

Animals

Naïve adult male mice, *Mus musculus* Swiss, 22 ± 2 g and two months old obtained from the animal laboratory of our university were used. Animals were housed in standard cages at 25°C, on a 12/12 hour-light-dark cycle. They were supplied with food and water *ad libitum*. For each test, mice were divided into 6 groups. One negative control group received distilled water as vehicle, one positive control group received an appropriate well known anxiolytic substance as a reference, and four test groups received different doses of plant extracts. Treatments were administered in a volume of 10 ml/kg of mice body weight. Specific substances were used as positive control according to the methods. Trials were conducted randomly with respect to treatment condition. Blinded animal experiments were carried out in accordance with the nationally (N° FWA-IRB00001954) accepted principles for laboratory animal use and care and the USNRC (1996) Guide for the care and use of Laboratory Animals, US National Research Council, Washington, D.C., United States of America.

Elevated Plus Maze (EPM) test

The apparatus comprise two open arms (16 cm x 5 cm) and two closed arms (16 cm x 5 cm x 10 cm) that extended from a common central platform (5 cm x 5 cm). The entire maze was elevated to a height of 50 cm above floor level. Group I (negative control) was treated with vehicle. Groups II to V (test groups) were treated with 4 doses of the plants extracts. Group VI treated with diazepam, 3 mg/kg ip was used as positive control. One hour after treatment, the mice were individually placed on the EPM centre platform facing an open arm and observed for 5 min (Bourin et al., 2001; Lister, 1987, Ngo Bum et al., 2009b). The number of entries by each animal into open or closed arms and the time spent by each animal in either open or closed arms (conventional parameters) were recorded with stopwatches. The inner faces of closed arms and the platform were cleaned with alcohol after each mouse recording.

Stress-Induced Hyperthermia (SIH) test

Naïve animals were marked and housed 10 per treatment or cage. Mice were removed from the cage one after the other in a precise order and treated with vehicle for the negative control group, phenobarbital (20 mg/kg, i.p.) for the positive control group, and one of the four doses of plant extracts for the test groups. Animals within a given cage were consecutively treated at 1 min intervals. After 60 min, mice were again consecutively removed from the cage (1-minute interval) and their body (rectal) temperature was recorded. This experiment is based on the fact that among animals in the same cage, mice removed later have a higher body temperature than those removed earlier (Borsini et al., 1989; Lecci et al., 1990). SIH (ΔT°) was defined as the difference between the mean temperature of the first three mice and the mean temperature of the last three mice.

Statistical analysis

The values of the negative control group were compared to the values of the tested groups and positive control group in each test. The analyses of variance (ANOVAs) followed by Dunnett's (HSD) test were used. A p value <0.05 was considered significant.

Chemicals

Diazepam: (Valium®, was from Roche, Neuilly, France); Phénobarbital (Sigma Aldrich Inc., St Louis, MO, USA)

Table 1: Details of preparation of plant for intraperitoneal administration.

Name of the plant	Part of the plant used	Quantity plant powder (g)	Quantity of water (ml)	Yield
<i>Afromosia laxiflora</i>	Dried leaves	6.67	50	16.4%
<i>Chenopodium ambrosioides</i>	Dried barks	15	50	4%
<i>Microglossa pyrifolia</i>	Dried leaves	6.67	50	9.7%
<i>Mimosa pudica</i>	Dried leaves	20	50	6%

Results

Effect of *A. laxiflora*

In EPM, *A. laxiflora* increased significantly the number of entries into open arms from 0.0 in the control group to 1.9 at the dose of 220 mg/kg [F(6,29) = 4.8; p < 0.003] (Table 2). The percentage of entries into open arms was dose dependently increased from 0.0% in the control group to 30.6% at the dose of 220 mg/kg [F(6,29) = 6.1; p < 0.002], as well as the percentage of time spent in open arms from 0.0% in the control group to 15.7% at the dose of 220 mg/kg [F(6,29) = 113; p < 0.0001] (Fig 1). As expected for a positive control group, diazepam induced an increase in the percentage of entries and time in open arms. *A. laxiflora* also induced a light reduction in the percentage of entries and time in closed arms [F(6,29) = 4.4; p < 0.002] and [F(6,29) = 6.1; p < 0.001], respectively (Fig 2). In addition, the ratio of open entries/total

entries versus closed entries/total entries strongly and significantly increased with the doses of the decoction [F(6,29) = 4; p < 0.001] (Table 2).

