

## EVALUATION OF THE SEDATIVE AND ANTICONVULSANT PROPERTIES OF THREE CAMEROONIAN PLANTS

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## Abstract

*Milletia thonningii*, *Ocimum sanctum* and *Securitaca longepedunculata* are used in traditional medicine in Cameroon to treat epilepsy, insomnia and headaches. Animal models of epilepsy (maximal electroshock (MES), n-methyl-d-aspartate (NMDA), pentylenetetrazol (PTZ), isonicotinic hydrazide acid (INH), picrotoxine (PIC) and strychnine (STR)-induced convulsions or turning behavior were used to evaluate anticonvulsant activity while diazepam-induced sleep test was used to evaluate sedative activity of the plants. Four doses of extracts were used for each plant (100, 200, 500 and 1000 mg/kg). At a dose of 1000 mg/kg, *Milletia thonningii* protected 60 and 90% of mice against MES and PTZ-induced convulsions, respectively. At the same dose, *Milletia thonningii* also protected 80% of mice against NMDA-induced turning behavior. At a dose of 1000 mg/kg, *Ocimum sanctum* provided complete protection against MES, PIC and STR- induced convulsions and 83.3% of protection in PTZ test. *Securitaca longepedunculata* completely protected (100%) mice in PIC test at a dose of 200 mg/kg, in MES test at a dose of 500 mg/kg and in PTZ test at a dose of 1000 mg/kg. 66.7% of mice were protected against STR-induced convulsions. All the three plants showed also sedative properties for they increased significantly and in a dose dependent manner the total sleep time induced by diazepam. The total sleep time of the control groups was multiplied by a factor of 3 at least by each extract. The presence of sedative and anticonvulsant activity in the three plants could explain their use in traditional medicine in the treatment of epilepsy and insomnia in Cameroon.

**Keywords:** Epilepsy; Insomnia; Traditional medicine.

## Introduction

*Milletia thonningii* Baker (Fabaceae) (*M. thonningii*) and *Securitaca longepedunculata* Fres (Polygalaceae) (*S. longepedunculata*) are deciduous trees of 3-10 meter high, often found in savannah and forest areas in Cameroon and other countries in Africa, Ghana, Ivory Coast, Nigeria, Senegal and Togo (Arbonnier, 2000; Hutchinson and Dalziel, 1958). *Ocimum sanctum* Lam (Lamiaceae) (*O. sanctum*) is a soudano-sahelian herb of 80 cm high also found in Africa (Geetha et al., 2004; Gupta et al., 2007). *M. thonningii*, *O. sanctum* and *S. longepedunculata* are used empirically in many countries in Africa and particularly in Cameroon to treat various diseases like epilepsy, insomnia, headaches, pains, fevers, heartaches, pneumonia, cold, stomachaches, allergy, worms, dysentery, rheumatism, jaundice, bronchitis, itch, intestinal obstruction, tuberculosis, leprosis (Abbiw, 1990; Arbonnier, 2000; Geetha et al., 2004; Gupta et al., 2007; Hutchinson and Dalziel, 1958; Irvine, 1961; Singhal et al., 1982). Knowing that in Sub-Saharan Africa, 70% of the populations are not able to pay for a modern Doctor or to buy a modern medicine, the use and the development of plants in traditional medicine are very essential in keeping the populations healthy. In addition, by using plants extracts, the side effects after treatment would be reduced, since plant extracts could possess fewer side effects (Vyawahare, et al., 2007).

