Evaluation of Anticonvulsant Activity and HPLC–DAD Profiling of *Achillea fragrantissima* (Gaisoom) Extracts Growing in Saudi Arabia

Mahmoud M. E. Mudawi¹, Mohammed F. Abd El-wahab²³⁴, Abdelhadi Y. A. Yassin¹, Rami S. Habeballa¹ and Mohammed M. Alshehri⁴

¹Department of Pharmacology and Toxicology, Faculty of Pharmacy, Northern Border University, Kingdom of Saudi Arabia; Mahmoud.Eltahir@nbu.edu.sa
²Department of Phytochemistry and Natural products, Faculty of Pharmacy, Northern Border University, Rafha, Kingdom of Saudi Arabia; mohabdelwahab@yahoo.com
³Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt
⁴Department of Clinical Pharmacy, Faculty of Pharmacy, Northern Border University, Rafha, Kingdom of Saudi Arabia; Moh.nbu.1995@hotmail.com

Abstract

In Kingdom of Saudi Arabia *Achillea fragrantissima* is a fragrant, perennial herb has long been used medicinally for its analgesic properties among Rafha residents. The present study concluded that *Achillea fragrantissima* (Gaisoom) possess a potent anticonvulsant activity against PTZ induced seizures which may be due to GABAA agonistic activity and /or antioxidant activity. The results of the current study showed that the ethanolic extract of Gaisoom at doses of 300 mg/kg and 600 mg/kg has anticonvulsant activity in pentylenetetrazole and maximum electroshock induced seizures models on mice. The Hexane extract have protected the mice against pentylenetetrazole – induced seizures with 100% protection rate and 100% animal survival similar to the anticonvulsant drug phenobarbital. HPLC–DAD fingerprints of different fractions were also carried out to find the pharmacological active compound. The studies are in progress to isolate the compounds by HPLC technique. Supplementary studies are required to explain the exact mechanism of action by which Gaisoom act as an anticonvulsant agent.

Keywords: *Achillea fragrantissima*, Anticonvulsant Activity, Epilepsy, Gaisoom, HPLC Profile

1. Introduction

Epilepsy is one of the serious diseases that affect a wide range of people throughout the world¹². Epilepsy may be associated with factors such as trauma, oxygen deprivation, tumors and infection³. There are many antiepileptic drugs presently in clinical use like phenytoin, carbamazepine, Ethosuximide, phenobarbital and clonazepam⁴. The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose related and chronic toxicity and teratogenicity⁵, and around 30% of the patients continue to have seizures with current antiepileptic drugs⁶⁷. Medicinal herbs had been used traditionally in the treatment of epilepsy as one alternative to synthetic drugs to avoid their side effects⁸. *Achillea fragrantissima* is a medicinal shrub plant that belongs to the Asteraceae family (formerly Compositae)⁹. *Achillea fragrantissima* is a desert plant that has been used for many years in traditional medicine in the Arabia region for the treatment of respiratory diseases and gastrointestinal disturbances⁹. In Saudi Arabia it is known locally as Gaisoom. It is used as carminative, anthelmintic, antiseptic to various infections for the urinary tract and also has antiviral and antioxidant¹⁰. Since antiquity, *Achillea* species have been used in traditional medicine of several civilizations to

*Author for correspondence*
alley pain, spasms and inflammation. Numerous studies describe the ethno-pharmacological value and various pharmacological effects of the *Achillea* extracts and hydro-distilled volatile oils in management of several diseases, used topically and orally\(^3\)\(^-\)\(^1^)\(^1^)\(^1^)\(^1^).

