pain mediators, such as cytokines, have also been shown to directly activate neurons via specific receptors on neuronal cells [16].

Traditional medicine practitioners enjoy patronage and success in the treatment of pain disorders; some herbal plants might well be suitable candidates for the treatment of neuropathic pain. As a result, the investigation of traditionally used plant species as analgesics and anti-inflammatory drugs should be encouraged as a viable strategy in the search for new analgesics and anti-inflammation drugs [17-19].

About 80% of population in the world utilized medicinal plants as antinociceptive or analgesic drugs in traditional therapy. Three plants from Eastern Nuba mountains- Western Sudan; with traditional use as analgesic had been selected for this study [20].

Acacia gerrardii Benth is a member of the family Fabaceae. *A. gerrardii* is distributed in Western Sudan Nuba mountains in lowland plains [20,21], from Benin eastwards to Ethiopia, and from there southwards to South Africa. A second subspecies is distributed in Israel, Iraq, Jordan, and the Arabian Peninsula [20,21]. *A. gerrardii* leaves were found to be rich in phenolic compounds and flavonoids, which reduced liver and kidney damage, improved the lipid profile, and lowered blood sugar in diabetic rats with renal failure [22]. The water extract of the *A. gerrardii* leaves was used for stomach pain in Eastern Nuba Mountains, while the decoction of the bark was used for swelling in West Kordofan [20,23]. In other parts of Africa, it is used to treat stomach-pain, schistosomiasis, enema, cough, and stomach and upper respiratory system infections, for infections of the as well as emetic [24-28].

Asparagus flagellaris (Kunth) Baker (family Asparagaceae), is a widespread plant in tropical Africa, including West Kordofan in Sudan [20,23]. The screening of *A. flagellaris* bark, stem, and leaves showed presence of flavonoids, cardiac glycoside, carbohydrate, and saponin [29]. Odeja et. al. quantifies and qualifies the *A. flagellaris* leaf essential oil composition, the predominate components were thymol and its derivatives, which are responsible for its antioxidant and antimicrobial activities. Also, the essential oil of the root was predominated by 84.54 % thymol derivatives and can be classified as bacteriostatic and antioxidant [30]. It has many traditional uses, the roots in Eastern Nuba Mountains are used as a fumigant for rheumatism [20] and in West Kordofan; the root decoction is used for snake bite and rabies [23], the root is used for the treatment of bilharziasis in Tanzania and tuberculosis in Ghana and the roots` decoction is used for toothache by drinking and as mouth wash [31,32]. In Ethiopia, dried or fresh stem pieces of *Asparagus flagellaris* tied around the waist or neck as a treatment for depression [33].

Tephrosia uniflora Pers. (Fabaceae) is distributed in the world subtropical and tropical zones, it is native range is Africa, from Arabian Peninsula to India [20,34]. It contains rotenoids, glucosides, isoflavones, flavanones, chalcones, prenylated flavonoids, flavanols, terpenoids, and sterols [20,35]. The ethnobotanical use of *T. uniflora* in Sudan includes tooth-ache, orally for diarrhea, and urine retention, as well as tonic [8,20,36]. Also, in Pakistan, *T. uniflora* has the same uses as antidiarrheal and for toothache [37]. Many different tests have been used in the literature to assess the analgesic and antinociceptive effects of plant extracts [38]. The formalin-produced paw licking, acetic acid-caused writhing, tail flick, and hot plate methods were selected to assess the analgesic and antinociceptive influence of the ethanolic extracts of *Acacia gerrardii* Benth, *Asparagus flagellaris* (Kunth) Baker and *Tephrosia uniflora* Pers.

2. Materials and methods

2.1. Plant materials

The plants were collected during Autumn (2019) from Abu Jubaiha and Rashad (Southern Kordofan). The taxonomy for the collected plants was done at MAPRI (Medicinal and Aromatic Plants Research Institute) and specimens numbered AG-19, AF-19, and TU-19 had been deposited at the Herbarium of MAPRI. Then the plants were cleared from any other impurities and air-dried under shelter.

2.2. Preparation of the extracts

Extraction was performed according to the method previously described [39]. 200 g of each part of the selected plants coarsely powdered and soaked in dichloromethane for 48 h with frequent shaking then filtered. Then, the plant residue was extracted with 80% ethanol for 48 h and then filtered. The filtrate was concentrated. The extracts were left to air dry in Petri dishes and the yield of each solvent was recorded [40].

2.3. Animals and maintenance

Male and female albino rats weighing (110–210) grams were released from the University of Khartoum's Faculty of Pharmacy's animal house. They were kept in the same facility, under standard conditions; 25°C temperature, controlled humidity, and 12h light/dark cycle. Ethics Committee for Animal Experimentation (Faculty of Pharmacy/University of Khartoum/Sudan) approved the experimental protocol (KU-02/2020), which follows the internationally recognized 3 Rs rule (replacement, refinement, reductions) [41,42].