In SIH test, like phenobarbital, *A. laxiflora* reduced ΔT° the difference of temperature between the first three mice and the last three mice in a dose dependent manner from 0.75°C in the control group to 0.36°C at the dose of 110 mg/kg [F(6,29) = 18.5; p < 0.0001] (Fig 3). But *A. laxiflora* did not affect the body temperature (Table 2).

Effect of *C. ambrosioides*

In EPM, *C. ambrosioides* induced a significant increase in the number of open arms entries from 2.0 in the control group to 3.4 at the dose of 120 mg/kg [F(6,29) = 28; p < 0.0001] (Table 3). The percentage of entries into open arms was dose dependently increased from 26.4% in the control group to 51% at the dose of 120 mg/kg [F(6,29) = 65; p < 0.0001], as well as the percentage of time spent in open arms from 7.9 % in the control group to 31.8% at the dose of 120 mg/kg [F(6,29) = 78; p < 0.0001] (Fig 4). Diazepam also induced an increase in the percentage of entries and time in open arms. Like diazepam, *C. ambrosioides* induced a significant reduction in the percentage of entries and time in closed arms from 73.5% and 62.5% in the control group to 48.9% and 24.7% at the dose of 120 mg/kg [F(6,29) = 9.2; p < 0.001] and [F(6,29) = 102; p < 0.0001], respectively (Fig 5). Here also, the ratio of open entries/total entries versus closed entries/total entries strongly and significantly increased with the doses of the decoction [F(6,29) = 14; p < 0.001] (Table 3). In SIH, like phenobarbital, *C. ambrosioides* strongly reduced ΔT° from 1.1°C in the control group to -1.1°C at the dose of 120 mg/kg [F(6,29) = 88; p < 0.0001] (Fig 6). In addition, *C. ambrosioides* strongly decreased the body temperature from 36.3°C in the control group to 31.0°C at the dose of 40 mg/kg [F(6,53) = 92; p < 0.0001] (Table 3).

Effect of *M. pyrifolia*

In EPM *M. pyrifolia* increased the number of open arms entries from 0.0 in the control group to 1.2 at the dose of 64 mg/kg [F(6,29) = 89; p < 0.0001] (Table 4). The percentage of entries into open arms was increased from 0.0% in the control group to 50.0% at the dose of 64 mg/kg [F(6,29) = 15; p < 0.0001], as well as the percentage of time in open arms from 0.0% in the control group to 6.3% [F(6,29) = 108; p < 0.0001] (Fig 7). Diazepam also induced an increase in the percentage of entries and time in open arms. The percentage of time in closed arms was not affected by *M. pyrifolia* (Fig 8). In SIH, *M. pyrifolia* produced in a dose dependent manner a very strong reduction of ΔT° from 1.7°C in the control group to 0.2°C at the dose of 128 mg/kg [F(6,29) = 52; p < 0.0001] (Fig 9). In addition, *M. pyrifolia* decreased the body temperature from 29.4°C in the control group to 22.6°C at the dose of 128 mg/kg [F(6,53) = 42; p < 0.0001] (Table 4).