Documented evidence revealed antimalarial, molluscicidal, trypanocidal, antischistosomal, insecticidal, hypotensive, myorelaxant, antidepressant, analgesic, anti-inflammatory, hypoglycemic activities (Adebiyi et al., 2006; Aderbauer et al., 2008; Ancolio et al., 2002; Belmain et al., 2001; Bhargava and Singh, 1981; Geetha et al., 2004; Jayasekara et al., 2005; Meyer et al., 2008; Ojewole, 2008; Perrett et al., 1994; Perrett et al., 1995a; Perrett et al., 1995b; Rakuambo et al., 2006). Phytochemical characterization showed that these plants contain alkaloids, coumarin, isoflavones, methyl salicylate, phenol, saponins, tannins, terpenoids, xanthenes (Ancolio et al., 2002; Asomaning et al., 1998; Jayasekara et al., 2002; Kandeda, 2007; Martinez et al., 1982; Meyer et al., 2008) (Table 1). Though a lot of pharmacological studies were done with these plants, very few were done to study their sedative and anticonvulsant activity (Adeyemi et al., 2010), for Adeyemi only worked with *S. longepedunculata*, and in addition he did not use the two main anticonvulsant tests (maximal electroshock and pentylenetetrazol) that are very predictive for the type of epilepsy. Furthermore natural health products of vegetable origin have shown promise for the prevention of chronic diseases (Haddad et al., 2005; Hazem and AlaaEldin 2008). The present study was undertaken to look for the anticonvulsant and sedative properties of the three plants used in traditional medicine in Cameroon to treat insomnia and epilepsy.

## Materials and Methods

### Plant material

A voucher specimen of each plant: *M. thonningii* (20134/SRF/Cam), *O. sanctum* (46702/HNC/Cam) and *S. longepedunculata* (10410/SRF/Cam) was authenticated and deposited at the National Herbarium of Cameroon in Yaoundé.

### Methanolic extract of *M. thonningii*

Dried seeds of *M. thonningii* were ground. 1.45 kg of powder was macerated at room temperature in 3 liters of methanol for 72 hours. The mixture was filtered with Watman n°1 filter paper and evaporated at 80°C. 230 g of the methanolic extract were obtained (yield 15.86%). The *M. thonningii* methanolic extract was diluted in dimethyl sulfoxide (DMSO) 20% in distilled water before use and administered intraperitoneally (ip).

### Ethanol extract of *O. sanctum*

The powder of dried root barks of *O. sanctum* (1200 g) was macerated for 72 hours in 3 liters of ethanol (95°C). The supernatant filtered and evaporated to dryness at 50°C with a Rota vapor gave 147 g of extract (yield: 12.25%). The extract of *O. sanctum* was diluted in distilled water before use (ip administration).

### Aqueous extract of *S. longepedunculata*

200 g of powder of dried roots of *S. longepedunculata* were macerated in 200 ml of distilled water for 48 hours at room temperature. After filtration with a Watman n°1 filter paper, the supernatant was evaporated to dryness using a Rota vapor at a temperature of 45°C. 11 g of the aqueous extract was obtained (yield: 5.5%). The extract was diluted in distilled water before use (oral administration). All the extracts were prepared according to traditional healers and chemists, and were administered 1 hour before the tests at the following doses 100, 200, 500 and 1000 mg/kg.

### Animals

Adult male mice: *Mus musculus* Swiss 22 ± 3 g, 2 months old and obtained from the animal laboratory of our University were used for this study. Animals were housed in standard cages at 25°C, on a 12/12 h light-dark cycle. They were supplied with food and water *ad libitum*. Mice were divided into 6 groups (except for the diazepam-induced sleep test that had 5 groups). One negative control group received a vehicle (distilled water or DMSO 20% in distilled water), one positive control group received an appropriate well-known anticonvulsant substance as a reference and four test groups received different doses of the plant extracts. Drugs were administered in a volume of 10 ml/kg of body weight. All animal experiments were carried out in accordance with the National (N°FWA-IRB0001954) and the United States Guide for the Care and Use of Laboratory Animals, US National Research Council (USNRC, 1996).

### Diazepam-induced sleep test

Mice were divided into five groups of 6 or 10 mice and received different treatments. Group I (negative control) was treated with distilled water or DMSO 20% in distilled water. Groups II to V (test groups) were treated with 4 doses of the plant extracts. The sleep potentiating effects of the plant extracts were studied in mice that had received diazepam (ip) at a dose of 50 mg/kg 1 hour after treatment. The time taken from the loss of the straightening reflex to its regain gave the sleeping time (Beretz et al., 1978; Ngo Bum et al., 2009ab; Rakotonirina et al., 2001). The straightening reflex (defined as a movement of the forehead that is on the same side as the stimulated ear) was obtained in the awakening mice by stimulating the external ear of the mouse with horsehair.

