Antiepileptic potential of *Anisomeles indica* (Linn.) Kuntze aerial parts in pentylenetetrazole-induced experimental convulsions in Wistar rats

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Received 5 October 2012; revised 3 June 2013

The chloroform (4.20% w/w), ethyl acetate (4.23% w/w) and aqueous decoction (12.11% w/w) extracts of the aerial parts of *A. indica* were screened for the antiepileptic activity against maximal electroshock (MES) model and pentylenetetrazole (PTZ) model, respectively. Further, ethyl acetate extract (active extract) was fractionated into flavonoid and tannin fraction, which were subsequently evaluated for the antiepileptic potential against both MES and PTZ models at a dose of 50 mg/kg, po. Pretreatment with ethyl acetate extract 200, 400 mg/kg, po, for 1 week showed significant antiepileptic activity against PTZ induced convulsions only. Isolated flavonoid fraction showed more potent antiepileptic activity as compared to ethyl acetate extract, without any neurotoxic effect. However, tannin fraction did not produce antiepileptic activity against PTZ induced convulsions. It may be concluded that the flavonoids fraction of ethyl acetate extract of aerial parts of *A. indica*, but not the aqueous decoction has antiepileptic potential, without producing neurotoxic effects.

**Keywords:** *Anisomeles indica*, Epilepsy, Flavonoids, Maximal electroshock, Pentylenetetrazole

Epilepsy is a common neurological disease that causes disturbances of the normal electrochemical functions of the brain. Epilepsy, like a seizure, is a symptom of abnormal brain function\(^1\). About 0.5–1% of world population in the developed world suffer from epilepsy and due to higher risk factors the figures are higher in the developing world\(^2\). Various antiepileptic drugs are available in market, but the side effects and the drug interactions are major problems associated with their clinical utility. Herbal medicines are widely used globally due to their wide acceptance, therapeutic effectiveness with minimum side effects\(^3\). *Anisomeles* is a genus of herbs or under shrubs distributed in tropical Asia and Australia. *Anisomeles indica* (Linn.) Lamiaceae is an erect herb or woody undershrub found throughout India up to an altitude, 1800 m as a weed in waste places, roadsides and forests clearing\(^4\).

Ethnobotanically the herb possesses aromatic, astringent, carminative and tonic properties and is employed as a cure in gastric catarrh and intermittent fevers. A decoction of the herb is used to treat convulsions\(^5\). The essential oil present in the herb is useful in urine infection\(^4\). The leaves are used as anti-inflammatory\(^5\), antiseptic, antibiotic\(^6\), stomachache\(^3\) and to cure cough and cold\(^7\). AI is well documented to possess phytoconstituents such as glycosides, flavonoid, terpenoids, and steroids\(^9\). Earlier work on a species of *Anisomeles* i.e *Anisomeles malabarica* has already been proved as antiepileptic\(^10,11\). Therefore, the present study has been undertaken to evaluate the *A. indica* for its possible antiepileptic potential.

**Materials and Methods**

**Collection and identification of plant material**—The aerial parts of *Anisomeles indica* were procured along with authentication certificate from Dr. K. Madhava Chetty, Asst. Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The material was washed, shade dried, powdered, passed through sieve no. 60 and stored in air tight containers for experimental work.

**Preparation of extracts and fractions**—The decoction of aerial plant material (100 g) was prepared\(^12\). Further, the plant material (500 g) was defatted by continuous hot extraction process using soxhlet apparatus with petroleum ether (40-60 °C) and then the dried marc was extracted with chloroform.
and ethyl acetate by successive extraction technique for 72 h. Both extracts and decoction were concentrated in rotary evaporator (Medica Instrument, Mumbai, India) under vacuum and dried over anhydrous sodium sulphate. The extracts were stored in a vacuum desiccator till further use. The dried ethyl acetate extract (21.15 g) was suspended in water and treated with chloroform. After removal of impurities, 10% NaCl solution was added drop wise to precipitate out the tannin by centrifugation (2.46 g). The supernatant liquid was partitioned with ethyl acetate and the solvent was evaporated to get flavonoid fraction (2.18 g)\(^1\).

**Preliminary phytochemical screening and estimation of total phenolics content and total flavonoid content of chloroform and ethyl acetate extracts**—The extracts were subjected to preliminary qualitatitative chemical screening\(^2\). Estimation of total phenolic content was done by the Folin Ciocalteu’s method and flavonoid content was determined by aluminium chloride colorimetric assay, using gallic acid and quercetin as standards respectively\(^3\).