2.4. Acute toxicity

The acute toxicity test (LD_{50}) for plant extracts was performed to assess any possible toxicity by the Lorke method [43,44]. The acute toxicity and lethality (median lethal dose, LD_{50}) of the extracts were done in two stages procedure in rats. In the first stage, three groups of rats (n=3) in each group were given orally 10, 100, and 1000 mg/kg, respectively, and 10 mL/kg normal saline for the control group. Following oral administration of the extracts, the animals were monitored for 24 h to see how many died and for general behavior changes. There was no death recorded at the end of the 24-h period. As a result, the second stage was carried out on another three groups, each group in the new batch of rats (n=1) received plant extracts of doses 1,600, 2,900, and 5000 mg/kg, respectively, deaths and general behavioral changes were monitored for 24 h. General behavior changes include observation of skin and fur, eyes, nose, motor activity, respiration, lacrimation, and feces were monitored. Then, the LD_{50} was calculated for the extracts that showed mortality.

2.5. Acetic acid-induced writhing test

0.8% Acetic acid intraperitoneal injection (10 mg/kg) was utilized to assess the antinociceptive effect of the extracts [38,45]. The rats were treated with the ethanolic extracts of *A. gerrardii* and *A. flagellaris* leaves and roots and *T. uniflora* roots (doses 100, 200, and 400 mg/kg); 5 mg/kg morphine was utilized as a reference standard, and normal saline as control (10 mL/kg). All the treatments were given by the oral route. 60 min after extract administration, each of the tested groups of animals was treated i.p. with acetic acid (0.8%, 10 mg/kg). Then, the number of abdominal writhes as abdomen contraction, body elongation, and trunk twisting were counted for 30 min, then the analgesic effect was calculated.

2.6. Formalin Test

The assessment of formalin-induced flinching behavior was carried out as formerly stated [45]. Into the animals right hind paw plantar surface, 0.05 mL formaldehyde (2.5%) was injected one hour after oral treating with the ethanolic extracts of *A. gerrardii* and *A. flagellaris* leaves and roots and *T. uniflora*

roots at doses 100 mg, 200 mg, and 400 mg/kg. 5 mg/kg Morphine (s.c.) and 10 mg/kg diclofenac sodium were employed as standard drugs and normal saline as control. The observations were recorded; phase I (0–5 min) subsequent phase II (15–30 min). Then, the effect was calculated as follows:

$$\text{Percent inhibition} = \frac{\text{number of licks (control)} - \text{number of licks (treated)}}{\text{number of licks (control)}} \times 100$$

2.7. Hot plate test

The rats` thermal nociception was examined by putting them on a hot metal plate at 55±0.05 ^{o}C (Hot plate - Panlab LE 7406) [46,47]. The control and reference groups received normal saline (10 mL/kg) and 5 mg/kg s.c. morphine, respectively. Test groups received doses (100, 200, and 400 mg/kg p.o.) of the selected thermal nociception was assessed by withdrawal response latency measurement in the form of paws withdrawal, jumping, or paws licking at 0, 30, 60, 90, and 120 min after treatment with a 30 s cut-off period to avert the paw damage in the response absence [48].

The ethanolic extracts *A. flagellaris* leaves and roots showed positive responses on the hot plate test, so an additional test was performed to find out if opioid receptors were included or not. In a separate group of animals (6 animals for each group), all test animals were pre-treated with 0.5 mg/kg naloxone (s.c.) and after 10 min, the hot plate test prescribed above was repeated.

2.8. Tail flick test

The tail-flick test was performed by directing radiant heat to the proximal third of the tail. The reaction time was observed when the rats tried to pull away their tails using CEIEC Tail-flick test instrument. The influence of extracts was estimated in terms of tail flick latency period at 0, 30, 60, 90, and 120 min after administering the doses. Before the experiments, baseline tail-flick latencies of all animals were determined. Rats with a reaction time of >6 sec. were excluded. A cut-off time was maintained at 20s to avert tissue injury [49,50]. The control group received 10 mL/kg of normal saline while the reference group received morphine (5 mg/kg, s.c.). Test groups received extracts (doses 100/200/400 mg/kg, p.o.) of *A. gerrardii* and *A. flagellaris* leaves and roots and *T. uniflora* roots. Maximum possible analgesia (MPA) was calculated.

3. Results

3.1. Acute toxicity

The result showed that no death was displayed by the ethanolic extracts of all tested plants in the first 24 h among the three rats` groups (doses 10, 100, and 1000 mg/kg) in stage one. Upon increasing the dose of all extracts in stage two to 1600, 2900, and 5000 mg/kg of the extracts, the results showed that *A. gerrardii* and *A. flagellaris* showed no death in the first 24 h among the three groups; hence, the extracts are safe and had a wide range of effective dose (Table 1). However, *T. uniflora* Pers. roots showed mortality at 5000 mg/kg. So, the LD_{50} for *T. uniflora* was 3800 mg/kg.

$LD_{50} = \sqrt{\boldsymbol{D0 \times D100}}$ where D100 = Lowest dose that produced mortality; D_0 = Highest dose that gave no mortality.

$LD50 = \sqrt{\boldsymbol{2900 \times 5000}} = 3800$ mg/kg

3.2. Acetic acid-induced writhing test

Morphine (5 mg/kg, reference analgesic drug) produced a significant reduction in the number of writhing ($P < 0.001$) as compared to the control normal saline. *A. flagellaris* leaves and roots similarly demonstrated a significant dose-dependent reduction in the writhing number ($P < 0.001$) as compared to the control normal saline (Figure 1).