### Maximal electroshock (MES) test

Mice were divided into six groups of 6 or 10 mice each and received different treatments. Group I (negative control) was treated with distilled water or DMSO 20% in distilled water. Groups II to V (test groups) were treated with 4 doses of the plant extracts. Group VI treated with diazepam, 5 mg/kg ip. was used as positive control. Tonic convulsions of the hind extremities of mice were induced by passing an alternating electrical current (50 Hz, 30 mA, 0.2 s) through eye electrodes (Bernasconi et al., 1988; Lehmann et al., 1988; Ngo Bum et al., 2001; Ngo Bum et al., 2009ab; Schmutz et al., 1990; Wamil et al., 1994). The number of animals protected from tonic hind limb extension was determined in each dose group.

### N-methyl-D-aspartate (NMDA) test

Mice were divided into six groups of 10 mice and treated as above. Except that here the positive control group received 33  $\mu$ mol/kg of d-2-amino-7-phosphonoheptanoate (D-AP7). Turning behaviour was induced in mice by the subcutaneous (s.c.) injection of NMDA 75 mg/kg, 1 h after treatment's administration. Mice were observed for 30 min. Animals which did not exhibit turning behaviour within 30 min were declared protected. Turning behaviour was characterised by two consecutive 360° cycles fulfilled by the same animal (Ngo Bum et al., 2001; Ngo Bum et al., 2004; Ngo Bum et al., 2010).

### Strychnine (STR) test

Six groups of 6 or 10 mice each were treated as above. Except that the positive control group received 3 mg/kg clonazepam (ip). STR convulsions followed by death were induced in mice by the ip injection of 2.5 mg/kg STR nitrate. A protective effect of the different treatments given 1 h prior to STR was recorded. Animals which survived more than 10 min were qualified protected (Bernasconi et al., 1988; Lehmann et al., 1988; Ngo Bum et al., 2004; Ngo Bum et al., 2005).

### Pentylentetrazol (PTZ) test

Six groups of 6 or 10 mice each were treated as above. However the positive control group received 0.1 mg/kg clonazepam ip. Clonic seizures were induced in mice by the ip injection of 70 mg/kg PTZ. The protective effect of the different treatments given 1 h before PTZ injection was recorded. Animals that did not convulse within the 10 min of observation were qualified protected (Bernasconi et al., 1988; Lehmann et al., 1988; Ngo Bum et al., 2001; Ngo Bum et al., 2010; Schmutz et al., 1990; Wamil et al., 1994).

### Picrotoxine (PIC) test

Six groups of 6 or 10 mice each were treated as above. However the positive control group received 0.4 mg/kg clonazepam ip. Clonic seizures were induced in mice by the ip injection of 7.5 mg/kg PIC. Mice were observed for 15 min. A protective effect of the different treatments given 1 h before PIC-induced clonic seizures was recorded. Animals that did not convulse within 15 min of observation were qualified protected (Bernasconi et al., 1988; Lehmann et al., 1988; Ngo Bum et al., 2001; Ngo Bum et al., 2005).

### Isonicotinic hydrazide acid (INH) test

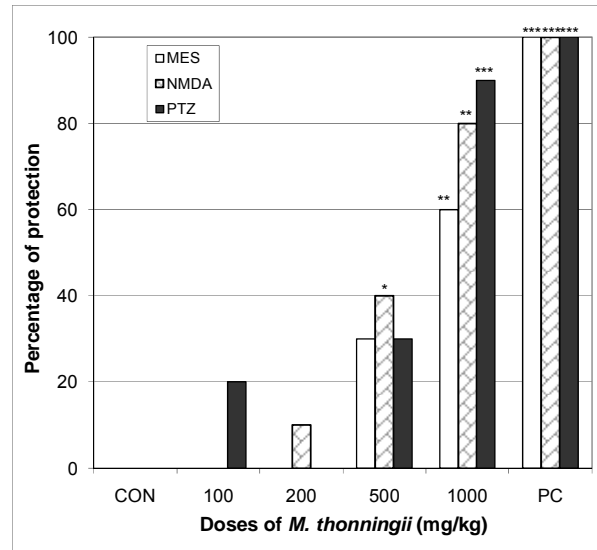
Six groups of 6 mice each were treated as above. Except that the positive control group received diazepam 10 mg/kg p.o. Animals were injected ip with INH 250 mg/kg 1 h after the different treatment's administration and the time to the onset of clonic or tonic seizures was recorded (Bernasconi et al., 1988; Ngo Bum et al., 2001; Ngo Bum et al., 2010).