**Thin layer chromatography (TLC) and HPTLC of the flavonoid fraction and EA extract**—Analytical thin layer chromatography of the chloroform, ethyl acetate extract, flavonoid and tannin fraction was performed on pre-coated silica gel 60 F254 aluminium sheets (E-Merck) and R\(_f\) values were calculated\(^4\). HPTLC profile of flavonoid fraction was performed on precoated aluminium plates of E-Merck by spotting of the sample and standard. Flavonoid fraction was compared by R\(_f\) values and spectral comparison with various flavonoid standards i.e quercetin, rutin, and narigenin. The flavonoid fraction (sample) and standard flavonoid were dissolved in methanol and diluted to 100 µg/mL concentration for the application of the test and standard on precoated aluminum plates by help of Linomat 5 applicator. Twin trough chambers were used for the development of the HPTLC plate. Solvent system was toluene:ethyl acetate:formic acid at a ratio of (5:3.5:0.1). Bands of flavonoid fraction and standard flavonoid were visualized at 254 and 366 nm, respectively in Camag U.V cabinet and scanned in Camag TLC scanner.

**Experimental animals**—Adult male and female Wistar rats (200-250 g body weight) obtained from the Animal House of, ISF College were housed in groups of 2-3 animals per cage and maintained at an ambient temperature (25±1 °C), 50±10% RH, and 12:12 h dark: light cycle. The experiments were carried out during 10.00-13.00 hrs. Animals had free access to food and water. The experimental protocol was approved by the Institutional Animal Ethics Committee. Experiments were conducted according to the guidelines of the Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), Government of India.

**Drugs and chemicals**—Pentylenetetrazole (PTZ) quercetin, rutin, and narigenin were purchased from Hi-Media Laboratories, Mumbai, India. Phenytoin was obtained from Sun pharmaceuticals Ltd. (India) and diazepam and CMC were from Ranbaxy Laboratories (India) obtained. All standard, chemicals used in the study were freshly prepared and were of analytical grade.

**Drug administration**—Phenytoin (25 mg/kg, ip) and Diazepam (2 mg/kg, ip) were used as standards\(^5\). All the extracts and fractions were suspended in 0.5% CMC and diluted with normal saline. The drug was administered orally 1 h before the induction of seizures in both MES and PTZ models.

**Maximal electroshock seizure (MES) model**—The development of generalized tonic-clonic seizures has been reported to be induced by applying current (150 mA, for 0.2 s) through corneal electrodes using electroconvulsometer (INCO, Ambala, India). Different parameters such as tonic flexion, tonic hindlimb extensor (THE) and extensor/flexion ratio were measured to assess the effectiveness of the different extracts, and the standard drug\(^6\).

**Pentylenetetrazole (PTZ) induced convulsions**—The absence seizures were induced by administration of PTZ at a dose of 50 mg/kg, ip once 1 h after the last dose of the drug extracts on the 7th day\(^7\). To assess the antiepileptic activity of different extracts, fractions and standard drug parameters observed after PTZ administration included, number of jerks, onset of jerks, onset and duration of generalized tonic flexion, onset and duration of generalized tonic extensor.

**Assessment of locomotor activity**—The locomotor activity is an index of wakefulness (alertness) of mental ability and can be used in the assessment of the sedative effect of the drugs. The locomotor activity was assessed by using digital actophotometer (INCO, Ambala, India). Each animal was observed over a period of 5 min in a square (30 cm) closed arena equipped with infrared light-sensitive.
photo cells and values expressed as counts per 5 min. The light beams cut by the animal in actophotometer were taken as measure of movements. The apparatus was placed in a dark, sound-attenuated and ventilated testing room\textsuperscript{11,18}. Locomotor activity was evaluated after 1 day (flavonoid and tannin fraction) and 7 days of treatment with different extracts but before inducing convulsions by PTZ and MES.

Assessment of rotarod performance—The animals were placed on the rotarod after 50 min of the drug administration and the latency to fall from the rotarod was noticed\textsuperscript{18}. Rotarod activity was evaluated after 1 day (flavonoid and tannin fraction) and 7 days (ethyl acetate extract) of treatment with different extracts but before inducing convulsions by PTZ and MES. Rats which were able to remain on the rotating rod, with a speed of 15 rpm for 3 min or more were selected and divided in different groups, each group containing 6 animals.

Experimental design—Two (MES and PTZ) experimental protocols were employed, each consist of 8 groups and each group having 6 rats. The treatment was given 1 h before induction of generalized tonic-clonic convulsions by corneal electroconvulsometer in MES model and induction of absence seizure by chemoconvulsant PTZ.