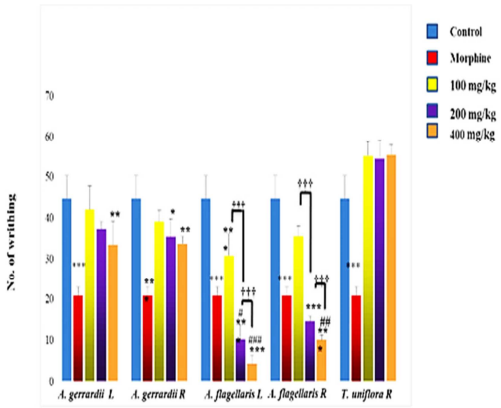

Figure 1: Acetic acid induced writhing test of the ethanolic extracts of tested plants of rats. Values were expressed as mean ± SEM (n=6). * $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs vehicle control group; # $P \leq 0.05$, ## $P \leq 0.01$, ### $P \leq 0.001$ vs the standard morphine, † $P \leq 0.05$, †† $P < 0.01$, ††† $P < 0.001$, between treatment groups, ANOVA followed by Turkey`s test.

Table 1
Acute oral toxicity for the ethanolic extracts of the tested plants in rats

Extracts	Dose	Mortality	General Condition
Control (Normal saline)	10 ml/kg	-	
A. gerrardii leaves	10 mg/kg	0/3	Normal
	100 mg/kg	0/3	Normal
	1000 mg/kg	0/3	Normal
	1600 mg/kg	0/1	Normal
	2900 mg/kg	0/1	Normal
	5000 mg/kg	0/1	Normal
A. gerrardii roots	10 mg/kg	0/3	Normal
	100 mg/kg	0/3	Normal
	1000 mg/kg	0/3	Normal
	1600 mg/kg	0/1	Normal
	2900 mg/kg	0/1	Normal
	5000 mg/kg	0/1	Normal
A. flagellaris leaves	10 mg/kg	0/3	Normal
	100 mg/kg	0/3	Normal
	1000 mg/kg	0/3	Normal
	1600 mg/kg	0/1	Normal
	2900 mg/kg	0/1	Normal
	5000 mg/kg	0/1	Normal
A. flagellaris roots	10 mg/kg	0/3	Normal
	100 mg/kg	0/3	Normal
	1000 mg/kg	0/3	Normal
	1600 mg/kg	0/1	Normal
	2900 mg/kg	0/1	Normal
	5000 mg/kg	0/1	Normal
T. uniflora roots	10 mg/kg	0/3	Normal
	100 mg/kg	0/3	Normal
	1000 mg/kg	0/3	Normal
	1600 mg/kg	0/1	Normal
	2900 mg/kg	0/1	Normal
	5000 mg/kg	1/1	Dead

The writhing-inhibition and analgesic influences were increased with the dose in a significant noteworthy manner (41.82%, 54.31%, and 87.92% for leaves and 25.01%, 62.50%, and 70.70% for roots) at the doses 100mg/kg, 200mg/kg, and 400 mg/kg, respectively. *A. flagellaris* leaves (400 mg/kg) recorded the highest percentage inhibition on the acetic acid-induced writhing among all tested doses. On the other hand, *T. uniflora* roots extract showed no analgesic and antinociceptive performance while *A. gerrardii* leaves and roots extracts demonstrated analgesic and writhing- inhibitory effects only at the higher doses.

3.3. Formalin test

Diclofenac significantly reduced the licking activity with a more potent inhibitory effect on the late phase (P<0.001). On the other hand, the analgesic morphine equally potently attenuated the licking behavior in both phases (P<0.001). The ethanolic extracts of the leaves and roots of *A. flagellari*s showed a significant (p<0.001) and dose-dependent decrease of nociceptive behavior triggered by formalin compared with the control group, with the leaves at a dose of 400 mg/kg manifested notable potent analgesic activity than both standards diclofenac and morphine. The leaves recorded 51.32% and 86.51% reduction in the number of lickings in phase1 for the doses 200 and 400 mg/kg, while 33.52%, 60.34%, and 79.89% reduction in lickings in the second phase with 100, 200, and 400 mg/kg, respectively (Table 2).

Table 2
Analgesic effect of ethanolic extracts of the tested plants, diclofenac, and morphine by formalin test (Phase I and II) in rats