Effect of *M. pudica*

In EPM, *M. pudica* significantly increased the number of open arms entries from 0.0 in the control group to 1.1 at the dose of 60 mg/kg [F(6,29) = 3.3; p < 0.05] (Table 5). The percentage of entries into open arms was dose dependently increased from 0% in the control group to 53.3% at the dose of 180 mg/kg [F(6,29) = 10; p < 0.0001], as well as the percentage of time in open arms from 0.0% in the control group to 38.3% at the dose of 60 mg/kg [F(6,29) = 10.2; p < 0.0001] (Fig 10). *M. pudica* at the dose of 180 mg/kg induced a significant reduction in the percentage of entries and time in closed arms from 100% and 95.6% in the control group to 46.6% and 5.8% at the dose of 180 mg/kg [F(6,29) = 9.2; p < 0.001] and [F(6,29) = 39.2; p < 0.0001], respectively (Fig 11). In addition, the ratio of open entries/total entries versus closed entries/total entries strongly and significantly increased with the doses of the decoction [F(6,29) = 4; p < 0.001] (Table 5).

In SIH, *M. pudica* reduced ΔT° in a dose dependent manner from 1.3°C in the control group to 0.5°C at the dose of 180 mg/kg [F(6,29) = 39.2; p < 0.0001] (Fig 12). The extract also decreased the mice body temperature from 30.5°C in the control group to 28.4°C at the dose of 18 mg/kg (Table 5).

Discussion

The extracts of *A. laxiflora*, *C. ambrosioides*, *M. pyrifolia* and *M. pudica* antagonized in a dose-dependent manner the hyperthermia induced by stress. This antagonism, close to the effect of phenobarbital, suggests the presence of anxiolytic-like activity in the four plants, since anxiolytic drugs induce inhibition of SIH (Bouwknicht et al., 2007; Ngo Bum et al., 2009b; Olivier et al., 2003; Vinkers et al., 2008). The anxiolytic-like activity was also found in the EPM test where the four plants increased the number of entries into, the percentage of entries and time in open arms, and *A. laxiflora*, *C. ambrosioides* and *M. pudica* reduced in addition the percentage of closed arms entries and time (Lister, 1987; Ngo Bum et al., 2009b; Rodgers et al., 1997). The correlation of the increase in time spent in open arms with the increase in the number of entries in open arms supported the anxiolytic-like activity of the four plants (Lister, 1987; Rodgers et al., 1997). With the four plants, the fact that the entries into open arms were increasing, while the total entries were decreasing or not changing suggested an increase of exploratory activity not related to the locomotion. The increase of the exploration activity, revealed also by the increase of the ratio OE/TE vs CE/TE suggested anxiolytic activity as anti-anxiety drugs decrease the stress-induced inhibition of exploratory behaviour (File and Wardill 1975; Jenck et al., 1997; Ngo Bum et al., 2009b; Rodgers et al., 1997). The anxiolytic-like activity of these plants could be explained by the presence of some components in the plant extracts interacting with the benzodiazepine/GABA_A receptors as agonists, or with the 5-HT_{1A} receptors as agonists, or with the NMDA receptors as antagonists, or with any other mechanisms (Olivier et al., 2003; Tunnicliff, 1991; Vinkers et al., 2008).

Table 2: The effect of *A. laxiflora*, Open arms entries, closed arms entries, total arm entries and ratio open arms entries/total arms entries (OE/TE) versus closed arms entries/total arms entries (CE/TE) in EPM test; and body temperature in SIH test.

	Distilled water	Doses of <i>A. laxiflora</i> (mg/kg)				DIAZ/PHO
		22	55	110	220	
Open arms entries	0.0 ± 0.0	0.4 ± 0.4 ^a	1.4 ± 0.5 ^c	1.6 ± 0.4 ^c	1.9 ± 0.4 ^c	1.5 ± 0.5 ^c
Closed arms entries	5.1 ± 1.6	4.0 ± 1	3.7 ± 2.2	4.0 ± 1.7	4.2 ± 1.6	2.2 ± 0.7 ^b
Total arms entries	41 ± 7	35 ± 6	41 ± 7	45 ± 7	49 ± 8	30 ± 5 ^a
Ratio OE/TE vs CE/TE	0 ± 0	9 ± 4 ^a	61 ± 21 ^b	64 ± 23 ^b	62 ± 28 ^b	87 ± 23 ^c
Body temperature °C	37.6 ± 1.1	37.8 ± 1.1	38.4 ± 0.7	38.3 ± 1.0	38.2 ± 0.8	37.2 ± 1.6

Data are mean ± S.E.M, n = 6 or 10, ^ap < 0.05, ^bp < 0.01, ^cp < 0.001, ANOVA followed by Dunnett (HSD). Diaz = diazepam in EPM test, PHO = phenobarbital in SIH test.

