Original Article:
Assessment of Anticonvulsant Activities of Petroleum Ether Extract of Anacyclus pyrethrum Roots on Experimental Rats

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ABSTRACT

Background: Epilepsy is one of the most common neurological conditions and a significant cause of morbidity and mortality.

Objectives: The present study aimed to evaluate the anticonvulsant activity of the petroleum ether extract of the root of Anacyclus pyrethrum on Pentylenetetrazole (PTZ)-induced seizure model in Wistar rats.

Methods: The composition of the petroleum ether extract of A. pyrethrum was first analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Subsequently, the anticonvulsant activities of these extracts (70 and 140 mg/kg, intraperitoneal injection) were evaluated on PTZ-induced seizures in rats. The protection rate against induced seizures, latency, and duration of seizures, as well as neurological symptoms, were assessed and compared to those protected by phenobarbital.

Results: GC/MS analysis of the petroleum ether extract showed that the main components were octadecadienoic acid, hexadecanoic acid, diheptylcyclopropene, naphthalene, and methyl stearate. The extract (70 and 140 mg/kg) was found to provide significant protection against PTZ-induced seizures. Moreover, compared to the negative control, the extracts increased the latency of induced-convulsion and reduced the duration of epilepsy. Interestingly, the extracts showed a reduction in neurological symptoms and the severity of seizures compared to the negative control. All of these outcomes manifested in a dose-dependent manner.

Conclusion: The petroleum ether extract of A. pyrethrum may produce anticonvulsant effects by reducing the duration of seizures and delaying the latency of seizures induced by PTZ.

Keywords:
Anacyclus pyrethrum, Gas chromatography-mass spectrometry (GC/MS), Pentylenetetrazole, Anticonvulsant activity

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Introduction

Epilepsy is one of the most common neurological disorders characterized by abnormal electrical discharges in the central nervous system that, in turn, causes recurrent seizures with or without convulsions. Epilepsy may also be associated with loss of consciousness [1]. Epidemiologically, epilepsy affects around 70 million people worldwide [2] and has a substantial effect not only on the health/wellbeing of people but also on the economic productivity and burden on health care services [3]. Currently, epilepsy is managed by using many antiepileptic drugs, such as phenytoin, carbamazepine, and phenobarbital [4]. However, using these drugs is associated with severe side effects that may limit its utilization in epilepsy management [5]. Moreover, around 20% to 30% of epileptic patients are resistant to conventional antiepileptic therapy [6, 7]. Therefore, the discovery and development of new, effective, and safe antiepileptic agents are continually required. Such new antiepileptic drugs should be explored based on original ideas to open new avenues for adequate control of this devastating disease [8, 9]. Recently, plants have attracted more attention as primary sources for biologically active natural compounds with various activities, including anticonvulsant activity [10].

Anacyclus pyrethrum (A. pyrethrum) (Figure 1A) belongs to the Asteraceae family and is mainly distributed in North Africa and India [11]. The roots of A. pyrethrum, also called Akarkara, have been long used traditionally for toothache, stimulating salivary glands, rheumatic and neuralgic affections of teeth, and rhinitis. A. pyrethrum showed various pharmacological effects such as anti-inflammatory, immunostimulating, antimicrobial, enhancing male sexual functions, antidiabetic and hepatoprotective [12-17]. Besides, it has been shown as a tonic agent to the nervous system in traditional medicine [13, 18].

In the current study, the petroleum ether extract of A. pyrethrum root was analyzed for its content and composition. The potential anticonvulsant activities of the extract were also evaluated using the Pentylentetrazole (PTZ)-induced seizure model.

Materials and Methods

Plant material

The roots of A. pyrethrum (Figure 1B) were obtained from the local market, then identified and authenticated by the medicinal and aromatic plants research institute, Khartoum, Sudan. A voucher specimen was placed in the Department of Pharmacognosy, Faculty of Pharmacy, University of Khartoum.

Chemicals

Standard convulsive agents, pentylentetrazole from Sigma Aldrich (St. Louis, MO, USA), and phenobarbital were obtained from Shanghai Pharmaceutical Company (Khartoum, Sudan).