Groups	No. of Licks Phase I: 0-5 min (Mean ±SEM)	% of inhibition Phase I	No. of Licks Phase II: 15-30 min	% of inhibition Phase II
Control N. S.	13.8±0.80		32.40±3.23	
Diclofenac Na 10mg/kg	9.80±0.58*	28.98%	15.33±1.26***	48.61%
Morphine 5 mg/kg	9.00±0.52***	34.78%	8.00±2.55***#	67.58%
A.g. L 100 mg/kg	11.00±0.26	20.29%	23.67±1.23***	20.65%
A.g. L 200 mg/kg	16.17±1.14	+11.33%	23.17±0.79***	22.32%
A.g. L 400 mg/kg	7.40±0.24***$	55.02%	20.60±1.25***	27.35%
A.g. R 100 mg/kg	9.83±0.40*	33.72%	22.17±1.14***	25.68%
A.g. R 200 mg/kg	16.75±0.48	+36.01%	32.60±1.21	+11.73%
A.g. R 400 mg/kg	11.40±1.29	17.94%	26.20±1.36	4.46%
A. f. L 100 mg/kg	15.83±0.60	+6.74%	19.83±0.60***	33.52%
A. f. L 200 mg/kg	6.33±0.21***$$$	57.32%	11.83±0.31***$$$	60.34%
A. f. L 400 mg/kg	2.40±0.40***###†††$$$	86.51%	6.00±0.00***###$$$	79.89%
A.f. R 100 mg/kg	8.33±0.33***	43.83%	16.00±1.26***	43.58%
A.f. R 200 mg/kg	7.00±1.22***	39.31%	12.00±1.22***	54.17%
A.f. R 400 mg/kg	6.00±.84***	65.14%	10.00±0.32***	60.34%
T. u. R 100 mg/kg	19.60±0.93	+25.89%	37.83±0.70	+26.82%
T. u. R 200 mg/kg	5.00±0.37***###††$$$	66.28%	12.40±1.03***	51.39%
T. u. R 400 mg/kg	21.20±1.07	+38.23%	33.40±0.93	+14.55%

Values were expressed as mean ± SEM (n = 6), *, P ≤ 0.05, **, P ≤ 0.01, ***, P≤ 0.001 versus vehicle control (NS), #, P ≤ 0.05, ##, P ≤ 0.01, ###, P≤ 0.001 versus the standard diclofenac , † P ≤ 0.05, ††, P ≤ 0.01, †††, P≤ 0.001 versus morphine , $, P ≤ 0.05, $$, P ≤ 0.01, $$$, P≤ 0.001 between treatment groups, ANOVA followed Tukey's test. L=leaves, R=roots.

Regarding *A. gerrardii* leaves, only the concentration 400 mg/kg produced a significant antinociceptive potential in both phases. On the other hand, the ethanolic extract from *T. uniflora* roots showed analgesic effects at the dose of 200 mg/kg, while an enhanced nociceptive response in both phases was observed at the low and high doses, 100 and 400 mg/kg, respectively.

3.4. Hot plate test

Analgesic and antinociceptive effect of the plants' understudy were assessed in rats exposed to thermal nociception using a hot metal plate maintained at 55±0.05 ˚C. The results of the analgesic and nociceptive effects of tested plants extracts using the hot plate method were presented in Table 3. There was no notable difference in the withdrawal response time to the thermal stimuli in rats treated with normal saline throughout the whole experimental time. Morphine administration remarkably ($p<0.001$) raised the animal response time to reach 20.97 seconds after 0.5 hr. This analgesic influence kept significant even after 6 hrs ($p<0.001$). All doses of *A. flagellaris* leaves showed the most significant ($p<0.001$) and pronounced increase in the latency time of treated rats at 120 minutes when compared to control group and standard morphine (24.38, 26.96, and 27.31 seconds for 100, 200, and 400mg/kg respectively in comparison to 16.29 seconds for morphine). Similar responses were observed for *A. flagellaris* roots (Table 3).

A. gerrardii roots (200 and 400mg/kg) demonstrated a significant increase ($p<0.001$) in the latency period. On the hand, *A. gerrardii* leaves and *T. uniflora* roots demonstrated no analgesic activity in the hot plate method.

The MPA (maximum possible analgesia) which represented the analgesic activity compared to morphine (positive control) displayed that *A. flagellaris* extracts at 120 minutes had higher MPA than morphine as shown in Figure 2.

Table 3
Analgesic effect of ethanolic extracts of the tested plants and morphine by hot-plate test in rats

Groups	Retention time in (sec) Time after drug administration				
	0 min	30 min	60 min	90 min	120 min
Control N.S.	8.92±0.19	8.47±0.31	10.77±0.63	13.41±0.68	11.03±0.82
Morphine 5mg/kg	10.69±0.27	20.97±0.44 *** $^{\$\$\$}$	18.66±1.34***	16.80±1.35	17.12±1.17***
A.g. L100 mg/kg	6.69±0.15	8.25±0.37	9.89±0.71	10.62±0.54	9.87±0.51
A.g. L 200 mg/kg	7.81±0.13	8.57±0.24	10.52±0.59	11.14±0.22	10.02±0.18
A.g. L 400 mg/kg	8.49±0.39	8.66±0.14	11.62±0.77	11.57±0.33	10.56±0.54
*A.g. R*100 mg/kg	8.22±0.17	9.45±0.47	10.18±0.65	12.15±0.87	12.49±0.84
A.g. R 200 mg/kg	8.04±0.13	12.26±0.59**†	13.54±1.21	14.65±1.48	19.03±2.40***†††
*A.g. R*400mg/kg	8.31±0.24	13.23±0.51***††	12.62±0.40	16.08±1.26	17.10±1.04***†
A. f. L 100 mg/kg	12.00±0.58	13.17±0.90***	16.06±1.01**	16.30±0.98	24.38±0.64***###
A. f. L 200 mg/kg	12.31±0.74***	15.43±0.61***	20.18±1.16***	26.40±1.48***###†††	26.96±0.69***###
A. f. L 400 mg/kg	10.15±0.56	14.16±0.51***	16.79±0.56***	26.66±1.57***###†††	27.31±0.79***###
A.f. R 100 mg/kg	10.19±0.49	10.70±0.63	16.62±1.13***	18.08±0.17*	17.98±0.53***
A.f. R 200 mg/kg	11.13±0.42*	12.66±1.68***	16.65±0.72***	20.50±0.39***	21.70±0.83***#
A.f. R 400 mg/kg	10.41±0.47	14.61±0.41***†††	19.64±0.88***	22.02±0.91***##	26.43±1.7***###†††
T. u. R 100 mg/kg	6.69±0.15*	8.25±0.37	9.89±0.71	10.62±0.54	9.87±0.51
T. u. R 200 mg/kg	7.81±0.13	8.57±0.24	10.52±0.59	11.14±0.22	10.02±0.18
T. u. R 400 mg/kg	8.49±0.38	8.66±0.14	10.93±0.94	11.57±0.33	10.56±0.54