Protocol-I (MES model):
The animals were divided into following 8 groups:
Group I: (vehicle control); group II: phenytoin (25 mg/kg, ip) treated; group III: chloroform extract (200 mg/kg, po once) treated; group IV: chloroform extract (400 mg/kg, po once) treated; group V: chloroform extract (400 mg/kg, po 1 week) treated; group VI: ethyl acetate extract (200 mg/kg, po once) treated; group VII: ethyl acetate extract (400 mg/kg, po once) treated; group VIII: ethyl acetate extract (400 mg/kg, po 1 week) treated.

Protocol-II (PTZ model):
The animals were divided into following 8 groups:
Group I: (vehicle control); group II: diazepam (2 mg/kg, ip) treated; group III: ethyl acetate extract (200 mg/kg, po 1 week) treated; group IV: ethyl acetate extract (400 mg/kg, po 1 week) treated; group V: chloroform extract (200 mg/kg, po 1 week) treated; group VI: chloroform extract (400 mg/kg, po 1 week) treated; group VII: flavonoid fraction (50 mg/kg, po once) treated; group VIII: tannin fraction (50 mg/kg, po once) treated.

Acute toxicity studies—Single dose administration of ethyl acetate and chloroform extract of aerial parts of AI (2000 mg/kg) was given to adult rats (n=3) using oral rat feeding catheter. Then, the behavioural manifestations and mortality were observed up to 14 days\textsuperscript{29}.

Statistical analysis—The behavioural data obtained in the present study were analyzed using one-way ANOVA followed by Tukey’s test and results were expressed as mean ± SD, $P < 0.05$ was considered statistically significant.

Results

Preliminary phytochemical analysis—The qualitative chemical tests revealed the presence of triterpenoids in chloroform extract. Tannin and flavonoid were found to be present in ethyl acetate extract whereas decoction shown the presence of phenolics and carbohydrates.

TLC and HPTLC profile of flavonoid fraction—Chloroform extract of AI showed 8 spots in TLC with mobile phase using toluene: methanol (4.5:0.5). Ethyl acetate, extract showed 9 spots with toluene:chloroform:methanol (4:4:1) and presence of quercetin flavonoid has been confirmed by superimposed UV spectra.

Effect of chloroform and ethyl acetate extracts on MES induced convulsions in rats—Administration of chloroform and ethyl acetate extracts (200, 400 mg/kg, po once) was failed to produce any significantly effect on tonic flexion, tonic hind limb extensor and extensor/flexion ratio as compared with vehicle control. Further, both the extracts i.e chloroform and ethyl acetate extract (400 mg/kg, po for 1 week) treatment also did not significantly affected any phase of MES model (Table 1).

Effect of chloroform, ethyl acetate extract, flavonoid and tannin fraction on PTZ induced seizures—The chloroform extract (200, 400 mg/kg, for po 1 week) treated group of animals did not show any significant activity, as compared with vehicle control, but ethyl acetate extract (200, 400 mg/kg, for po 1 week) treatment significantly showed effect on all phases, onset and number of jerks, onset and duration of generalized tonic flexion and onset and duration of generalized tonic extensor. Further, the flavonoid fraction of ethyl acetate extract (50 mg/kg, po, once), but not tannin fraction (50 mg/kg, po, once) showed significant ($P< 0.05$) effect on PTZ treated rats as compared with vehicle control and ethyl acetate extract (Table 2).
Effect of chloroform, ethyl acetate extract, flavonoid, and tannin fraction on locomotor activity and rotarod performance—Administration of chloroform and ethyl acetate extract (400 mg/kg, po for 1 week) treated and flavonoid, and tannin fraction (50 mg/kg, po, once) treated didn’t significantly affect locomotion and grip strength as compared to vehicle (0.5% CMC, po) control (Table 3).

Acute toxicity studies—The single dose of chloroform and ethyl acetate extract of AI did not produce any behavioral or toxic manifestations at the highest dose (2000 mg/kg) of both ethyl acetate, and chloroform extracts.