### Chemicals

Clonazepam: (Rivotril®, was from Roche Pharma, Reinach Schweiz); Diazepam: (Valium®, was from Roche, Neuilly, France); d-2-amino-7-phosphonoheptanoate, Isonicotinic hydrazide acid, n-methyl-d-aspartate, pentylentetrazol, picrotoxine and strychnine: (Sigma Aldrich Inc., St Louis, MO, USA).

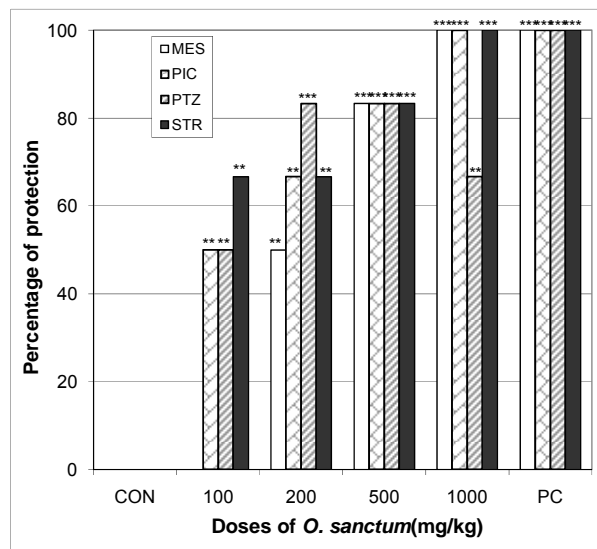
### Statistical analysis

Three parameters were measured: the protection against MES and chemically-induced seizures, the latency to the onset of seizures in INH test and the sleeping time. The percentage of protected animals were analysed using the Fisher Exact Test (two-tail). The analyses of variance (ANOVA) followed by Dunnett (HSD) were done for the latency to the onset of seizures in INH test and the sleeping time. Data were  $p < 0.05$  were qualified significant. The ED<sub>50</sub> were determined with Statgraphics Plus (confident limits at 95%).



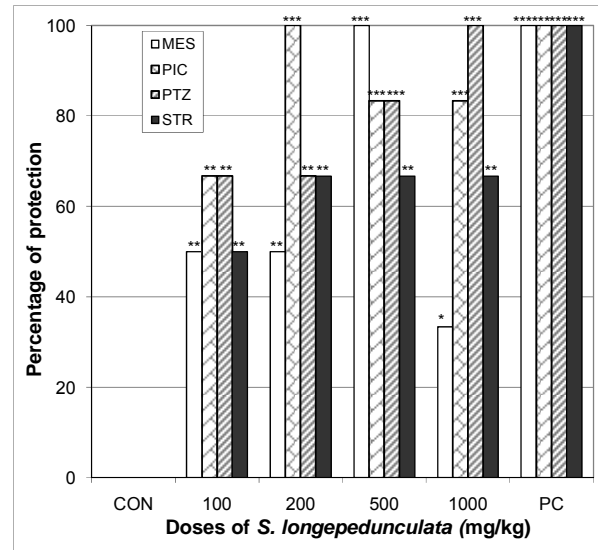
**Figure 1:** Effect of *M. thonningii* on electrically and chemically-induced convulsions in mice.

The results are expressed as the percentage of mice protected against convulsions. CON = DMSO 20% in distilled water, PC = diazepam 5 mg/kg for MES test, d-2-amino-7-phosphonoheptanoate 33  $\mu$ mol/kg for NMDA test and clonazepam 0.1 mg/kg for PTZ test. N = 10 per dose, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs CON, Fisher Exact Test (two-tail).



