Anticonvulsant effect of methanolic extract and isolation of active constituents from *Cnestis ferruginea* Vahl ex DC (Connaraceae)

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**ABSTRACT**

**Background:** The root decoction of *Cnestis ferruginea* Vahl ex DC (Connaraceae) is used in traditional African medicine (TAM) in the treatment of epilepsy.

**Objectives:** This study sought to investigate the anticonvulsant effect of the methanolic root extract of *Cnestis ferruginea* (CF) in mice as well as isolation of its phytoconstituents responsible for the observed effect.

**Methods:** Anticonvulsant activity of CF (50-400 mg/kg, p.o.) was assessed using maximal electroshock- (MES), strychnine- (STR) (4 mg/kg, i.p.), picrotoxin- (PTX) (7.5 mg/kg, i.p.), bicuculline- (BIC) (2.7 mg/kg, i.p.), isoniazid- (INH) (250 mg/kg, i.p.) and yohimbine (YHB) (45 mg/kg, s.c.)- induced seizure models in mice.

**Results:** CF (50-400 mg/kg, p.o.) produced significant reduction in the duration of MES-induced seizure with peak effect 20% protection at 200 mg/kg. However, peak effect 60% protection of tonic seizure was obtained at 50 mg/kg following CF pretreatment in strychnine model. Clonazepam (0.5 mg/kg, p.o.) completely abolished picrotoxin-induced seizure while the extract produced 40, 20 and 20% protection, respectively, at 100, 200 and 400 mg/kg. More importantly, CF (100-400 mg/kg) produced 100% inhibition of bicuculline-induced seizure. CF enhanced Gamma amino butyric acid (GABA) activity as observed in significant delay in the onset of tonic/clonic convulsion in isoniazid- and yohimbine-induced seizure, respectively, which was comparable to the effect of clonazepam.

**Conclusion:** The results of study suggest that CF possesses anticonvulsant activity possibly mediated through glycinergic and GABAergic neurotransmission. The results justify the use of the extract in TAM for the treatment of epilepsy and reinforce the value of studying traditional resources as sources of new drug leads.

**Key words:** bicuculline; isoniazid; picrotoxin; strychnine; yohimbine
**RÉSUMÉ**

**Contexte:** La décoction de racine de Cnestis ferruginea Vahl ex DC (Connaraceae) est utilisé dans la médecine traditionnelle africaine (TAM) dans le traitement de l’épilepsie.

**Objectifs:** Cette étude visait à étudier l’effet anticonvulsivant de l’extrait de racine méthanolique de Cnestis ferruginea (FC) chez la souris ainsi que l’isolement de ses phytoconstituents responsables de l’effet observé.

**Méthodes:** activité anticonvulsivante de FC (50-400 mg / kg, po) a été évaluée à l’aide maximales électrochoc (MES), la strychnine (STR) (4 mg / kg, ip), PTX (PTX) (7,5 mg / kg, ip), bicuculline (BIC) (2,7 mg / kg, ip), l’isoniazide (INH) (250 mg / kg, ip) et yohimbine (YHB) (45 mg / kg, sc) - modèles de saisie induits chez la souris.

**Résultats:** FC (50-400 mg / kg, po) produit une réduction significative de la durée de MES-induites saisie avec effet maximal de protection de 20% à 200 mg / kg. Cependant, l’effet maximal de 60% la protection de tonique saisie a été obtenu à 50 mg / kg après un prétraitement FC dans le modèle de la strychnine. Le clonazépam (0,5 mg / kg, par voie orale) a complètement aboli saisie picrotoxine induite produite alors que l’extrait de 40, 20 et la protection de 20%, respectivement, à 100, 200 et 400 mg / kg. Plus important encore, CF (100-400 mg / kg) a produit 100 % d’inhibition de la saisie induite bicuculline. CF renforcé gamma amino butyrique (GABA) de l’activité observée en tant que retard important dans l’apparition de la tonique / clonique convulsion dans l’isoniazide et à la saisie induite yohimbine, respectivement, ce qui est comparable à l’effet du clonazépam.