## 2. Materials and Methods

### 2.1 Plant Material

Fresh parts of the plant *Achillea fragrantissima* were collected from Northern Border Region, Kingdom of Saudi Arabia. The plant was identified, authenticated and extracted in Northern Border University. Three hundred grams of the grinded plant was exhaustively extracted at room temperature with 80% ethyl alcohol (2 x 2.5 L). The combined ethanolic extracts were concentrated under vacuo at 40°C to dryness (55 g). The 45 gram of concentrated alcoholic extract was then suspended in distilled water (300 ml). The water was partitioned first with hexane (3 x 1.5 L) then methylene chloride (3 x 1.5 L) then with ethyl acetate (3 x 1.5 L) and finally with n-butanol (3 x 1.5 L). Each fraction was pooled and concentrated under vacuo at a temperature not exceeding 40°C to afford (12, 10, 8 and 15 g); respectively. All plant extracts and the reference drugs used in this study were suspended in distilled water each day at the beginning of each experiment.

### 2.2 Animals

Male, albino mice weighing 25-30 g were used in this study. The animals were kept and maintained under laboratory conditions in Northern Border University, KSA; and were allowed free access to food (standard pellet diet) and water ad libitum. The animals were divided into 4 groups of 6 animals per cage for each method as: Two doses of alcoholic extract (300mg/kg and 600mg/kg), reference anticonvulsant drug-treated ‘test’, and distilled water-treated ‘control’ group. Food was forbidden for all the animals, but still animals allowed free access to water for 12 hours, preceding the experiment.

Following induction of convulsions in the ‘test’ mice, the animals were observed for 30 min and 24 hours for signs of neurological shortages\(^1^)\(^3\).

### 2.3 Drugs and Equipment

Standard convulsive agents, pentylenetetrazole (PTZ, 90mg/kg i.p.), Strychnine (STR 2mg/kg i.p.), picrotoxin (PCT, 10mg/kg i.p.), Isonicotinic acid Hydrazide (INH, 250mg/kg i.p.) and Maximum Electroshock (MES, 30mA, 20 Hz for 0.2 s) were used to induce convulsions in mice. Phenobarbitone (PBT, 40mg/kg i.p.), Diazepam (DZP, 0.5 mg/kg i.p.) and Phenytoin (25mg/kg i.p.) were used as reference anticonvulsant drugs for comparison.

### 2.4 PentyleneTetrazole Induced Convulsion in Mice:

Pentylenetetrazole (90mg/kg i.p.) produces petitmal type of epilepsy. It exerts its action by acting as an antagonist at the GABA\(_A\) receptor complex\(^1^)\(^2\). The anticonvulsant testing method of Vellucci and Webster, modified by Amabeoku and Chikuni, was used to assess the anticonvulsant activity of the plant extracts in mice. Phenobarbitone (40 mg/kg i.p.) and distilled water (3 ml/kg i.p) each was administered 30 min prior to Pentylenetetrazole 90 mg/kg i.p. for comparison. Following induction of convulsions in the ‘test’ mice, the animals were observed for 30 mins. and 24 h for signs of neurological deficits\(^1^)\(^3\).

**Group I:** Animals were maintained as a negative control, which was given distilled water (3 ml/kg p.o.) before Pentylenetetrazole (90 mg/kg i.p), then anticonvulsant activity was recorded.

**Group II:** Phenobarbitone 40 mg/kg i.p. was given once 30 mins before Pentylenetetrazole (90 mg/kg i.p.), and then anticonvulsant activity was recorded.

**Group III:** Animals were treated with 300 mg/kg p.o alcoholic extract once, 30 mins before Pentylenetetrazole (90 mg/kg i.p.), then anticonvulsant activity was recorded.

**Group IV:** Animals were treated with 600 mg/kg p.o alcoholic extract once, 30 mins before Pentylenetetrazole (90 mg/kg i.p.), then anticonvulsant activity was recorded.

### 2.5 Picrotoxin Induced Convulsion in Mice

Picrotoxin is a noncompetitive antagonist at GABA\(_A\) receptors and it blocks the GABA-activated chloride ionophore. Diazepam significantly protects animals against PIC\(^1^)\(^2\). Diazepam (0.5 mg/kg i.p.) was used as reference anticonvulsant drug that administered 30 min prior to Picrotoxin (10 mg/kg i.p.) for comparison. Following induction of convulsions in the ‘test’ mice, the animals were observed for 30 min for signs of neurological deficits\(^1^)\(^3\).
**Group I:** Animals were maintained as a negative control, which was given distilled water (3 ml/kg p.o.) before Picrotoxin (10 mg/kg i.p.), then anticonvulsant activity was recorded.