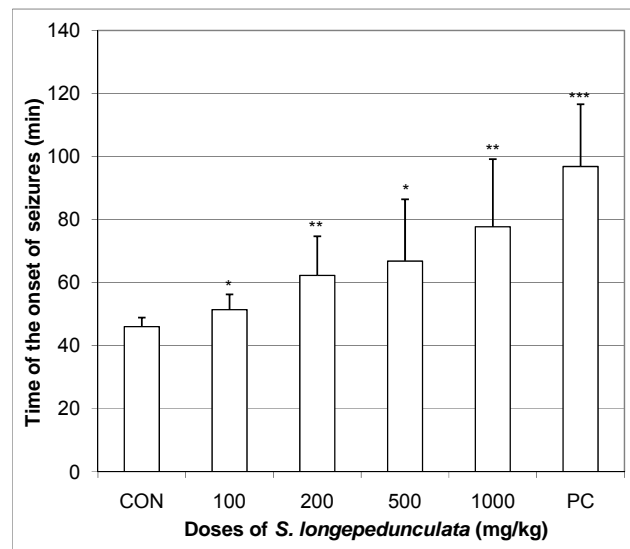
**Figure 2:** Effect of *O. sanctum* on electrically and chemically-induced convulsions in mice.

The results are expressed as the percentage of mice protected against convulsions. CON = distilled water, PC = diazepam 5 mg/kg for MES test, clonazepam 0.4 mg/kg for PIC test, clonazepam 0.1 mg/kg for PTZ test and clonazepam 3 mg/kg for STR test. N = 6 per dose, \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs CON, Fisher Exact Test (two-tail).



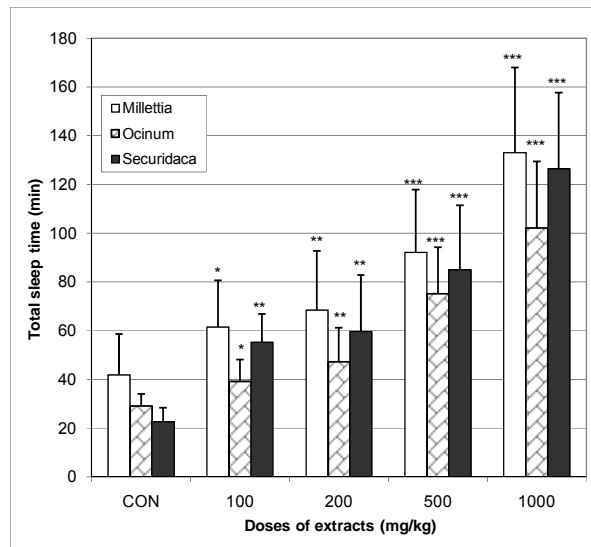
**Figure 3: Effect of *S. longepedunculata* on electrically and chemically-induced convulsions in mice.**

The results are expressed as the percentage of mice protected against convulsions. CON = distilled water, PC = diazepam 5 mg/kg for MES test, clonazepam 0.4 mg/kg for PIC test, clonazepam 0.1 mg/kg for PTZ test and clonazepam 3 mg/kg for STR test. N = 6 per dose, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 vs CON, Fisher Exact Test (two-tail).



**Figure 4: Effect of *S. longepedunculata* on INH-induced convulsions in mice.**

The results are the time (min) to the onset of seizures. They are expressed as means  $\pm$  SEM. CON = distilled water, PC = diazepam 10 mg/kg. N = 6 per dose. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 vs CON, Anova followed by Dunnett (HSD).



**Figure 5:** Effect of *M. thonningii*, *O. sanctum* and *S. longepedunculata* on diazepam-induced sleep in mice.

The results are the total sleep time (min) induced by diazepam (50 mg/kg, i.p.) in the presence of different extracts in mice. They are expressed as means  $\pm$  SEM. CON = distilled water or DMSO 20% in distilled water. N = 6 or 10 per dose. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs CON, Anova followed by Dunnett (HSD).

## Results

### Effects of *M. thonningii*

The methanolic extract of *M. thonningii* at a dose of 1000 mg/kg protected 60% of mice against MES-induced seizures ( $p < 0.05$ ) and 90% of mice against PTZ-induced seizures ( $p < 0.001$ ). *M. thonningii* also protected 80% of mice against NMDA-induced turning behavior ( $p < 0.01$ ) (Fig. 1). The  $ED_{50}$  were 646(574-717) mg/kg in NMDA test and 596(189-1004) mg/kg in PTZ test. Controversially, *M. thonningii* failed to protect mice in PIC and STR tests. In diazepam-induced sleep test, the extract of *M. thonningii* multiplied by a factor of 3 the sleeping time of the control group (from  $41 \pm 16$  min to  $133 \pm 34$  min at a dose of 1000 mg/kg) [ $F(5,44) = 94$ ;  $p < 0.001$ ] (Fig. 5).