Preparation of the plant extract

Roots of A. pyrethrum were collected and dried in the shade. Then it was ground by using mortar and pestle to produce a coarse powder. Then, 200 g of powder was divided into four amounts. The weight of each one equals 50 g and is put into a Soxhlet apparatus; then 250 mL of petroleum ether is added for each the Soxhlet at temperature 70°C for 7 hours. Next, the extract was cooled, and the solvent was evaporated to dryness by rotary evaporator at 40°C. The final crude extract was 4 g, and then each 1 g was dissolved in 1 mL of olive oil. Based on the previous studies for different pharmacological activities for the A. pyrethrum extract on the central nervous system [19, 20], 70 and 140 mg/kg doses of the extract were tested for the anticonvulsant activities.

Sample Preparation for Gas Chromatography-Mass Spectrometry (GC/MS)

About 2 mL of the extract was mixed thoroughly with 7 mL of alcoholic sodium hydroxide, and then 7 mL from alcoholic sulfuric acid was added. The mixture was shocked well for 5 minutes and then was left to stand overnight. The next day, 1 mL of super-saturated sodium chloride was added and shocked, then 2 mL of n-hexane was added, and the contents were shocked thoroughly for 3 minutes. Then the n-hexane layer was taken using a disposable syringe. Afterward, 5 µL of n-hexane extract was diluted with 5 mL of diethyl ether. Then the mixture was filtered through a syringe 0.45-µm filter and dried with 1 g of anhydrous sodium sulfate as a drying agent. Finally, 1 µL of the diluted sample was injected in the Gas Chromatography-Mass Spectrometry (GC/MS) instrument.
GC/MS Conditions

The qualitative and quantitative analysis of the sample was carried out using GC/MS technique (GC/MS-QP2010-Ultra model from Japan’s Shimadzu Company, with serial number 02052510156S101) and capillary column (Rtx-5MS, 30m× 0.25 mm× 0.25µm). The sample was injected using the split mode, helium, as the carrier gas passed with a flow rate of 1.61 mL/min. The temperature program was started from 60°C with a rate of 10°C/min to 300°C as the final temperature with 5 minutes holding time. The injection port temperature was 300°C, the ion source temperature was 200°C, and the interface temperature was 250°C. The sample was analyzed using scan mode in the m/z range of 40-500, and the total run time was 29 minutes. Identification of components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST).

Experimental animals

Wistar albino rats of both sexes, weighing about 130–170 g, were housed under standard environmental conditions of temperature (12 hours light/dark cycle; 24±2°C, 30-70% humidity) and had ad libitum access to purified water and food. All animals were fasted overnight before the experiment with free access to water. Animals were adapted to the laboratory condition for a week before initiating the experiments. Before starting the investigation, all experimental animal protocols were approved by the Institutional Animal Ethical Committee of the Faculty of Pharmacy, University of Khartoum.

Evaluation of anticonvulsant activity

This investigation was performed following the anti-epileptic drug development program protocol [21]. Two concentrations (70 and 140 mg/kg) of the A. pyrethrum extract were investigated for anticonvulsant activity in albino rats and compared with the activities produced from 30 mg/kg of phenobarbital as a positive control. The rats were divided into four groups; each group contained 5 rats. All groups were injected subcutaneously in a loose fold of skin on the back of the neck PTZ (70 mg/kg) as a chemical epileptic inducer. The first group of rats was orally administered olive oil as vehicle control (negative control). The second and third groups were treated orally (PO) with A. pyrethrum extract 70 and 140 mg/kg, respectively. The fourth group was treated orally with 30 mg/kg of phenobarbital. All treatments were administered one hour before the induction of seizure by subcutaneous PTZ injection. Then, all animals were placed in isolation cages, then observed for 30 min., 1 hour, and 24 hours for signs of neurological deficits and anticonvulsant activities.

Measuring of anticonvulsant activities

The protection rate % against PTZ-induced seizure was measured by the following equation: (Protection rate % = NC–NT/NC × 100), where NC= the number of seizure attacks during 1 hour after seizure induction in the control group, and NT = the number of seizure attacks during 1 hour after seizure induction in the treated group. Furthermore, convolution latency was measured as the onset time (seconds) of convolution after seizure induction. Moreover, the duration of convolution (seconds) was calculated by the following formula:

Time after completion of convolution – the onset time of convolution.