**Conclusion:** Les résultats de l’étude suggèrent que les FC possède une activité anti convulsivant éventuellement médiée par glycinerigique et la neurotransmission GABAergique. Les résultats justifient l’utilisation de l’extrait de TAM pour le traitement de l’épilepsie et de renforcer la valeur de l’étude des ressources traditionnelles comme sources de nouvelles pistes de drogue.

**Mots clés:** bicuculline; isoniazide; Picrotoxine; strychnine; yohimbine.
INTRODUCTION
Epilepsy is a major neurological disorder and up to 5% of the world population develop epilepsy in their lifetime and it remains a major medical and social problem. Although different antiseizure medications are available to alleviate epileptic disorders in patients, however, the current antiepileptic drugs (AEDs) are associated with adverse effects, dose-related neurotoxicity and teratogenic effects, and approximately 30% of the patients continue to have seizures with current AED therapy. Therefore, investigation of new antiseizure drugs which allow more efficient control seizure and its associated problems seems imperative.

In the traditional African Medicine, roots of *Cnestis ferruginea* Vahl ex DC. (Connaraceae) are useful in the treatment of infantile illness, epilepsy, dysmenorrhoea and cough. We have previously reported the analgesic, anti-inflammatory, antidepressant, anxiolytic and nootropic effects of CF in rodents. The objective of the present study was to investigate the anticonvulsive activity of CF against different convulsants like maximal electroshock, picrotoxin, bicuculline, strychnine, isoniazid and yohimbine.

MATERIALS AND METHODS
Plant material
The dried roots of *Cnestis ferruginea* were purchased from a traditional herbal practitioner in Mushin, Lagos State, Nigeria. The botanical identification and authentication of the plant was done by Prof. J.D. Olowokudejo of the Department of Botany and Microbiology, Faculty of Science, University of Lagos, Lagos, Nigeria and Mr. Joseph Ariwaodo of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The voucher specimen of the plant was deposited at the herbarium of the Institute (FHI 108219).

Preparation of extract
Powdered root of *C. ferruginea* (5.2 kg) was loaded into a glass percolator containing methanol (20L). It was allowed to stand at room temperature (28ºC) for about 16 h (overnight). The filtrate was collected and the process of extraction was repeated five times. The combined extract was concentrated on Buchi Rotavapor at 40ºC and was further dried under vacuum pump. The weight of the extract obtained was 560 g (brownish extract).

General experimental procedures
1H NMR (300 MHz) and 13C NMR (75 MHz) were recorded in CDCl3 using Bruker Avance ARX 300MHz (Bruker, Germany) with TMS as internal standard. ESI-MS: (direct inlet, 70 eV) were recorded using Micromass Quattro II. Column chromatography was conducted using flash Si gel 60-230 µm (Amersham Pharmacia Biotech). Medium pressure liquid chromatography (MPLC) was performed with a Büchi model 688 apparatus on columns containing R18 Si gel 60 Merck 20-40 µm (Amersham Pharmacia Biotech). TLC was carried out on precoated silica gel plates 60 F254 or RP-18 F254 Plates (Merck). Spots were visualized by UV light or by spraying with H2SO4-MeOH or anisaldehyde-H2SO4 and vanillin-H2SO4 reagents.

Isolation of compounds
The extract was suspended in distilled water then partitioned between ethyl acetate and water. The ethyl acetate phase was concentrated again under reduced pressure to afford a dark brown extract (146 g). The residue was further partition between chloroform and water then n-butanol. This gave chloroform (114g), n-butanol (160g) and an aqueous fraction (140g).