### Effects of *O. sanctum*

The aqueous extract of *O. sanctum* protected mice in a dose dependant manner against convulsions. Like the known anticonvulsant compounds, *O. sanctum* at dose of 1000 mg/kg provided full protection against MES, PIC and STR-induced convulsions ( $p < 0.001$ ). *O. sanctum* protected 83.3% of mice in PTZ test at the doses of 200 and 500 mg/kg ( $p < 0.01$ ) (Fig. 2). The  $ED_{50}$  were 396(194-598) mg/kg in MES test, 283(27-538) mg/kg in PIC test, 308(0-705) mg/kg in PTZ test and 263(0-561) mg/kg in STR test. In diazepam-induced sleep test, the extract of *O. sanctum* multiplied by a factor of 3 the sleeping time of the control group (from  $29 \pm 5$  min to  $102 \pm 27$  min at a dose of 1000 mg/kg) [ $F(5,24) = 104$ ;  $p < 0.001$ ] (Fig. 5).

### Effects of *S. longepedunculata*

The aqueous extract of *S. longepedunculata* strongly protected mice against convulsions. Like clonazepam, *S. longepedunculata* at a dose of 200 mg/kg provided full protection against PIC-induced seizures ( $p < 0.001$ ). At doses of 500 and 1000 mg/kg, *S. longepedunculata* completely protected mice against MES and PTZ-induced convulsions ( $p < 0.001$ ) and ( $p < 0.001$ ), respectively. 66.6% of mice were protected in STR test ( $p < 0.01$ ) (Fig. 3). The  $ED_{50}$  were 351(0-824) mg/kg in MES test, 264(0-674) mg/kg in PIC test and 263(0-561) mg/kg in PTZ test. In addition, *S. longepedunculata* significantly delayed the time of the onset of seizures in INH test [ $F(6,41) = 103$ ;  $p < 0.001$ ] (Fig. 4). In diazepam-induced sleep test, the extract of *S. longepedunculata* multiplied by a factor superior to 5 the sleeping time of the control group (from  $22 \pm 5$  min to  $126 \pm 31$  min at a dose of 1000 mg/kg) [ $F(5,24) = 154$ ;  $p < 0.001$ ] (Fig. 5).

**Table 1:** Parts of the plant, form of the medicine and diseases treated in traditional medicine, chemical characterization and pharmacological activity.

Name of the plant	Part of the plant used	Form of the medicine	Diseases	Country	Chemical characterization	Pharmacological activity	References
<i>M. thonningii</i>	Roots Barks Leaves Seeds	Decoction Washing Infusion	Epilepsy, fevers, heartaches, pneumonia, cold, stomach aches, rheumatism, headaches, pains, allergy, worms, anti-venomous, dysentery, insomnia.	Cameroon Central Africa West Africa	Coumarin, Isoflavones	Molluscicidal Antischistosomal Insecticidal	Ancolio et al., 2002; Arbonnier, 2000; Belmain et al., 2001; Hutchinson and Dalziel 1958; Geetha et al., 2004; Perrett et al., 1994; Singhal et al., 1982; Vyahare et al., 2007
<i>O. sanctum</i>	Leaves Roots Root barks	Decoction	Epilepsy, insomnia headaches, fever, stomachaches, asthma, allergy, itch, cough	Cameroon Central Africa Vietnam Thailand	Terpenoids Phenol, Tannins Alkaloids Saponins	Hypotensive Myorelaxant Anti-stress Cardiac depressant	Abbiw, 1990; Gupta et al., 2007; Kandeda, 2007; Martinez et al., 1982; Perrett et al., 1995a
<i>S. longepedunculata</i>	Roots Barks Leaves		Epilepsy, insomnia, pains, anti-venomous, worms, rheumatism, jaundice, bronchitis, itch, intestinal obstruction, tuberculosis, leprosy, cough, fever, constipation, conjunctivitis, cataract, anti-venomous	Cameroon Central Africa East Africa	Xanthones, Methyl salicylate Alkaloids	Antinociceptive, antidepressant, sedative, insecticidal, trypanocidal, analgesic, tonic, anti-malarial, anti-inflammatory hypoglycemic, anticonvulsant	Adebiyi et al., 2006; Aderbauer et al., 2008; Adeyemi et al., 2010; Asomaning et al., 1998; Bhargava and Singh, 1981; Irvine, 1961; Jayasekara et al., 2005; Meyer et al., 2008; Ojewole, 2008; Perrett et al., 1995b; Rakuambo et al., 2006