### Table 1—Effect of chloroform extract and ethyl acetate extract on tonic flexion, tonic hind limb extensor (THE) and E/F ratio [Values are mean ± SD from 6 rats each]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tonic flexion (sec)</th>
<th>THE(sec)</th>
<th>E/F ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>2.50±0.46</td>
<td>5.16±0.62</td>
<td>2.25±0.64</td>
</tr>
<tr>
<td>PHN (25 mg/kg, ip once)</td>
<td>1.21±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.39±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.90±0.55</td>
</tr>
<tr>
<td>CE (200 mg/kg, po once)</td>
<td>2.80±0.52</td>
<td>5.24±0.68</td>
<td>1.90±0.55</td>
</tr>
<tr>
<td>CE (400 mg/kg, po once)</td>
<td>2.80±0.36</td>
<td>5.76±1.02</td>
<td>1.96±0.59</td>
</tr>
<tr>
<td>CE (400 mg/kg, po 1 week)</td>
<td>2.61±0.68</td>
<td>4.82±0.75</td>
<td>1.86±0.50</td>
</tr>
<tr>
<td>EAE (200 mg/kg, po once)</td>
<td>2.49±0.81</td>
<td>4.92±0.59</td>
<td>2.18±0.73</td>
</tr>
<tr>
<td>EAE (400 mg/kg, po once)</td>
<td>2.23±0.57</td>
<td>5.13±0.83</td>
<td>2.45±0.57</td>
</tr>
<tr>
<td>EAE (400 mg/kg, po 1 week)</td>
<td>2.15±0.79</td>
<td>4.95±1.15</td>
<td>1.86±0.52</td>
</tr>
</tbody>
</table>

CE=chloroform extract; EAE=ethyl acetate extract; MES=maximal electroshock; PHN=phenytoin; VC=Vehicle control <sup>a</sup>P < 0.05 vs VC

### Table 2—Effect of chloroform extract, ethyl acetate extract (200, 400 mg/kg, po for (1 week) and flavonoids, and tannins fraction (50 mg/kg, po, once a day) on PTZ induced convulsion in rats [Values are mean ± SD from 6 rats each]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Myoclonic jerks</th>
<th>Generalized tonic flexion(s)</th>
<th>Generalized tonic extensor(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Onset(s)</td>
<td>duration</td>
<td>onset</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>78.77±7.71</td>
<td>41.33±2.58</td>
<td>166.70±3.80</td>
</tr>
<tr>
<td>DIA (2 mg/kg, ip)</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CE (200 mg/kg, po 1 week)</td>
<td>74.79±7.28</td>
<td>35.16±2.92</td>
<td>166.28±5.36</td>
</tr>
<tr>
<td>CE (400 mg/kg, po 1 week)</td>
<td>70.45±12.16</td>
<td>37.33±2.65</td>
<td>160.57±3.99</td>
</tr>
<tr>
<td>EAE (200 mg/kg, po 1 week)</td>
<td>155.79±16.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.66±1.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>288.12±10.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EAE (400 mg/kg, po 1 week)</td>
<td>244.67±23.60&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.33±1.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>418.72±9.96&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>FF (50 mg/kg, po once)</td>
<td>537.9±15.32&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>3.66±1.03&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>TF (50 mg/kg, po once)</td>
<td>71.59±9.16</td>
<td>38.33±3.26</td>
<td>158.28±5.95</td>
</tr>
</tbody>
</table>

CE= chloroform extract; DIA= Diazepam; EAE= ethyl acetate extract; FF= flavonoid fraction; TF= tannin fraction; VC= vehicle control; P values: <sup>a</sup>P < 0.05 vs VC; <sup>b</sup>P < 0.05 vs EAE 200mg/kg; <sup>c</sup>P < 0.05 vs EAE

**Effect of chloroform, ethyl acetate extract, flavonoid, and tannin fraction on locomotor activity and rotarod performance**—Administration of chloroform and ethyl acetate extract (400 mg/kg, po for 1 week) treated and flavonoid, and tannin fraction (50 mg/kg, po, once) treated didn’t significantly affect locomotion and grip strength as compared to vehicle (0.5% CMC, po) control (Table 3).

**Acute toxicity studies**—The single dose of chloroform and ethyl acetate extract of AI did not produce any behavioral or toxic manifestations at the highest dose (2000 mg/kg) of both ethyl acetate, and chloroform extracts.

### Table 3—Effect of chloroform, ethyl acetate extract (400 mg/kg, po for 1 week), and flavonoids, tannins fraction (50 mg/kg, po once), on rotarod performance and locomotor activity of rats [Values are mean ± SD from 6 rats each]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Locomotor activity (counts)</th>
<th>Rotarod activity(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>172±4.42</td>
<td>154.66±11.96</td>
</tr>
<tr>
<td>Diazepam (2 mg/kg, ip)</td>
<td>42.83±7.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.83±4.62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CE (400 mg/kg, po 1 week)</td>
<td>152.0±7.61</td>
<td>141.33±12.61</td>
</tr>
<tr>
<td>EAE (400 mg/kg, po 1 week)</td>
<td>156.33±7.08</td>
<td>146.66±5.92</td>
</tr>
<tr>
<td>FF (50 mg/kg, po once)</td>
<td>161.83±4.35</td>
<td>143.66±5.00</td>
</tr>
<tr>
<td>TF (50 mg/kg, po once)</td>
<td>159.83±7.54</td>
<td>146.33±8.16</td>
</tr>
</tbody>
</table>