**Group II:** Diazepam (0.5 mg/kg i.p.) was given once 30 mins before Picrotoxin (10 mg/kg i.p.), and then anticonvulsant activity was recorded.

**Group III:** Animals were treated with 300 mg/kg p.o. alcoholic extract once, 30 mins before Picrotoxin (10 mg/kg i.p.), then anticonvulsant activity was recorded.

**Group IV:** Animals were treated with 600 mg/kg p.o. alcoholic extract once, 30 mins before Picrotoxin (10 mg/kg i.p.), and then anticonvulsant activity was recorded.

**2.6 Strychnine Induced Convulsion in Mice**
Strychnine is a potent spinal cord convulsant; it blocks glycine receptor selectively to induce excitatory response in CNS\(^{12}\). The test alcoholic extract at the doses of 300 and 600 mg/kg p.o., standard drug phenobarbitone sodium (40 mg/kg i.p.) and vehicle control (3 ml/kg p.o) were administered 30 min prior to Strychnine (2 mg/kg). Anticonvulsant activity was recorded. Mice that survived 30 min after strychnine administration were considered protected\(^{14}\).

**Group I:** Animals were maintained as a negative control, which was given distilled water (3 ml/kg p.o.) before strychnine (2 mg/kg), then anticonvulsant activity was recorded.

**Group II:** Phenobarbitone sodium (40 mg/kg i.p.) was given once 30 mins before strychnine (2 mg/kg), and then anticonvulsant activity was recorded.

**Group III:** Animals were treated with 300 mg/kg p.o. alcoholic extract once, 30 mins before strychnine (2 mg/kg), then anticonvulsant activity was recorded.

**Group IV:** Animals were treated with 600 mg/kg p.o. alcoholic extract once, 30 mins before strychnine (2 mg/kg), and then anticonvulsant activity was recorded.

**2.7 Isonicotinic acid Hydrazide Induced Convulsion in Mice**
Isonicotinic acid Hydrazide (INH) is thought to inhibit GABA synthesis in CNS\(^{12,15}\).

**Group I:** Animals were maintained as a negative control, which was given distilled water (3 ml/kg p.o.) before INH 250mg/kg i.p., then anticonvulsant activity was recorded.

**Group III:** Animals were treated with 300 mg/kg p.o. alcoholic extract once, 30 mins before INH 250mg/kg i.p., then anticonvulsant activity was recorded.

**Group IV:** Animals were treated with 600 mg/kg p.o. alcoholic extract once, 30 mins before INH 250mg/kg i.p. and then anticonvulsant activity was recorded.

**2.8 Maximum Electroshock Induced Convulsion in Mice**
A current of 30 mA, electroshock was delivered to each mouse via ear electrodes as a single train of pulses (20 Hz for 0.2 s) using an electro-convulsiometer\(^{16}\).

MES is also one of the commonly used models for preliminary testing of anticonvulsant drugs that produces generalized tonic-clonic seizures i.e., hind limb tonic extensor, tonic flexion and clonic convolution\(^{15}\).

**Group I:** Animals were maintained as a negative control, which was given distilled water (3 ml/kg p.o.) before MES then anticonvulsant activity was recorded.

**Group II:** Phenytoin 25 mg/kg i.p. was given once 30 mins before MES, then anticonvulsant activity was recorded.

**Group III:** Animals were treated with 300 mg/kg p.o. plant extract once, 30 mins before MES, then anticonvulsant activity was recorded.

**Group IV:** Animals were treated with 600 mg/kg p.o. plant extract once, 30 mins MES and then anticonvulsant activity was recorded.

**2.9 Acute Toxicity Study**
Acute toxicity study for the alcoholic extract of Gaisoom was done according to the OECD guidelines No: 423 (2001), then low and high doses were selected for treatment. The alcoholic extract of Gaisoom was administered orally in the escalating dosages, up to 2000 mg/kg to different groups\(^3\) of mice (n = 1, in each). The animals were observed for behavioral and physiological variations initially continuously for 30 minutes and 24 hours\(^{17}\).