## Discussion

*O. sanctum* and *S. longepedunculata* protected 66.7 to 100% of STR-induced convulsions with *O. sanctum* being the most effective. The inhibition of STR-induced seizures suggested the presence of anticonvulsant properties (Adeyemi et al., 2010; Mustafa and Ali, 2008) and the involvement of glycine receptors (Findlay et al., 2002) in the two plants. The lack of effect of *M. thonningii* in STR test reveal that this extract might not mediate its anticonvulsant effect via glycine receptors. The extract of *M. thonningii* strongly antagonized NMDA-induced turning behaviour. Since NMDA receptor antagonists have been shown to possess anticonvulsant and antiepileptic properties in several animal models of epilepsy (Davies et al., 1986; Ngo Bum et al., 1996), it can be suggested that the anticonvulsant properties of *M. thonningii* could be mediated by NMDA receptors. The extracts of *O. sanctum* and *S. longepedunculata* completely protected mice against the convulsions induced by PIC while *M. thonningii* failed to protect mice in PIC test. This result suggested that, the anticonvulsant activity of *M. thonningii* could not be related to the picrotoxin site of the GABA<sub>A</sub> receptor complex (Mustafa and Ali, 2008). In INH test, *S. longepedunculata* prolonged the time of the onset of convulsions. The extracts of *M. thonningii*, *O. sanctum* and *S. longepedunculata* protected mice against PTZ-induced convulsions, with *S. longepedunculata* being the most effective. The antagonism of INH and PTZ-induced seizures suggested the presence of anticonvulsant properties and the interaction of the plant extracts with the GABA-ergic neurotransmission (Mustafa and Ali, 2008; Pérez-Saad and Buznego, 2008). Finally, all three extracts inhibited MES-induced convulsions by probably prolonging the inactivation of sodium channels (Holmes, 2007). The MES and PTZ tests are of predictive relevance considering the clinical spectrum of activity of experimental compounds (Kupferberg and Schmutz, 1997). They are assumed to identify anticonvulsant drugs effective against generalized tonic-clonic/partial seizures and generalized clonic seizures, respectively (Holmes, 2007; Kupferberg and Schmutz, 1997). Therefore the effect of the extracts of *M. thonningii*, *O. sanctum* and *S. longepedunculata* in these tests could suggest anticonvulsant efficacy against the afore-mentioned seizures types in man. In addition, *M. thonningii*, *O. sanctum* and *S. longepedunculata* seemed to possess sedative properties for they strongly increased, and in a dose-dependent manner the sleeping time induced by diazepam (Rakotonirina et al., 2001; Ngo Bum et al., 2009a). The sedative properties of these plants could be related to the presence of some components in the extracts activating the benzodiazepine and/or GABA receptors in the GABA<sub>A</sub> receptor complex (Rang et al., 1999). In conclusion, this study allowed showing that the purported sedative and anticonvulsant activity of *M. thonningii*, *O. sanctum* and *S. longepedunculata* are real and that they could be helpful in the treatment of insomnia and epilepsy in traditional medicine in Africa and particularly in Cameroon. In the future, isolation of active constituents of the plant extracts could pave the way for the standardization of biologically active compound as in the case of *Turnera aphrodisiac* Ward (Kumar and Sharma, 2005; Rabbani et al., 2008) and could be helpful to cure insomnia, epilepsies, intractable epilepsies or other brain diseases.

**Abbreviations:** Maximal electroshock (MES), N-methyl-D-aspartate (NMDA), Pentylene-tetrazol (PTZ), Picrotoxin (PIC), Strychnine (STR), Negative control (CON), Positive control (PC).

## Acknowledgement

This work was supported by the University of Ngaoundéré in Cameroon who provided for animals, drugs and equipments.

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