Values were expressed as mean ± SEM (n = 6), *, $P \leq 0.05$, **, $P \leq 0.01$, ***, $P \leq 0.001$ versus vehicle control (N.S.), #, $P \leq 0.05$, ##, $P \leq 0.01$, ###, $P \leq 0.001$ versus the standard morphine , † $P \leq 0.05$, ††, $P \leq 0.01$, †††, $P \leq 0.001$ versus doses with in a group , $^{\$}$, $P \leq 0.05$, $^{\$\$}$, $P \leq 0.01$, $^{\$\$\$}$, $P \leq 0.001$ between time points with the group, ANOVA followed Tukey's test. L=leaves, R=roots.

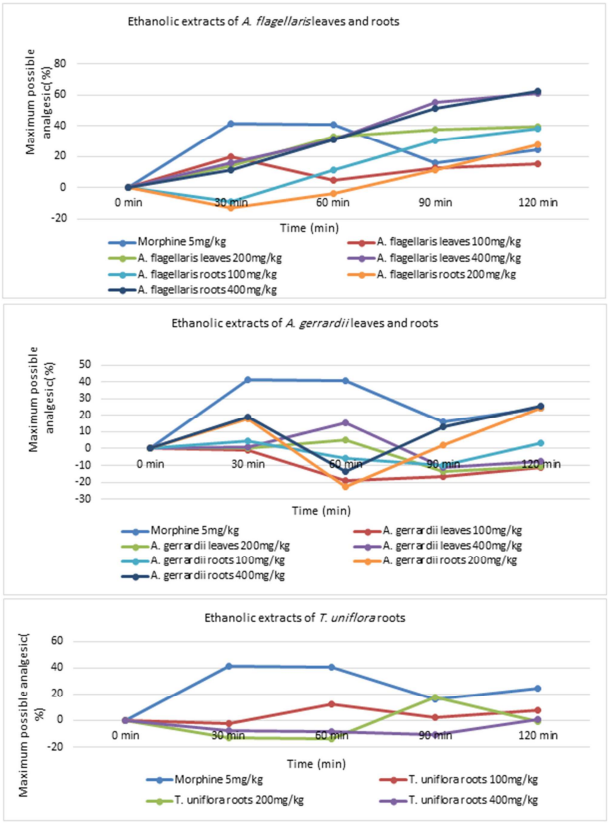

Figure 2: Maximum possible analgesia (MPA) (%) representing the effect of Alcoholic extracts against Morphine (positive control) on Hot plate test in Rats.

To investigate whether or not, the opioids receptors were involved in the potent analgesic effect exhibited by *A. flagellaris* leaves and roots, the rats were pretreated with naloxone (non-selective opioid-receptor antagonist, 0.5 mg/k, i.p.) 15 min before the administration of morphine and the tested ethanolic extracts. Pretreatment with naloxone completely reversed the analgesic activity of morphine and the tested extracts at all doses (Figures 3 and 4).

3.5. Tail-flick test

The pain was produced by giving radiant heat to the proximal third of the rat's tail (using the CEIEC Tail flick apparatus). Reaction time was recorded as the interval between exposing the tail to the light beam and the tail withdrawal. The effect of extracts was obtained in terms of tail-flick latency period at 0, 30, 60, 90, and 120 min. The results of analgesic activity of the ethanolic extracts of the studied plants are shown in Table 4. Tail flick retention time for morphine as standard was highly significant ($P < 0.001$) and time-dependent with highest retention time 13.68 seconds achieved in 90 minutes. *A. gerrardii* leaves and roots at all doses did not display any analgesic and antinociceptive effects in the tail-flick test.