The ethylacetate extract (114 g) was fractionated on column chromatography on silica gel by gradient elution with n-hexane (100%), n-hexane/EtOAC mixtures, EtOAc (100%) and CHCl3/Methanol mixtures. Fractions were collected in 150 ml portions, monitoring their profiles using TLC with aid of H2SO4/vanillin as visualizing reagent. As a result 200 fractions were obtained and combined on the basis of TLC profiles to give 10 fractions. Fractions 4 was further purified through recrystallization in MeOH/CH2Cl2 to afford stigmastanol (103 mg) (Fig. 1). Fraction 6 was basically a single spot that yielded oleanolic acid (84 mg) (Fig. 1) after recrystallization from MeOH/CH2Cl2. Fraction 8 and 9 eluted with n-hexane/EtOAc (1:4) and EtOAc (100%) were combined and subjected to column chromatography on silica gel to furnish 10 fractions. Purification of sub-fraction 4 yielded Ursolic acid (4) (80 mg) (Fig. 1) as white crystalline needles in methanol. Further purification of fraction 10 (3 g) on R-18 column chromatography using water-methanol (from 100:0 to 0:100 gradients) on MPLC resulted into 5 fractions. Fractions 4 furnished stigmastrol3-O-α-D-glucopyranoside (5) (87 mg) (Fig. 1) as a white amorphous solid from CH2Cl2/n-hexane mixture. Acid hydrolysis stigmastrol3-O-α-D-glucopyranoside; a
portion of the compound (50 mg) was dissolved in MeOH (10 ml) containing 2N HCl (10 ml) and refluxed on boiling water bath for 6 h. After concentration at reduced pressure, the reaction product was diluted with water and extracted with ethyl acetate. The aqueous phase was neutralized with Ag₂CO₃, filtered and evaporated in vacuo to give a whitish residue. It was identified as glucose by co-TLC with an authenticated sample (n-BuOH:AcOH:H₂O; 4:1:5) and visualizing the spots with aniline phthalate reagent. The observed optical rotation of the glycone (sugar) [α]₀ = +41.6°C (0.5 in H₂O, 26°C) revealed that it was D-glucose.

![Compounds isolated from methanolic root extract of C.ferruginea. Compounds 1-5 were identified as stigmasterol (1) (Jamal et al., 2009), oleanolic acid (2) (Seebacher et al., 2003), ursolic acid (3) (Seebacher et al., 2003), betulinic acid (4) (Chandramu et al., 2003) and stigmastrol-3-O-β-D-glucopyranoside (5) (Mandal et al., 2006) by spectroscopic methods and comparison with the literature data.](image)

**Fig. 1:** Compounds isolated from methanolic root extract of *C.ferruginea*. Compounds 1-5 were identified as stigmasterol (1) (Jamal et al., 2009), oleanolic acid (2) (Seebacher et al., 2003), ursolic acid (3) (Seebacher et al., 2003), betulinic acid (4) (Chandramu et al., 2003) and stigmastrol-3-O-β-D-glucopyranoside (5) (Mandal et al., 2006) by spectroscopic methods and comparison with the literature data.

**Laboratory animals**

Albino mice (2025 g) and rats (100-200 g) of either sex used in this study were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria. The animals were kept in well-ventilated and hygienic compartments, maintained under standard environmental conditions and fed with standard rodent pellet (Livestock Feed PLC, Lagos, Nigeria) and water ad libitum. The experimental procedures adopted in this study were in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research. Equal numbers of male and female animals were used in all the groups in this study.

**Drugs**

Phenytoin (Acme formulation pvt. Ltd, India), carbamazepine, clonazepam, strychnine, yohimbine, bicuculline, (Sigma Aldrich, USA), isoniazid (Macleods Pharmaceuticals Ltd, Mumbai, India).

**Anticonvulsant activity**

**Maximal electroshock-induced seizure**

The method described by Swinyard and Kupferberg as modified by Sayyah et al was used in the study. Forty mice were randomly divided into eight groups (n = 5). Group I = normal saline (10 ml/kg, p.o), group II-V = CF (50, 100, 200 and 400 mg/kg, p.o.), respectively, and group VI-VIII = clonazepam (0.5 mg/kg, p.o.), carbamazepine (50 mg/kg, p.o), phenytoin (20 mg/kg, p.o.), respectively. One hour after saline or CF administration, an electrical stimulus, was applied through ear clip electrodes connected to the pineal of the mice using Ugo Basile electroconvulsive machine (CAT no 57800-001). The shock duration, frequency, current and pulse width were set and maintained at 90 s, 200 pulse/s, 48mA and 1 ms, respectively. A current of about 48 mA was applied to induce acute generalized tonic-clonic seizure which produced seizures in 99% of the negative control mice was used throughout the
study. The ability to prevent this feature or prolong the latency and/or onset of the tonic hind limb extension was considered as an indication of anticonvulsant activity.