Table 3: The effect of *C. ambrosioides*, Open arms entries, closed arms entries, total arm entries and ratio open arms entries/total arms entries (OE/TE) versus closed arms entries/total arms entries (CE/TE) in EPM test; and body temperature in SIH test.

	Distilled water	Doses of <i>C. ambrosioides</i> (mg/kg)				DIAZ/PHO
		4	12	40	120	
Open arms entries	2.0 ± 0.3	2.4 ± 0.9	2.6 ± 0.6	3.0 ± 0.8	3.4 ± 0.6 ^b	4.0 ± 0.8 ^c
Closed arms entries	5.6 ± 1.8	4.8 ± 1.6	4.1 ± 0.8	4.4 ± 1.2	3.3 ± 0.9 ^a	3.6 ± 0.5 ^a
Total arms entries	53 ± 2	51 ± 2	47 ± 1	52 ± 2	47 ± 1	53 ± 1
Ratio OE/TE vs CE/TE	43 ± 16	67 ± 28	69 ± 29	84 ± 28 ^a	119 ± 30 ^b	117 ± 36 ^b
Body temperature °C	36.3 ± 0.5	34.9 ± 0.8	33.8 ± 1.2 ^b	31.0 ± 1.5 ^c	31.3 ± 1.5 ^c	34.9 ± 1.3

Data are mean ± S.E.M, n = 6 or 10, ^ap < 0.05, ^bp < 0.01, ^cp < 0.001, ANOVA followed by Dunnett (HSD). Diaz = diazepam in EPM test, PHO = phenobarbital in SIH test.

Table 4: The effect of *M. pyrifolia*, Open arms entries, closed arms entries, total arm entries and ratio open arms entries/total arms entries (OE/TE) versus closed arms entries/total arms entries (CE/TE) in EPM test; and body temperature in SIH test.

	Distilled water	Doses of <i>M. pyrifolia</i> (mg/kg)				DIAZ/PHO
		12.8	32	64	128	
Open arms entries	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	1.2 ± 0.4 ^c	0.0 ± 0.0	1.6 ± 0.6 ^c
Closed arms entries	6.2 ± 0.8	4.2 ± 0.8 ^c	3.7 ± 0.8 ^c	1.2 ± 0.4 ^c	1.09 ± 0.0 ^c	2.0 ± 0.5 ^c
Total arms entries	50 ± 1	34 ± 1	31 ± 1 ^b	20 ± 1 ^c	8 ± 1 ^c	29 ± 1 ^c
Ratio OE/TE vs CE/TE	0 ± 0	0 ± 0	3 ± 1	117 ± 42 ^c	0 ± 0	100 ± 22 ^c
Body temperature °C	29.4 ± 0.9	28.5 ± 0.3	25.7 ± 0.7 ^b	23.9 ± 0.7 ^c	22.6 ± 0.2 ^c	30.1 ± 0.6

Data are mean ± S.E.M, n = 6 or 10, ^bp < 0.01, ^cp < 0.001, ANOVA followed by Dunnett (HSD). Diaz = diazepam in EPM test, PHO = phenobarbital in SIH test.

Table 5: The effect of *M. pudica*, Open arms entries, closed arms entries, total arm entries and ratio open arms entries/total arms entries (OE/TE) versus closed arms entries/total arms entries (CE/TE) in EPM test; and body temperature in SIH test.