CE= Chloroform extract; EAE= Ethyl acetate extract; FF= Flavonoids fraction; TF= Tannins fraction; VC= Vehicle control <sup>a</sup>P < 0.05 vs VC
Discussion

Another species of genus Anisomeles i.e. Anisomeles malabarica (AM) has antiepileptic potential against both MES and PTZ induced convulsions in rats\(^1\). Further the flavonoid fraction of AM proved to be responsible for antiepileptic potential against both MES and PTZ induced convulsions in rats. However, the effective dose was also shown to produce a significant neurotoxic side effects\(^1\). Ethnobotanically the decoction of plant AI is used to treat convulsions. Therefore, initial preliminary studies were conducted to evaluate the antiepileptic potential of decoction obtained from AI aerial parts. Neither acute nor chronic treatment of decoction produced any significant effect in both PTZ and MES model (unpublished data). Therefore aerial parts of plant were subjected to successive extraction scheme for the present study.

Chloroform and ethyl acetate extract (200, 400 mg/kg, po) in both acute and chronic treatment were found to be ineffective in MES model (Table 1). On other hand, one week treatment with ethyl acetate extract, but not chloroform extract (200, 400 mg/kg, po) selectively produced significant antiepileptic effects against PTZ induced convulsions (Table 2). On the basis of phytochemical screening profile of the ethyl acetate extract it was further fractionated into flavonoids and tannin fractions and was subsequently screened selectively using PTZ model for its antiepileptic potential. Interestingly single dose treatment with flavonoid fraction (50 mg/kg, po) but not tannin at a dose (50 mg/kg, po) significantly affected the onset and number of myoclonic jerks, and completely abolished the onset and duration of generalized tonic flexion, and generalized tonic extensor phases induced by PTZ, as compared to vehicle control, indicating that fractionation of ethyl acetate extract has potentiated antiepileptic effect. It is also interesting to note that the observed antiepileptic dose of flavonoid fraction (50 mg/kg, po once) did not show any significant effect on locomotor activity and rotarod performance (Table 3).

Locomotor activity is an index of wakefulness (alertness) of mental ability and was assessed in the present study to investigate the sedative effect of the extracts and fractions. Further, rotarod test is a well established model to assess central muscle relaxation property\(^6\). The present results suggest that flavonoid fraction produced acute antiepileptic activity against PTZ induced convulsions without producing neurotoxic effects. On the other hand, the AM flavonoid fraction showed significant neurotoxic effect\(^1\). Hence the plant AI is safer to use as antiepileptic as compared with AM.

Pentylenetetrazole, a GABA receptor antagonist at a dose of (50 mg/kg, i.p.) induced onset of myoclonic jerks, onset and duration of generalized tonic extension, onset and duration of generalized tonic flexion and mortality in animals which mimic absence seizures\(^17\). Diazepam is employed as a standard drug in PTZ model reported to suppress absence seizures by potentiating GABAergic system\(^20\). Hence, the antiepileptic activity of aerial parts of AI may be due to modulation of GABAergic system. The involvement of NMDA receptor activation has shown to mediate the PTZ seizures\(^21\). Further, NMDA receptor antagonist such as agmatine, ketamine, diazocilpine and 2-amino-5-phosphonovaleric acid (APV) have been reported effective against PTZ induced convulsions\(^22,23\). Experimental evidences clearly demonstrated that flavonoids exerts antiepileptic activity by modulating the GABAergic system, as they are structurally similar to benzodiazepines\(^24,25\). Hence, the observed antiepileptic effect of FF against PTZ-induced convulsions may be due to presence of flavonoid(s) other than quercetin, which may be either potentiating the GABAergic system and/or inhibition of NMDA receptors.

Conclusion

On the basis of results of the present study, it may be concluded that both chloroform and ethyl acetate extracts of AI are completely devoid of antiepileptic activity in MES induced convulsions. Ethyl acetate extract, but not chloroform extract of AI at the employed doses (200 and 400 mg/kg, po for 1 week) selectively produce antiepileptic activity against PTZ convulsions. In addition flavonoid fraction, but not tannin fraction (50 mg/kg, po once) also produced effective antiepileptic activity against PTZ convulsions. Both ethyl acetate and flavonoid fraction failed to produce neurotoxic side effects. Hence, the results provide support for the traditional use of AI as an antiepileptic drug.

Acknowledgement

We thank the Management and Shri Parveen Garg, Hon’able Chairman, I S F College of Pharmacy, Moga, India, for facilities.
References