**Strychnine-induced seizure**
Mice of either sex were randomly allotted to 5 groups (n = 8). Group I = normal saline (10 ml/kg, p.o), group II-IV = CF (50, 100, 200 mg/kg, p.o) and group V = clonazepam (0.5 mg/kg, p.o). 60 min post drug treatment, animal was given strychnine (4 mg/kg, i.p). Onset and duration of seizure were recorded. Mice that did not convulse 30 min after strychnine administration were considered to be protected.

**Picrotoxin-induced seizure**
Albino mice of either sex were randomly allotted to 5 groups (n=8). Group I = normal saline (10 ml/kg, p.o), group II-IV = CF (50, 100, 200 mg/kg, p.o), and group V = clonazepam (0.5 mg/kg, p.o). Picrotoxin (7.5 mg/kg, i.p) was injected 60 min post drug treatments. Onset and duration of seizures were recorded. Mice that did not convulse after 30 min of picrotoxin administration were considered to be protected.

**Bicuculline-induced seizure**
Albino mice of either sex were randomly allotted to 5 groups (n=8). Group I = normal saline (10 ml/kg, p.o), group II-IV = CF (50, 100, 200 mg/kg, p.o), and group V = clonazepam (0.5 mg/kg, p.o). Bicuculline (2.7 mg/kg, i.p) was administered 1 h after drug administration. The time to onset of clonic or tonic seizure was recorded. Animals that did not have seizures within a 30 min observation period were declared protected.

**Isoniazid-induced seizure**
Albino mice of either sex were randomly allotted to 8 groups (n=8). Group I = normal saline (10 ml/kg, p.o), group II-IV = CF (50, 100, 200 mg/kg, p.o), and group V-VII = clonazepam (0.5 mg/kg, p.o), phenytoin (20 mg/kg, p.o), carbamazepine (50 mg/kg, p.o), respectively. 1 h post-administration animals were given isoniazid (INH) (250 mg/kg, i.p). The latency for onset of clonic-tonic seizures and death was noted after the administration of INH during a 2 h test session.

**Yohimbine-induced clonic seizure in mice**
Yohimbine hydrochloride (45 mg/kg, s.c, n=8) was administered one hour after oral administration of normal saline (10 ml/kg), clonazepam (0.5 mg/kg), CF (50200 mg/kg). Animals were observed for 60 min for the latency for onset of clonic seizures. Animals that did not exhibit at least one clonic seizure within 60 min were considered protected.

**Pentobarbitone-induced sleeping time**
Albino mice of either sex were randomly allotted to 5 groups (n=8). Group I = normal saline (10 ml/kg, p.o.), group II-IV = CF (50, 100, 200 mg/kg, p.o.), and group V = clonazepam (0.5 mg/kg, p.o.). One hour later, pentobarbitone (100 mg/kg, i.p.) was administered to each mouse in turn. The mice were placed on their backs in separate chambers and the duration of loss of righting reflex starting at the time of hexobarbitone administration until they regained their righting reflexes were recorded. When there was any doubt, the animal was placed gently on its back again and if it righted itself within 1 min, this was regarded as the end point.

**RESULTS**

**Maximal electroshock-induced seizure**
Anticonvulsant studies with CF extract showed a significant protection in MES-induced convulsion model in a dose dependent manner. There was a significant (P < 0.001) decrease in the duration of tonic hind limb extension at all the four doses of extract (50, 100, 200 and 400 mg/kg) in MES model with maximum protection observed at 200 mg/kg dose, as compared to control group. The anticonvulsant activity of the extract at 200 mg/kg was found to be comparable to carbamazepine, clonazepam and phenytoin treated group (Table 1).
Table 1: Effect of Methanolic Root Extract of *C. ferruginea* on Maximal Electroconvulsive Shock Test in Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Onset of tonic (secs)</th>
<th>Duration of seizure (secs)</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10 ml/kg</td>
<td>2.60 ± 0.40</td>
<td>105.40 ± 12.79</td>
<td>0</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>0.5</td>
<td>NC</td>
<td>78.40 ± 17.47</td>
<td>40</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>20</td>
<td>NC</td>
<td>23.960 ± 3.78***</td>
<td>100</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>50</td>
<td>NC</td>
<td>33.82 ± 5.70***</td>
<td>100</td>
</tr>
<tr>
<td>CF</td>
<td>50</td>
<td>3.60 ± 0.25</td>
<td>31.20 ± 7.69***</td>
<td>0</td>
</tr>
<tr>
<td>CF</td>
<td>100</td>
<td>3.80 ± 0.20</td>
<td>28.00 ± 3.89***</td>
<td>0</td>
</tr>
<tr>
<td>CF</td>
<td>200</td>
<td>3.60 ± 0.98</td>
<td>45.00 ± 12.46***</td>
<td>20</td>
</tr>
<tr>
<td>CF</td>
<td>400</td>
<td>5.60 ± 0.40*</td>
<td>16.20 ± 0.97***</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M, *P* < 0.05; ***P* < 0.001 versus vehicle control group (one way ANOVA followed by Dunnett’s multiple comparison post hoc test. (NC = no convulsion)