Statistical analysis

The obtained data were expressed as protection rate %, seizure latency, and duration times in seconds ± SEM and analyzed by the two-tailed Student’s t test to determine the significance of the difference between the negative control group and treated group. The difference in results was considered significant when P value < 0.05. All data analyses were carried out using GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA, USA).
Results

Phytochemical composition

The composition of petroleum ether extract of *A. pyrethrum* roots was determined by GC/MS, as shown in Table 1. In the current study, we could identify and quantify 33 components; the majority of which are 9,12-octadecadienoic acid (conjugated linoleic acids) (37.2%), 1,2-diheptylcyclopropene (11.93%), hexadecanoic acid (palmitic acid) (10.8%), naphthalene (10.99%), 9-octadecenoic acid (10.77%), and methyl stearate (5.5%). The rest of the extract contained minor components in low concentrations (Table 1).

Anticonvulsant activity

Protection Rate % of *A. pyrethrum* Extract Against PTZ-induced Seizure

The protection rate % of different treatments (70 and 140 mg/kg of the *A. pyrethrum* extract and 30 mg/kg of phenobarbital) from PTZ-induced seizure were measured and compared to the negative control group administered olive oil only. The standard drug phenobarbital 30 mg/kg showed 48% protection against PTZ-induced seizure (Figure 2). Whereas 70 and 140 mg/kg of *A. pyrethrum* extracts were produced significant protections 26.7% and 55%, respectively (Figure 2). These data indicated that the anticonvulsant activity of the *A. pyrethrum* showed a dose-dependent relationship.

![Figure 2](image1.png)

*Figure 2.* Protection rate % of *A. pyrethrum* extract (70 and 140 mg/kg) and phenobarbital (30 mg/kg) against PTZ-induced seizure

The statistical differences in means between different treatments and the control group were analyzed by the two-tailed Student’s test (n=5), *P*<0.05, **P**<0.01

![Figure 3](image2.png)

*Figure 3.* The effect of *A. pyrethrum* extract (70 and 140 mg/kg) and phenobarbital (30 mg/kg) on the latency time of convulsions induced by PTZ

The statistical differences in means between different treatments and the control group were analyzed by two-tailed Student’s test (n=5), *P*<0.05, **P**<0.01

![Figure 4](image3.png)

*Figure 4.* The effect of *A. pyrethrum* extract (70 and 140 mg/kg) and phenobarbital (30 mg/kg) on the duration of convulsions induced by PTZ

The statistical differences in means between different treatments and the control group were analyzed by the two-tailed Student’s test (n=5), *P*<0.05, **P**<0.01
<table>
<thead>
<tr>
<th>S. No.</th>
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<th>Retention Time</th>
<th>Area</th>
<th>Area%</th>
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<td>1</td>
<td>Copaene</td>
<td>9.059</td>
<td>23400</td>
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<td>2</td>
<td>Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-[1S-(1.alpha.,2.beta.,4.beta.)]-</td>
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<td>45057</td>
<td>0.06</td>
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<tr>
<td>3</td>
<td>Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-[(1R-1R*,4Z,9S*)]-</td>
<td>9.658</td>
<td>9057</td>
<td>0.01</td>
</tr>
<tr>
<td>4</td>
<td>(-)-Spathulenol</td>
<td>11.663</td>
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<tr>
<td>5</td>
<td>Caryophyllene oxide</td>
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<td>104111</td>
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<td>6</td>
<td>1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-1-methylethenyl]-, [1R-1R,4a,beta.,8a.beta.]-</td>
<td>12.365</td>
<td>59326</td>
<td>0.09</td>
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<td>7</td>
<td>3,7-Cyclodecadiene-1-methanol, alpha.,alpha.,4,8-tetramethyl-, [s-(Z,Z)]</td>
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<td>20444</td>
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<td>8</td>
<td>Methyl tetradecanoate</td>
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<td>9</td>
<td>7-Hexadecenoic acid, methyl ester, (Z)-</td>
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<td>Hexacosane</td>
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<td>Tricosanoic acid, methyl ester</td>
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<td>35033</td>
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<td>15-Tetracosenoic acid, methyl ester, (Z)-</td>
<td>21.829</td>
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<td>Gamma-Sitosterol</td>
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<td>33</td>
<td>Lupeol</td>
<td>27.038</td>
<td>629590</td>
<td>0.90</td>
</tr>
</tbody>
</table>
The time in seconds of the onset of seizure expressed as the latency of convulsion and duration of PTZ-induced seizure was obtained for the *A. pyrethrum* extract and phenobarbital compared to the negative control. As illustrated in Figure 3, the low and high doses of *A. pyrethrum* extract increased the latency of convulsion compared to the negative control but only significant in 140 mg/kg dose. The onset of seizure for the high dose of extract was 582.5±124.7 s. Compared to 267±64.8 s, the standard drug phenobarbital exhibited the onset of convulsion after 622.0±83.4 s (Figure 3). Furthermore, *A. pyrethrum* extract showed a reduction in epileptic duration when compared with the negative control. Similarly, only statistical significance was observed in the high dose of the extract, and it produced a higher reduction in seizure duration than standard drug phenobarbital (Figure 4).