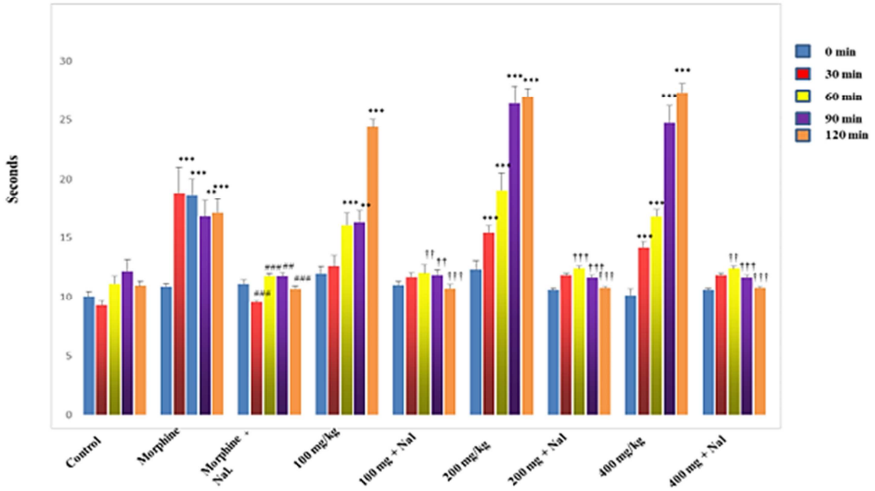

Figure 3: Effect of ethanolic extract of *A. flagellaries* leaves by hot plate test in naloxone-pretreated rats.
Values were expressed as mean ± SEM (n = 6), *, $P \leq 0.05$, **, $P \leq 0.01$, ***, $P \leq 0.001$ versus vehicle control (N.S.), #, $P \leq 0.05$, ##, $P \leq 0.01$, ###, $P \leq 0.001$ versus the standard morphine , † $P \leq 0.05$, ††, $P \leq 0.01$, †††, $P \leq 0.001$ versus doses with in a group , $^{\$}$, $P \leq 0.05$, $^{\$\$}$, $P \leq 0.01$, $^{\$\$\$}$, $P \leq 0.001$ between time points with the group, ANOVA followed Tukey's test. L=leaves, R=roots.

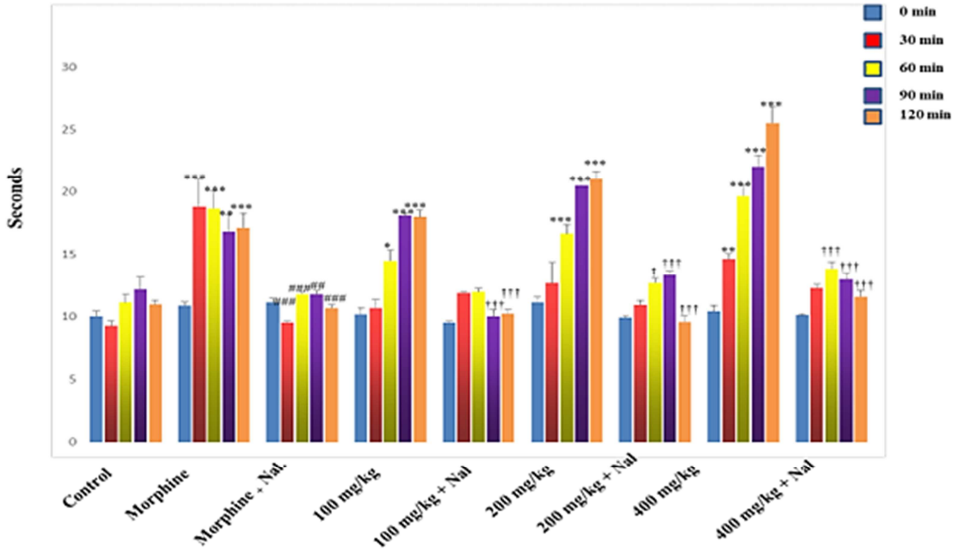

Figure 4: Effect of ethanolic extract of *A. flagellaries* roots by hot plate test in naloxone-pretreated rats.
Values were expressed as mean ± SEM (n = 6), *, P ≤ 0.05, **, P ≤ 0.01, ***, P≤ 0.001 versus vehicle control (N.S.), #, P ≤ 0.05, ##, P ≤ 0.01, ###, P≤ 0.001 versus the standard morphine , † P ≤ 0.05, ††, P ≤ 0.01, †††, P≤ 0.001 versus doses with in a group , $, P ≤ 0.05, $$, P ≤ 0.01, $$$, P≤ 0.001 between time points with the group, ANOVA followed Tukey's test. L=leaves, R=roots.

Similar insignificant responses were observed by the *T. uniflora* roots (Table 4). *A. flagellaris* leaves showed a marked (p< 0.001) time- and dose - dependent analgesic response manifested as an increase in the tail-flick retention times in rats to 12.96 and 14.38 seconds at 90 minutes for 200 and 400 mg/kg, respectively. Similar dose- and time-dependent noticeable (P < 0.001) antinociceptive responses were demonstrated by *A. flagellaris* roots extracts which were more pronounced at the dose of 400 mg/kg (Table 4). These results appeared clearly in maximum possible analgesic effect compared to morphine as shown in Figure 5.