**2.10 Phytochemical Screening**
Phytochemical screening of Achillea fragrantissima herb was carried out for the presence of carbohydrates (and / or glycosides), volatile substances, saponins, flavonoids, phenolic, steroids (and /or triterpenes), and alkaloids\(^{18,19}\).
2.11 Statistical Analysis
Data are expressed as mean ± S.E.M and the data were analyzed using one way ANOVA followed by Dunnett test. The level of significance was taken as p ≤ 0.05. All treatment groups were compared to the negative control group (the group which given distilled water).

2.12 HPLC Fingerprints of Gaisoom Extracts
The extracts were diluted and filter with HPLC grade methanol. Analysis of all samples was performed using RP-HPLC, Waters® 2545 Quaternary Gradient Module pump and equipped with Waters® 2998 Diode array detector, and chromatograms were recorded at 210-400 nm. The entire system was controlled using Empower 3 Software. The system has Waters® guard Column: Symmetry C18 (5µm, 4.6*250mm) and Hamilton microliter syringe using an injection volume of 10 µl.

RP-HPLC methods is consist of isocrical and gradient elution of two solvents - Solvent A (Methanol or Acetonitrile) and Solvent B (2% formic acid in water). Detector wavelengths range set at 210 nm and 400 nm. Flow rate adjusted at 1.4 ml/min (Waters®)20.

3. Results

3.1 Acute Toxicity Study
Acute toxicity study for the alcoholic extract of Gaisoom was done according to the OECD guidelines No: 423 (2001); toxic signs and lethality were not observed and the extract produced no mortality up to 2000 mg/kg.

3.2 Phytochemical Screening
Phytochemical screening of leaves were revealed the presence of flavonoids (and/or glycosides), carbohydrates, phenolic, alkaloids (and/or nitrogenous bases), and steroids (and/or triterpenes).

3.3 Pentylentetrazole Induced Seizures: (Ethanolic Extract)
Intraperitoneal administration of PTZ induced convulsions with 100% mortality in the control group. The anticonvulsant drug phenobarbital (40 mg/kg) completely protected the mice (100% protection) against PTZ (90 mg/kg) induced seizures. The dose of 300 mg/kg of Gaisoom ethanolic extract showed 50% protection rate and increased the latency time (sec.) of convulsions by (128.33 ± 8.31) compared to the negative control (59.00 ± 2.53). While the dose of 600 mg/kg of Gaisoom ethanolic extract produced 33.33% protection rate and increased the latency time (sec.) of convulsions by (144.17 ± 55.03) (Table 1 and Figure 1).

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Dose</th>
<th>Protection rate against (90 mg/kg i.p) PTZ %</th>
<th>Time (Sec.) of the latency of convulsions (Mean ± SEM)</th>
<th>Animals survived (30 minutes)(n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>3 ml/kg</td>
<td>0 %</td>
<td>59.00 ± 2.53</td>
<td>0</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>40 mg/kg</td>
<td>100 %</td>
<td>Nil (no convulsions)</td>
<td>6</td>
</tr>
<tr>
<td>Gaisoom extract</td>
<td>300 mg/kg</td>
<td>50 %</td>
<td>128.33 ± 8.31</td>
<td>3</td>
</tr>
<tr>
<td>Gaisoom extract</td>
<td>600 mg/kg</td>
<td>33.33 %</td>
<td>144.17 ± 55.03</td>
<td>2</td>
</tr>
</tbody>
</table>

* P < 0.05 significant difference (compared with negative control group)
3.5 Strychnine Induced Seizures: (Ethanolic Extract)
Gaisoom Ethanolic extract at doses of 300 mg/kg and 600 mg/kg did not protect the mice against the picrotoxin induced seizures with 100% mortality but it increased the onset of convulsions compared to the negative control group (Table 3).