Effect of *A. pyrethrum* extract on neurological signs of PTZ-induced seizure

The neurological signs and severity were observed for 1 hour after induction of seizures using PTZ-subcutaneous injection. As shown in Table 2, both doses of *A. pyrethrum* extract decreased neurological symptoms and seizure severity compared to the negative control, with better results in high doses. These results suggested that the extract of *A. pyrethrum* showed potent anticonvulsant activity.

**Discussion**

The findings of the current study revealed that petroleum ether extract of the roots of *A. pyrethrum* possessed anticonvulsant activity. *A. pyrethrum* extract at doses of 70 and 140 mg/kg significantly protected from convulsion, delayed the onset of induced-convulsions, and significantly reduced the duration of convulsion and neurological symptoms induced by PTZ in rats. We compared these results with standard antiepileptic drug phenobarbital (30 mg/kg) that produces its anticonvulsant effect through enhancing GABA transmission in the central nervous system and thereby antagonizing the seizures made by PTZ [22], as PTZ may induce convulsions by inhibiting the GABA activity [23]. Since the *A. pyrethrum* extract suppresses the convulsion induced by PTZ, it is probably may exert its anticonvulsant activities through activation of GABAergic transmission in a similar way to barbiturate. However, more extensive studies are required to evaluate the precise mechanism of action of this anticonvulsant activity. Interestingly, the anticonvulsant activities of chloroform and ethanolic extracts of *A. pyrethrum* roots were previously proved against PTZ, and electroshock models were used [24-26].

The phytochemical analysis of the *A. pyrethrum* extract revealed the presence of different terpenoid compounds. The phytoconstituents of the roots have reported containing volatile oils, gum, and traces of tannic acid, N-isobutyldienedynamide, and soluble polysaccharides [13, 27]. Hereby, GS/MS analysis revealed that the plant root is rich in fatty acids such as linoleic acid and palmitic acid; these molecules may contribute to their anticonvulsant activities. Several studies indicated the potential anticonvulsant effects of these polyunsaturated and straight-chain fatty acids [28-30]. Based on the current research, it is impossible to determine and attribute the anticonvulsant effect to any component. However, some previous reports have indicated that terpenoid saponins and terpenic steroids possess antiseizure potential in different electrical- and chemical-induced seizure models [31-33]. Thus, further fractionated-based studies to isolate the bioactive components are strongly recommended.

The petroleum ether extract of *A. pyrethrum* showed anticonvulsant effects by reducing the duration of seizures and delayed the latency of seizures induced by PTZ. However, more studies are needed to determine the

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Myoclonic Jerks</th>
<th>S-Shape in Tail</th>
<th>Upright Position</th>
<th>Head Nodding</th>
<th>Loss of Postural Control</th>
<th>Muscle Flaccid</th>
<th>A Tonic or Clonic</th>
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<tr>
<td>Olive oil</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Phenobarbital 30 mg/kg</td>
<td>+</td>
<td>++</td>
<td>Nil</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><em>A. pyrethrum</em> extract 70 mg/kg</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td><em>A. pyrethrum</em> extract 140 mg/kg</td>
<td>++</td>
<td>++</td>
<td>Nil</td>
<td>Nil</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

The severity of the neurological signs:
+ Mild;
++ Moderate;
+++ Sever.
precise mechanism(s) of action, active components that produce the anticonvulsant activities.

Ethical Considerations

Compliance with ethical guidelines

All experimental animal protocols were approved by the Institutional Animal Ethical Committee (IAEC) of Faculty of Pharmacy, University of Khartoum.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflicts of interest.

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[22] Huang RQ, Bell-Horner CL, Dibas MI, Covey DF, Drewe JA, Dillon GH. Pentylenetetrazole-induced inhibition of re-