	Distilled water	Doses of <i>M. pudica</i> (mg/kg)				DIAZ/PHO
		6	18	60	180	
Open arms entries	0.0 ± 0.0	0.2 ± 0.4	0.7 ± 0.4 ^c	1.1 ± 0.4 ^c	1.0 ± 0.5 ^b	1.5 ± 0.5 ^c
Closed arms entries	5.7 ± 2.0	5.6 ± 1.1	5.0 ± 1.2	3.6 ± 1.2 ^a	0.9 ± 0.9 ^c	0.6 ± 0.5 ^c
Total arms entries	46 ± 2	47 ± 1	46 ± 1	38 ± 1 ^b	15 ± 11 ^c	17 ± 1 ^c
Ratio OE/TE vs CE/TE	0 ± 0	4 ± 1	17 ± 7 ^b	50 ± 12 ^c	87 ± 21 ^c	100 ± 23 ^c
Body temperature °C	30.5 ± 0.6	30.1 ± 0.8	28.4 ± 0.6 ^a	28.7 ± 0.6 ^a	28.9 ± 0.4 ^a	29.5 ± 0.9

Data are mean ± S.E.M, n = 6 or 10, ^ap < 0.05, ^bp < 0.01, ^cp < 0.001, ANOVA followed by Dunnett (HSD). Diaz = diazepam in EPM test, PHO = phenobarbital in SIH test.

In addition, *C. ambrosioides*, *M. pyrifolia* and *M. pudica* showed antipyretic properties which allowed the body temperature to fall in SIH test (Olivier et al., 2003; Sundgren-Andersson et al., 1998). Some studies are ongoing in our laboratory to determine the mechanism of action of these plants.

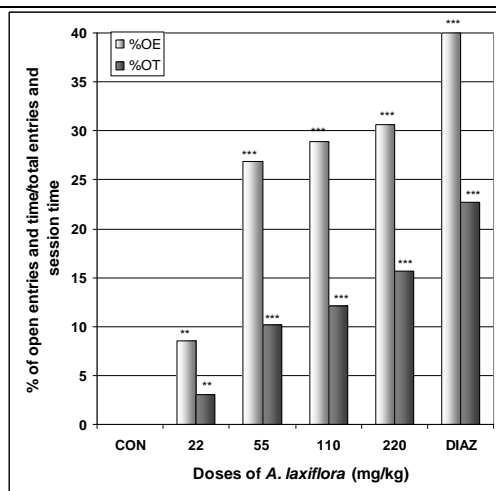


Figure 1: Effect of *A. laxiflora* in mice placed on EPM; Open arms entries and time

The figure represents the percentage of open arms entries/total arms entries and the percentage of open arms time/session time (5min). N = 6, ** p < 0.01, *** p < 0.001, ANOVA followed by Dunnett (HSD). CON = negative control, DIAZ = diazepam.

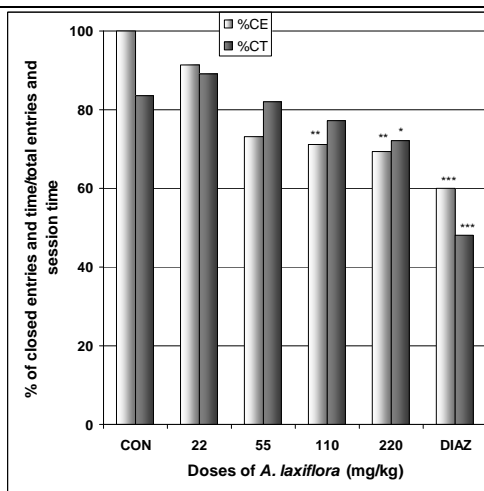


Figure 2: Effect of *A. laxiflora* in mice placed on EPM; Closed arms entries and time

The figure represents the percentage of closed arms entries/total arms entries and the percentage of open arms time/session time (5min). N = 6, * p < 0.05, ** p < 0.01, *** p < 0.001, ANOVA followed by Dunnett (HSD). CON = negative control, DIAZ = diazepam.