**Strychnine-induced seizure**

The extract (50–200 mg/kg) produced a dose dependent delay in time of onset of seizures (Table 2). The peak delay in the onset of seizure was produced by the extract at 50 mg/kg which was Similar to the effect of clonazepam (0.5 mg/kg).

Table 2: Effect of methanolic root extract of *C. ferruginea* on strychnine-induced seizure in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Latency to clonic seizure (mins)</th>
<th>Latency to tonic seizure (mins)</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10 ml/kg</td>
<td>5.03 ± 1.10</td>
<td>6.07 ± 1.30</td>
<td>0</td>
</tr>
<tr>
<td>CF</td>
<td>50</td>
<td>6.40 ± 0.75</td>
<td>17.60 ± 5.10*</td>
<td>40</td>
</tr>
<tr>
<td>CF</td>
<td>100</td>
<td>5.20 ± 0.73</td>
<td>8.40 ± 2.20</td>
<td>0</td>
</tr>
<tr>
<td>CF</td>
<td>200</td>
<td>5.80 ± 0.80</td>
<td>7.20 ± 0.58</td>
<td>0</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>0.5</td>
<td>14.20 ± 5.00*</td>
<td>22.60 ± 4.70*</td>
<td>60</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M, *P* < 0.05 versus vehicle control group (one way ANOVA followed by Dunnett’s post hoc multiple comparison test.

**Picrotoxin-induced seizure**

Intraperitoneal injection of picrotoxin (7.5 mg/kg) induced seizure in mice at 9.14 ± 0.99 min, 1 h post vehicle control treatment. However, oral administration of CF produced; 40, 20 and 20% protection, respectively, at 100, 200 and 400 mg/kg from picrotoxin induced convulsion. In comparison, oral administration of clonazepam (0.5 mg/kg) prevented picrotoxin induced seizure with 100% protection (Table 3).
Table 3: Effect of methanolic root extract of *C. ferruginea* on picrotoxin–induced seizure in mice

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Onset of Seizure (min)</th>
<th>% mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle 10 ml/kg</td>
<td>9.14 ± 0.99</td>
<td>100</td>
</tr>
<tr>
<td>Clonazepam 0.5</td>
<td>NC</td>
<td>0</td>
</tr>
<tr>
<td>CF 100</td>
<td>5.17 ± 0.09</td>
<td>80</td>
</tr>
<tr>
<td>CF 200</td>
<td>8.63 ± 1.06</td>
<td>20</td>
</tr>
<tr>
<td>CF 400</td>
<td>8.14 ± 1.88</td>
<td>40</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±S.E.M. P>0.05 (NC = no convulsion).

**Bicuculline-induced seizure**

Bicuculline (2.7 mg/kg, *i.p.*) induced seizure in vehicle treated group (6.00±0.71 min). Oral administration of CF completely inhibited occurrence of bicuculline-induced seizure in mice which was similar to the effect of standard antiepileptic drug (Clonazepam, 0.5 mg/kg; *p.o.*) (Table 4).

Table 4: Effect of methanolic root extract of *C. ferruginea* on bicuculline–induced seizure in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Onset of Seizure (min)</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10 ml/kg</td>
<td>6.04 ± 0.71</td>
<td>0</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>0.5</td>
<td>NC</td>
<td>100</td>
</tr>
<tr>
<td>CF</td>
<td>100</td>
<td>NC</td>
<td>100</td>
</tr>
<tr>
<td>CF</td>
<td>200</td>
<td>NC</td>
<td>100</td>
</tr>
<tr>
<td>CF</td>
<td>400</td>
<td>NC</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (NC = no convulsion).