3.6 Maximum Electroshock Induced Seizures: (Ethanolic Extract)
The Ethanolic extract of Gaisoom at doses of 300 mg/kg and 600 mg/kg protected the mice against MES induced convulsions with protection rate of 66.67% and 83.33% respectively. It also increased the latency time of convulsions significantly compared to the negative control (Table 4 and Figure 2).

3.7 Pentylenetetrazole Induced Seizures: (Butanol Extract)
The Butanol extract of Gaisoom at doses of 300 mg/kg and 600 mg/kg protected the mice against pentylenetetrazole induced seizures with 50% and 66.7% protection rate respectively and 100% animal survival (Table 5 and Figure 3).

3.8 Pentylenetetrazole Induced Seizures: (Hexane Extract)
The Hexane extract of Gaisoom at doses of 300 mg/kg and 600 mg/kg strongly protected the mice against pentylenetetrazole induced seizures with 100% protection rate and 100% animal survival similar to the anticonvulsant drug phenobarbital at dose of 40 mg/kg (Table 6 & Figure 4).

Table 2. Effect of Gaisoom ethanolic extract and Diazepam on Picrotoxin induced seizures on mice (n = 6)

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Dose</th>
<th>Protection rate against (10 mg/kg i.p) PIC %</th>
<th>Time (Sec.) of the latency of convulsions (Mean ± SEM)</th>
<th>Animals survived (30 minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>3 (ml/kg)</td>
<td>0%</td>
<td>373.83 ± 23.98</td>
<td>0</td>
</tr>
<tr>
<td>Diazepam</td>
<td>0.5 mg/kg</td>
<td>50%</td>
<td>514.17 ± 247.18</td>
<td>5</td>
</tr>
<tr>
<td>Gaisoom extract</td>
<td>300 mg/kg</td>
<td>0%</td>
<td>418.83 ± 33.98</td>
<td>0</td>
</tr>
<tr>
<td>Gaisoom extract</td>
<td>600 mg/kg</td>
<td>0%</td>
<td>374.67 ± 40.73</td>
<td>0</td>
</tr>
</tbody>
</table>

* P < 0.05 significant difference (compared with negative control group)

Table 3. Effect of Gaisoom Ethanol Extract and phenobarbital on strychnine induced seizures on mice (n = 6)

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Dose</th>
<th>Protection rate against STR %</th>
<th>Time (Sec.) of the latency of convulsions (Mean ± SEM)</th>
<th>Animals survived (30 minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>3 ml/kg</td>
<td>0%</td>
<td>156.33 ± 27.92</td>
<td>0</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>40 mg/kg</td>
<td>100%</td>
<td>Nil (no convulsions)</td>
<td>6</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>300 mg/kg</td>
<td>0%</td>
<td>285.50 ± 41.18 *</td>
<td>0</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>600 mg/kg</td>
<td>0%</td>
<td>214.83 ± 20.82</td>
<td>0</td>
</tr>
</tbody>
</table>

* P < 0.05 significant difference (compared with negative control group)
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3.9 HPLC Fingerprints of Gaisoom Extract Fractions

HPLC method is powerful importance for qualitative and quantitative analysis of phytochemical consentient of herbal drugs. The active entity was detected in this crude extract or fraction is carried out which make the isolation process simple. The HPLC fingerprints can be used as benchmarks for the purpose of comparison when doing the qualitative and quantitative analysis of unknown compounds present in Gaisoom extracts. HPLC analysis was performed using two different gradients of mobile phase in same run times.

Methods for analysis: Two HPLC methods using different mobile phases selected on the basis of varying gradations of two solvents in specific retention times and elute detections were used.

It was found that UV detection was marked at wavelengths of 210 nm and 400 nm using a flow rate of 1.4 ml/min.

The isocratically elution for Hexane and Chloroform fractions was started with 50% A for 5 minutes followed by Gradient elution to100% eluent A for next 20 minutes and then increasing B to 50% for next 10 minutes.

In Hexane fraction chromatogram, the major peaks appeared between 15.30 and 22.10 minutes of run.