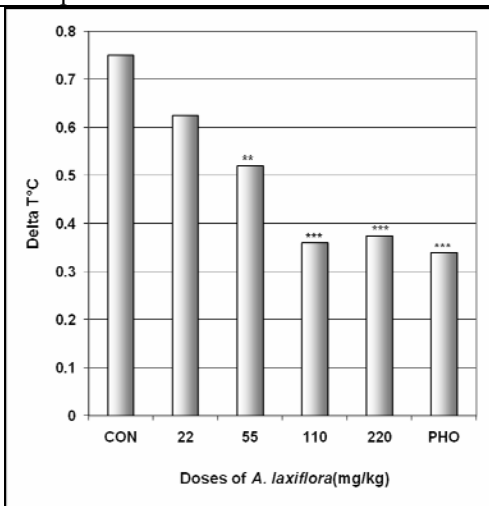


Figure 3: Effect of *A. laxiflora* in SIH

The figure represents the temperature difference ($\Delta T^{\circ}C$) between the first three mice and the last three mice. N = 10, ** p < 0.01, *** p < 0.001, ANOVA followed by Dunnett (HSD). CON = negative control, PHO = phenobarbital.

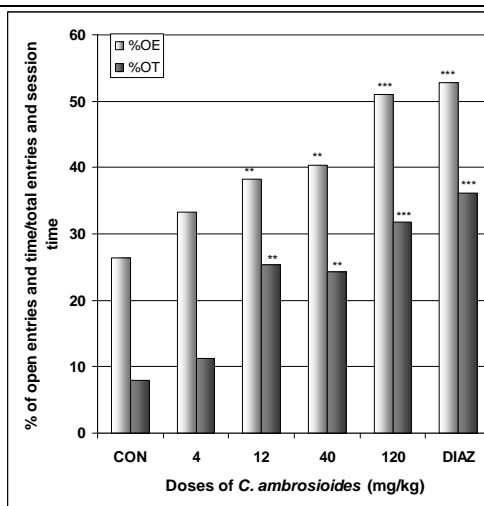


Figure 4: Effect of *C. ambrosioides* in mice placed on EPM; Open arms entries and time.

The figure represents the percentage of open arms entries/total arms entries and the percentage of open arms time/session time (5min). N = 6, ** p < 0.01, *** p < 0.001, ANOVA followed by Dunnett (HSD). CON = negative control, DIAZ = diazepam.

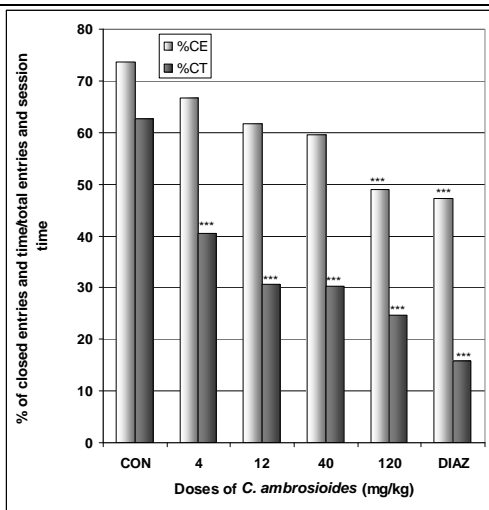


Figure 5: Effect of *C. ambrosioides* in mice placed on EPM; Closed arms entries and time.
The figure represents the percentage of closed arms entries/total arms entries and the percentage of open arms time/session time (5min). N = 6, *** p < 0.001, ANOVA followed by Dunnett (HSD). CON = negative control, DIAZ = diazepam.

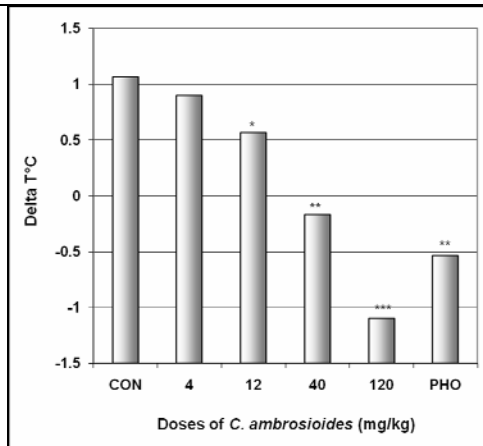


Figure 6: Effect of *C. ambrosioides* In SIH
The figure represents the temperature difference ($\Delta T^{\circ}C$) between the first three mice and the last three mice. N = 10, ** p < 0.01, ANOVA followed by Dunnett (HSD). CON = negative control, PHO = phenobarbital.