Table 4
Analgesic effect of ethanolic extracts of the tested plants and morphine by tail-flick test in rats

Groups	Retention time in (sec)				
	Time after drug administration				
	0 min	30 min	60 min	90 min	120 min
Control N.S.	8.57±0.75	8.00±0.43	7.83±1.02	7.50±1.18	6.58±0.80
Morphine 5 mg/kg	9.40±0.41	13.37±1.43*** $	12.77±0.91*** $$	13.68±1.42*** $	12.63±1.10*** $
A.g. L100 mg/kg	8.03±0.39	6.40±0.52	5.50±0.19	6.00±0.09	6.55±0.34
A.g. L 200 mg/kg	9.52±0.15	9.28±0.18	8.43±0.72	6.66±0.48	7.00±0.18
A.g. L 400 mg/kg	8.43±0.49	8.43±0.95	9.70±0.32†††	8.52±0.39	7.83±0.23
*A.g. R*100 mg/kg	9.08±0.23	10.00±0.67	7.12±0.24	5.05±0.16	5.23±0.16
A.g. R 200 mg/kg	8.90±0.46	6.65±0.32	5.10±0.28	4.98±0.55	4.82±0.34
*A.g. R*400mg/kg	6.72±0.17	7.07±0.29	6.18±0.63	4.88±0.11	4.32±0.16
A. f. L 100 mg/kg	8.85±0.27	10.35±1.49	8.43±0.25	9.07±0.48	8.62±0.51
A. f. L 200 mg/kg	8.70±0.6	10.24±1.22 $	12.32±1.27***†† $$	12.96±1.14***† $$$	12.44±1.14******†† $$$
A. f. L 400 mg/kg	9.12±0.34	9.87±0.23	11.57±0.28**†	14.38±0.28******†††	14.80±0.12******†††
A.f. R 100 mg/kg	7.5±0.51	6.92±0.48	7.57±0.40	9.56±0.54 $	10.42±0.55*** $
A.f. R 200 mg/kg	6.82±0.61	6.74±0.27	7.46±0.13	9.12±0.26 $	10.52±0.37*** $$
A.f. R 400 mg/kg	7.78±0.46	9.35±0.28 $	11.57±0.32*****†† $$	13.88±0.32******†† $$$	14.97±0.19******††† $$$
T. u. R 100 mg/kg	6.88±0.60	7.72±0.41	9.33±0.96	7.75±0.75	7.63±0.41
T. u. R 200 mg/kg	6.88±0.59	6.40±0.35	6.17±0.33	9.65±0.91	6.45±0.26
T. u. R 400 mg/kg	8.03±0.61	7.08±0.75	6.78±0.36	6.15±0.24	6.72±0.35

Values were expressed as mean ± SEM (n = 6), *, P ≤ 0.05, **, P ≤ 0.01, ***, P≤ 0.001 versus vehicle control (N.S.),= #, P ≤ 0.05, ##, P ≤ 0.01, ###, P≤ 0.001 versus the standard morphine , † P ≤ 0.05, ††, P ≤ 0.01, †††, P≤ 0.001 versus doses with in a group , $, P ≤ 0.05, $$, P ≤ 0.01, $$$, P≤ 0.001 between time points with the group, ANOVA followed Tukey's test.

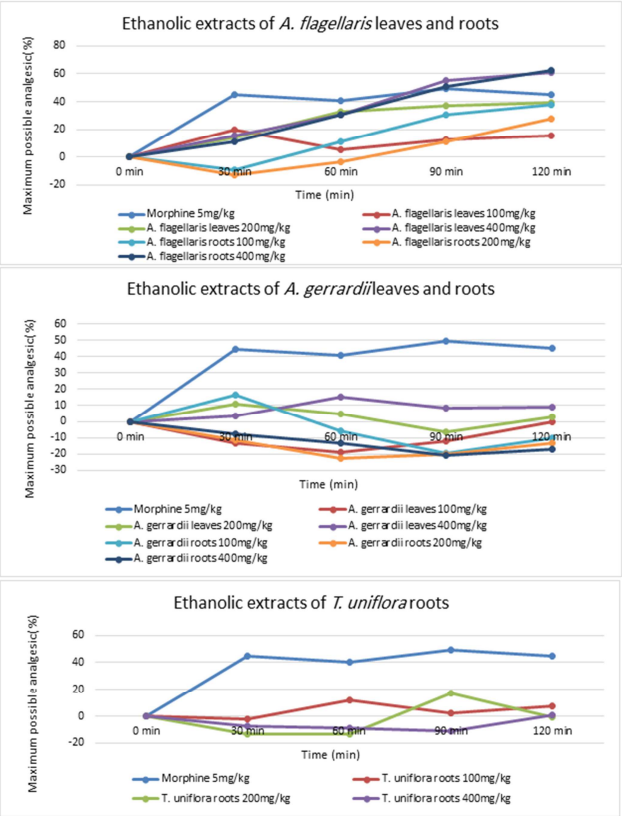

Figure 5: Maximum possible analgesia (MPA) (%) representing the effect of ethanolic extracts against morphine (positive control) in tail flick test in rats.