**Isoniazid–induced seizure**

Oral administration of *C.ferruginea* produced dose dependent significant (P <0.05) increase in onset of tonic seizure from 44.40 ± 1.03 min in vehicle control treated to 66.20 ± 9.74 min with peak effect 40% protection at 200 mg/kg following subcutaneous injection of isoniazid (250 mg/kg). This effect was comparable to the anticonvulsant effect of phenytoin and carbamazepine (standard antiepileptic) each producing 60% protection with non-significant (P >0.05) increase in onset of clonic and tonic seizure. However, clonazepam (0.5 mg/kg) produced 100% protection from isoniazid induced seizure (Table 5).

Table 5: Effect of methanolic root extract of *C. ferruginea* on isoniazid-induced seizure in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Clonic (minutes)</th>
<th>Tonic (minutes)</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>10 ml/kg</td>
<td>40.20 ± 0.86</td>
<td>44.40 ± 1.03</td>
<td>0</td>
</tr>
<tr>
<td>CF</td>
<td>50</td>
<td>42.60 ± 1.44</td>
<td>56.00 ± 0.63</td>
<td>0</td>
</tr>
<tr>
<td>CF</td>
<td>100</td>
<td>49.20 ± 6.35</td>
<td>61.20 ± 4.52</td>
<td>20</td>
</tr>
<tr>
<td>CF</td>
<td>200</td>
<td>56.40 ± 7.94</td>
<td>66.20 ± 9.74*</td>
<td>40</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>0.5</td>
<td>NC</td>
<td>NC</td>
<td>100</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>20</td>
<td>46.80 ± 1.36</td>
<td>56.20 ± 3.412</td>
<td>60</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>50</td>
<td>52.80 ± 3.96</td>
<td>61.00 ± 3.08</td>
<td>60</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M, *P<0.05 versus vehicle control group (one way ANOVA followed by Dunnet's post hoc multiple comparison test.)
Yohimbine-induced seizure
Subcutaneous injection of yohimbine (45 mg/kg) induced clonic seizure in mice (38.80 ± 3.76 min) in vehicle treated control with a loss of righting reflex and 100% mortality. Initially, mice became motionless, approximately 15-20 min following injection. Oral administration of CF produced dose dependent increase in percentage protection, the peak effect was observed at 100 mg/kg (80% protection). In comparison, oral administration of clonazepam (0.5mg/kg) produced significant ($P < 0.01$) increase in onset of clonic convulsion with 100% protection (Table 6).

Table 6: Effect of methanolic root extract of *C. ferruginea* on yohimbine-induced seizure in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Onset of clonic seizure (min)</th>
<th>% Protection</th>
<th>% mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10 ml/kg</td>
<td>38.80±3.76</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>CF</td>
<td>50</td>
<td>40.20±4.28</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>CF</td>
<td>100</td>
<td>42.00±1.76</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>CF</td>
<td>200</td>
<td>48.40±8.32</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>0.5</td>
<td>62.60±1.60**</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M, *$P<0.01$ versus vehicle control group (one way ANOVA followed by Dunnet’s post hoc multiple comparison test.

Pentobarbitone–induced sleeping time
*There was no significant ($P >0.05$) difference between extract treated group (100, 200, 400mg/kg) and pentobarbitone alone normal saline treated group. However, clonazepam prolonged the sleeping time significantly ($P<0.05$).

DISCUSSION
In this study, *Cnestis ferruginea* was investigated for anti-epileptic activity using the maximal electroshock-induced seizure (MES) and chemical-induced seizures using strychnine, picrotoxin, bicuculline, isoniazid and yohimbine. The electroshock assay in mice is used primarily as an indication for compounds which are effective in grand mal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by anti-epileptics. CF extract possesses anticonvulsant activity as evidenced by decrease duration of tonic hind limb extension in MES induced convulsions. This study further confirmed the activity of currently available antiepileptic drugs that are clinically effective in the management of generalized tonic–clonic and partial seizures.