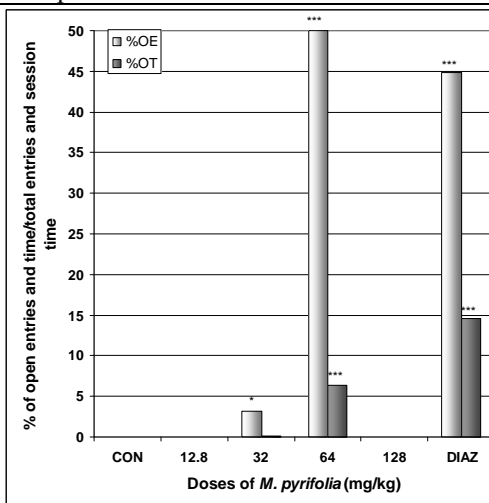


Figure 7: Effect of *M. pyrifolia* in mice placed on EPM; Open arms entries and time.
The figure represents the percentage of open arms entries/total arms entries and the percentage of open arms time/session time (5min). N = 6, * p < 0.05, *** p < 0.001, ANOVA followed by Dunnett (HSD). CON = negative control, DIAZ = diazepam.

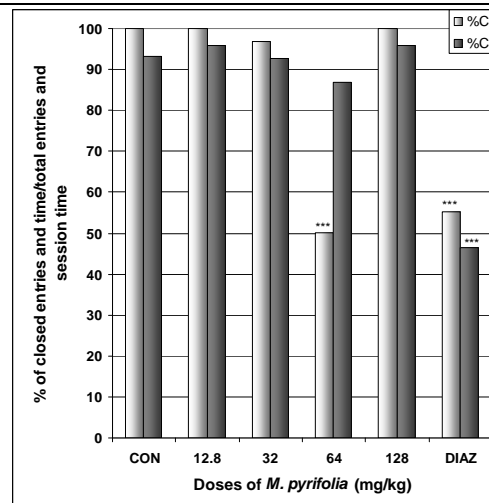


Figure 8: Effect of *M. pyrifolia* in mice placed on EPM; Closed arms entries and time.
The figure represents the percentage of closed arms entries/total arms entries and the percentage of open arms time/session time (5min). N = 6, *** p < 0.001, ANOVA followed by Dunnett (HSD). CON = negative control, DIAZ = diazepam.

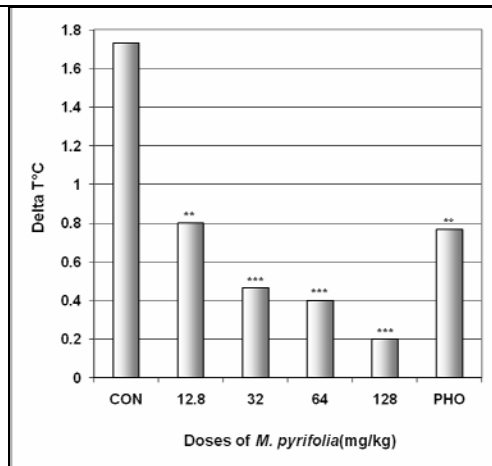


Figure 9: Effect of *M. pyrifolia* in SIH
The figure represents the temperature difference ($\Delta T^{\circ}\text{C}$) between the first three mice and the last three mice. N = 10, ** p < 0.01, *** p < 0.001, ANOVA followed by Dunnett (HSD). CON = negative control, PHO = phenobarbital.