4. Discussion

The extracts analgesic and antinociceptive activity were investigated by the writhing test and the formalin test which create visceral and deep pain. Thermal stimuli were tested by tail-flick and hot plate tests, in which the skin is stimulated [51]. The acetic acid intraperitoneal administration irritates serous membranes provoking a very stereotyped behavior in the rat and mouse, which is characterized by movements of the body, twisting of dorso-abdominal muscles, abdominal contractions, a motor activity reduction, and motor incoordination [52]. The ethanolic extracts of *A. flagellaris* leaves and roots demonstrated significant dose-dependent inhibition of writhings. The greatest inhibition of nociceptive behaviors was observed at a dose of (400 mg/kg) for *A. flagellaris* leaves (87.92%) as compared to morphine (55.18%). *A. flagellaris* roots (400 mg/kg) also showed a notable inhibition (70.70%). While the ethanolic extracts of *A. gerrardii* (leaves and roots) and *T. uniflora* roots did not increase the inhibition of writhes compared to normal saline and morphine (Figure 1). Injection of formalin causes an immediate, intense increase in the spontaneous activity of afferent C fibers, licking, and biting of the injected paw. Also, formalin test is utilized to indicate the analgesic agent's capability to treat chronic pain due to inflammation [53]. The results of tested extracts showed that the ethanolic leaf extract of *A. flagellaris* at a concentration 400 mg/kg inhibited both the early and the late phases of formalin - induced pain by 86.51% and 79.89%, respectively which was higher than that of morphine (34.78% and 67.58%) and diclofenac sodium (28.98% and 48.61%). In addition, the ethanolic *A. flagellaris* roots (400 mg/kg) inhibited the two phases by 65.14% and 60.34%, respectively (Table 2). Ethanolic extract *T. uniflora* roots at concentration (200 mg/kg) significantly inhibited phase I and II by 66.28% and 51.39%, respectively compared to the control. Hot plate test is a thermal test, which is sensitive to strong analgesics and limited tissue damage, the test is supraspinally mediated and therefore can be used as a test of central activity [54]. Ethanolic extracts *of A. flagellaris* leaves and roots produced significant prolongation of time latency in all concentrations, the highest was 27.31±0.79 seconds for 400 mg/kg of the leaves (Table 3). The use of naloxone before administering these extracts reduced the latency period to time like the control normal saline (Figures 3 and 4). These results suggested that the antinociceptive effect of *A. flagellaris* was reversed by naloxone, which indicated the central antinociceptive effect of *A. flagellaris*. According to the suggestion that δ- and μ2-opioid receptors are involved in spinal mechanism, while μ2/μ1- opioid receptors may principally mediate supraspinal analgesia [55]. This obviously suggested the activation of opioid receptors involvement in the antinociceptive action of *A. flagellaris.* Ethanolic extract of *A. gerrardii* roots (200 and 400 mg/kg) possessed a significant increase in latency period similar to that of morphine. Tail-flick model is used for the evaluation of central pain, indicative for analgesic drugs of opioid origin where pain mediates from the spinal region by spinal reflex. It has been suggested that the spinal mechanism involves the μ2- and δ-opioid receptors. Ethanolic extracts of *A. flagellaris* leaves and roots of dose 400 mg/kg at 120

minutes produced the highest retention at 14.80±0.12 and 14.97±0.19 seconds, respectively (Table 4). This result confirmed the centrally acting analgesic activity of the ethanolic extracts of *A. flagellaris* leaves and roots that was obtained by hot plate test [56]. *A. flagellaris* reported in the literature that it contained essential oils mainly thymol derivatives [30]; which had many pharmacological activities including analgesic, anti-inflammatory and sedative effects [57]. These contents could explain the results obtained for the analgesic and antinociceptive activities of *A. flagellaris*.

A. gerrardii was reported by being rich in phenolic compounds and flavonoids [22]. Flavonoids are polyphenolic compounds that are widely found in fruits and vegetables. They can block the activation and expression of various cellular regulatory proteins such as transcription factors and cytokines, leading to lessened cellular inflammatory responses and pain [58]. So, the reduction of nociceptive behaviors in the experiments by the extract of *A. gerrardii* could be assumed by flavonoids interference with any of the pains and inflammation induction pathways [53]. Narcotics analgesics are alkaloids with broad pharmacological actions, and their ability to interact with various neurotransmitter receptors and cross the blood-brain barrier determines their pharmacology. Several studies have shown that tannins, flavonoids, and other polyphenolics have analgesic properties in various experimental animal models.

Conclusion

Analgesic and antinociceptive activities of the selected plants; *A. flagellaris*, *A. gerrardii,* and *T. uniflora* were assessed by acetic acid writhing, formalin, hot plate, and tail-flick tests. All the tests carried on albino rats of both sexes and the doses of the extracts were given orally. LD_{50}s for *A. flagellaris* and *A. gerrardii* were greater than 5000 mg/kg and for *T. uniflora* was 3800 mg/kg. The ethanolic extracts of *A. flagellaris* leaves and roots had analgesic and antinociceptive activities in a dose-dependent manner. The analgesic and antinociceptive effect of *A. flagellaris* was reversed by naloxone, which suggested central activity. However, further investigations are needed to fully characterize the responsible active compounds present in *A. flagellaris* and to clarify the possible mode of action.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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