In Chloroform fraction chromatogram, the major peaks appeared at 14 and 18.4 minutes, and small peaks were obtained between 18.30-20.10 minutes of run.

The isocratically elution for Ethyl acetate and Butanol fractions was started with 5% A for 5 minutes followed by Gradient elution to100% eluent A for next 20 minutes and then increasing B to 5% for next 10 minutes.
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Figure 5. HPLC chromatogram of Gaisoom hexane fraction.

Figure 6. HPLC chromatogram of Gaisoom chloroform fraction.

Figure 7. HPLC chromatogram of Gaisoom ethyl acetate fraction.

Figure 8. HPLC chromatogram of Gaisoom butanol fraction.
In Ethyl acetate fraction chromatogram, the major peaks appeared at 14.30 and 19.50 minutes, and small peaks were obtained between 20.00-25.00 minutes of run.

In Butanol fraction chromatogram, the peaks appeared at 3.30 and 14.00 minutes of run.

4. Discussion

There are several plants used for the treatment of epilepsy in traditional medicine and have shown activity when tested for antiepileptic activity which will be of value to reduce the side effects and cost of synthetic anticonvulsant drugs.

Gaisoom is commonly used in the folk medicine in Saudi Arabia for the treatment of various diseases; therefore the aim of the present study was to evaluate its anticonvulsant activity and to prove the claim for its use in traditional medicine to treat various CNS disorders.

The results of the current study showed that the ethanolic extract of Gaisoom at doses of 300 mg/kg and 600 mg/kg has anticonvulsant activity in pentylentetrazole and maximum electroshock (MES) induced seizures models on mice (Table 1 and 4).

Table 5 indicates that the Hexane extract of Gaisoom at doses of 300 mg/kg and 600 mg/kg strongly protected the mice against pentylentetrazole induced seizures with 100 % protection rate and 100 % animal survival similar to the anticonvulsant drug phenobarbital at dose of 40 mg/kg.

The experiment was conducted in two phases. First, evaluation of the anticonvulsant activity of the ethanolic extract of Gaisoom in 5 models and later evaluation of the anticonvulsant activity of the butanol and hexane extract of Gaisoom against pentylentetrazole induced seizures.

The MES and PTZ are used for evaluation of antiepileptic activity in human generalized tonic – clonic seizures and absence seizures respectively. Moreover; MES and PTZ induced seizures are associated with oxidative damage.

MES induced seizures are prevented by drugs that blocks voltage gated sodium channels. Whereas the drugs that block T-type Ca\(^{2+}\) current in thalamus or the drugs which possess GABA\(_A\) agonistic activity prevents PTZ induced convulsions.

The strong activity of the hexane extract of Gaisoom against PTZ induced seizures may be due to a GABA\(_A\) agonistic activity of Gaisoom hexane extract. It is reported that pentylentetrazole induces seizure by the inhibition of GABA\(_A\) receptors and is widely used model for absence seizure.

The activity of Gaisoom against both PTZ and MES induced convulsions could be due the antioxidant activity of Gaisoom because it has been reported that Achillea fragrantissima exhibited antioxidant activity.

If we compare Hexane and Chloroform chromatograms have bear a resemblance to each other. Whereas in case of Ethyl acetate and Butanol, the chromatograms resemble each other but anticonvulsant activity of Hexane and Butanol differs totally. For this purpose further chemical studies should be directed in detail to discover the compounds in each fraction along with crude sample which will open the gates for pharmacological screening of pure compounds.

5. Conclusion

The present study concluded that Achillea fragrantissima possess a potent anticonvulsant activity against PTZ induced seizures which may be due to GABAA agonistic activity and/or antioxidant activity. Further studies are required to explain the exact mechanism of action by which Achillea fragrantissima act as an anticonvulsant agent. Additional chemical studies could be directed on crude sample and fractions of organic solvents.

6. References

5. Hegde K, Thakker SP, Joshi AB, Shastry CS, Chandrashekhar KS. Anticonvulsant activity of Carissa carandas Linn.