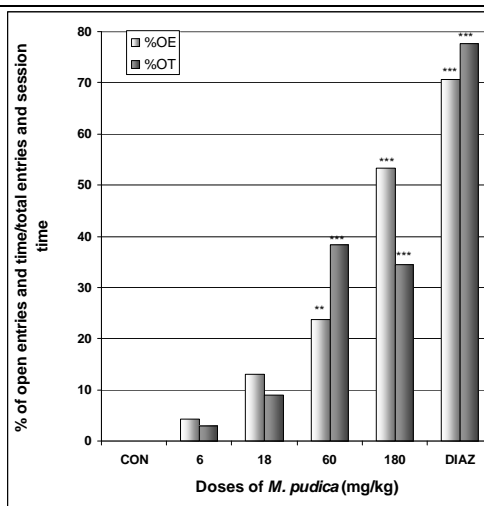


Figure 10: Effect of *M. pudica* in mice placed on EPM; Open arms entries and time.
The figure represents the percentage of open arms entries/total arms entries and the percentage of open arms time/session time (5min). N = 6, ** p < 0.01, *** p < 0.001, ANOVA followed by Dunnett (HSD). CON = negative control, DIAZ = diazepam.

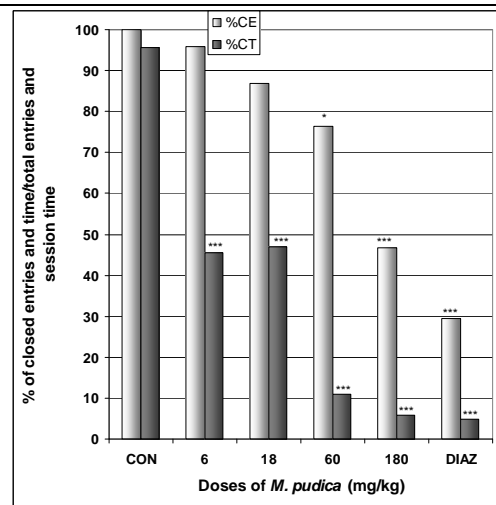


Figure 11: Effect of *M. pudica* in mice placed on EPM; Closed arms entries and time.
The figure represents the percentage of closed arms entries/total arms entries and the percentage of open arms time/session time (5min). N = 6, * p < 0.05, *** p < 0.001, ANOVA followed by Dunnett (HSD). CON = negative control, DIAZ = diazepam.

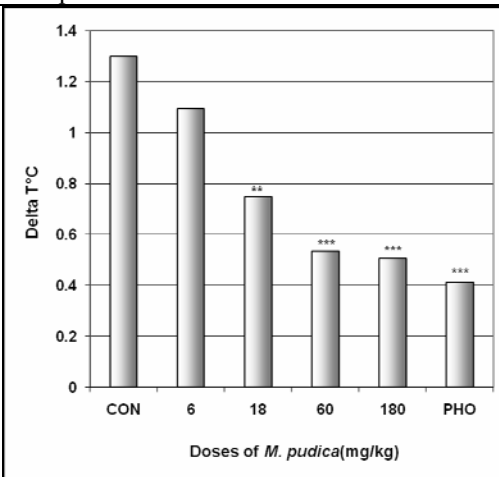


Figure 12: Effect of *M. pudica* in SIH
The figure represents the temperature difference ($\Delta T^{\circ}\text{C}$) between the first three mice and the last three mice. N = 10, ** p < 0.01, *** p < 0.001, ANOVA followed by Dunnett (HSD). CON = negative control, PHO = phenobarbital.

Conclusion

The purported anxiolytic and/or antipyretic activities of *A. laxiflora*, *C. ambrosioides*, *M. pyrifolia* and *M. pudica* used in traditional medicine in Cameroon to treat people were demonstrated scientifically. The extracts of *C. ambrosioides*, *M. pyrifolia* and *M. pudica* were shown to possess antipyretic and anxiolytic properties in mice; while the extract of *A. laxiflora* possesses only anxiolytic properties. These properties supported the pertinence of the utilization of these plants in traditional medicine in Cameroon in the treatment of fever, agitations and anxiety.

Abbreviations: Negative control (CON), closed arms entries (CE), closed arms time (CT), diazepam (DIAZ), Elevated plus maze (EPM), open arms entries (OE), open arms time (OT), phenobarbital (Pheno), stress-induced hyperthermia (SIH), total arms entries (TE).

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