To further evaluate the anticonvulsant action of CF, strychnine-induced seizure model was used. Strychnine has been demonstrated to have a well defined mechanism of convulsant action reported to be by directly antagonizing the inhibitory spinal cord and brainstem reflexes of glycine and thus increasing spinal reflexes. The extract at the lowest dose significantly delayed the onset of convulsion which was comparable to the effect of clonazepam. The activity observed in this model suggests that CF possibly possesses compounds which interact with glycine receptors to prevent strychnine interference with postsynaptic inhibition mediated by glycine. To evaluate the possible involvement of GABA in the anticonvulsant activity of the extract, the picrotoxin and bicuculline-induced seizure models were employed. In the picrotoxin-induced seizure test, the extract was unable to inhibit seizure but it produced dose-dependent decrease in mortality. In the bicuculline-induced seizure, the extract completely inhibited seizure (100% protection). Picrotoxin and bicuculline are regarded as GABA$_A$-receptor antagonist modifying the function of the chloride ion channel of the GABA$_A$ receptor complex. This shows that the extract produced its anticonvulsant effect partly by enhancing the affinity of GABA for its receptive site. In addition, the partial antagonism of isoniazid-induced seizure by CF showed that it slightly reversed isoniazid inhibition of GABA synthesis.
being a potent monoamine oxidase (MAO) inhibitor increases the brain monoamine content, leading to CNS excitation and convulsions. INH has been shown to lower brain GABA content in rats, it reduces the GABA content in the brain by inhibiting glutamic acid decarboxylase (GAD) activity (enzyme responsible for the synthesis of GABA), by combining with pyridoxal phosphate (a coenzyme for its reactions) to form hydrazones and, thus, inhibits the GAD activity. In this study, subcutaneous injection of INH induce severe clonic-tonic convulsion in mice which was reversed by clonazepam and CF. Furthermore, results obtained in this study further confirmed the non-involvement of phenytoin and carbamazepine in GABAergic activity enhancement. The extract exerted GABA-mimetic activity at 50 and 100 mg/kg with 60 and 80% protection, respectively, in the yohimbine-induced seizure model. However, it increased the onset of clonic seizure non-significantly when compared to control.

The methanolic root extract of *C. ferruginea* had very significant antiepileptic effect on bicuculline-induced seizures and modest effect on picrotoxin-induced seizures. Isoniazid and yohimbine have been shown to interact with the GABA neurotransmitter and the GABA receptor complex. Antagonism of yohimbine and isoniazid-induced seizures therefore suggests that CF possibly have effect on GABAergic neurotransmission. Moreover, these effects seem to be related to the GABA or bicuculline sites of the GABA receptor complex. The multiplicity of putative mechanisms of action and the broad spectrum of anticonvulsant activity of CF might be due to the presence of different active components in the methanolic extract interacting simultaneously.

Chromatographic separation of the active components of CF led to the isolation of five active constituents of the plant. The compounds 1-5 (Fig. 1) were identified as stigmasterol (1), oleanolic acid (2), ursolic acid (3), betulinic acid (4) and stigmasterol-3-O-β-D-glucopyranoside (5) by spectroscopic methods and comparison with the literature data. Oleanolic acid (2), ursolic acid (3), betulinic acid (4) and stigmasterol-3-O-β-D-glucopyranoside (5) have not been reported previously in this plant. Taviano and co-workers reported that oral administration of ursolic acid produces a significant reduction in spontaneous motor activity, a potentiation of pentobarbital-induced sleeping time, a protective action against PTZ-induced convulsion probably through interaction with the GABA-ergic system. Similarly, Oleanolic acid (a triterpenes) has been reported as a potential neuroprotective agent. In addition, we had previously isolated amentoflavone from CF and it has been shown to exhibit high affinity to brain benzodiazepine receptors *in vitro*, with Ki value of 6 nM. The ability of the extract to exhibit activity against different convulsants suggests that it may act through different mechanisms to elicit its anticonvulsant effects. CF probably produces its anticonvulsant activity via glycinegic and GABAergic systems.

**CONCLUSION**

The results obtained in this study have demonstrated that *Cnestis ferruginea* possesses anticonvulsant activity in animal models and this provides a rationale for its use in traditional medicine for the management of epilepsy. This study also reinforces the value of studying traditional resources (biologic and cultural) as sources of new drug leads. However, further study is required to evaluate the possible mechanism of anticonvulsant effect of *Cnestis ferruginea*.

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**REFERENCES**


