Anxiolytic and Antidepressant Effects of a Leaf Extract of *Palisota hirsuta* K. Schum. (Commelinaeae) in Mice

E. Woode, E. Boakye-Gyasi, N. Amidu, C. Ansaah and M. Duwiejua

Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

**Abstract:** The effect of a 70% (v/v) ethanolic leaf extract of *Palisota hirsuta*, a traditional West African plant used for CNS disorders and pain, in animal models of anxiety and depression—the open field test, the light/dark box, the Elevated plus Maze (EPM), the Forced Swimming Test (FST) and Tail Suspension Test (TST) has been reported. *P. hirsuta* (30-300 mg kg$^{-1}$) treated mice exhibited anxiolytic activity in all the anxiety models used by significantly increasing the percentage of center entries and the percentage time spent in the center of the open field. *P. hirsuta* also significantly increased the time spent in the lit area in comparison to the time spent in the dark area of the light/dark box. In the EPM, it significantly increased open arm activity which was completely reversed by flumazenil (3 mg kg$^{-1}$), a specific antagonist of the GABA$_A$ benzodiazepine receptor complex. In the antidepressant test, the extract also dose-dependently reduced the duration of immobility in both the FST (ED$_{50}$: 114.55±72.69 mg kg$^{-1}$) and TST (70.42±0.06 mg kg$^{-1}$). Pretreatment with α-methyldopa (400 mg kg$^{-1}$, 3 h; p.o.), reserpine (1 mg kg$^{-1}$; 24 h; s.c.) or a combination of the two drugs attenuated the anti-immobility effects of both imipramine and the extract but not fluoxetine. Neither the extract nor the standard drugs used modified motor performance on the rotarod test at all doses tested. These results suggest that the extract has anxiolytic and antidepressant-like effects in the models employed possibly by GABAergic activation and/or effect on monoamine levels in the CNS.

**Key words:** *Palisota hirsuta*, anxiolytic, antidepressants, mice

**INTRODUCTION**

Depression and anxiety are the most common mental disorders. More than 20% of the adult population suffers from these conditions at some time during their life (Buller and Legrand, 2001). According to the World Health report, roughly 450 million people experience a mental or behavioral disorder, yet only a small minority of them receive even the most basic treatment. Approximately two-thirds of anxious or depressed patients respond to presently available treatments but the extent of improvement is still below par (Buller and Legrand, 2001). This therefore brings to fore, the medical need for newer, better-tolerated and more efficacious treatments. In the search for new therapeutic products for the treatment of neurological disorders, research into medicinal plant worldwide has been intensified and thus, revealed the pharmacological effectiveness of different plant species in a variety of animal models (Zhang, 2004). An increasing number of herbal products have been introduced into psychiatric practice, as alternative or complementary medicines.

In West African and Ghanaian traditional medicine, an infusion is prepared from the leaves of *Palisota hirsuta* K. Schum. (Commelinaeae) and administered orally for treating various painful conditions (Burkill, 1985; Dokosi, 1998). Locally in Ghana, *Palisota hirsuta* is known as *someni* or *mpenten* (Twi), *sombenyin* (Fantu) and *sumbe* (Ewe). The central analgesic properties of this plant have been recently reported by Woode et al. (2009). Moreover, leaves decoction of this plant is also taken orally for CNS diseases (Abbiw, 1990). Despite the popular use of the plant, there is no information in literature on the probable CNS effect of this plant. Experimental paradigms such as elevated plus-maze, open field, light/dark box, tail suspension and forced swimming tests which are widely used for identifying putative candidates for new treatment obtained from natural sources for anxiety and depression were employed in this study. The objective of the present study therefore, was to evaluate the anxiolytic and antidepressant effect produced by the hydroalcoholic extract from *Palisota hirsuta* leaves in mice and its possible mechanism of action.

**Corresponding Author:** Eric Woode, Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
MATERIALS AND METHODS

**Plant material:** Leaves of the plant *Palisota hirsuta* were collected from the Botanic Gardens of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, between January and February, 2007. The leaves were authenticated by Mr. Amisah, the curator of the garden and a voucher specimen (No. FP 10081) has been kept in the Faculty of Pharmacy Herbarium, KNUST, Kumasi.

**Preparation of extract:** The leaves were air-dried indoors for a week and pulverized with a hammer-mill. The powder was extracted by maceration using 70% (v/v) ethanol over a period of 72 h. The resulting extract was concentrated under low temperature (60°C) and pressure to a syrupy mass in a rotary evaporator. The syrupy mass was then dried to a dark brown semi-solid mass using water bath and kept in a desiccator till it was ready to be used. The final yield was 10.5% (w/w). This is subsequently referred to as *Palisota hirsuta* extract (PHE) or extract.

**Animals:** Male ICR mice were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Accra and housed at the animal facility of the Department of Pharmacology, KNUST, Kumasi, Ghana. The animals were housed in groups of 6 in stainless steel cages (34×47×18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions. All behavioral experiments were carried out under dim light and therefore to acclimatize the animals to the test conditions, they were brought to the laboratory and exposed to dim light at the stipulated time of testing daily for 6 days before the experiment. All animals used in these studies were treated in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services publication No. 85-23, revised 1985) and were approved by the College Ethics Committee.

**Drugs and chemicals:** Fluoxetine hydrochloride (Prozac®), was bought from Eli Lilly and Co., Basingstoke, England, imipramine hydrochloride was from Phyto-Riker Pharmaceuticals, Acura, Ghana, α-methylDopa (Aldomet®), was from Merck Sharp Dohme, Herts, England, reserpine was also bought from BDH, Poole, England whilst diazepam and caffeine hydrochloride were obtained from Sigma Chemicals, St. Louis, MO, USA and flumazenil (Anexate®), from Roche Products Ltd., Herts, England. Fluoxetine hydrochloride, imipramine hydrochloride, α-methylDopa and the extract were suspended using 2% tragacanth suspension and given orally whilst diazepam, caffeine hydrochloride and flumazenil were prepared using normal saline and given intraperitoneally.

**Phytochemistry:** The presence of saponins, tannins, alkaloids, triterpenes, flavonoids, glycosides, reducing sugars and tannins were tested by simple qualitative and quantitative methods of Trease and Evans (1989).

**Open-field test:** The test was based on that described previously by other workers (Erdogan *et al.*, 2004; Kasture *et al.*, 2002). Testing was conducted in clear Plexiglas boxes (40×40×30 cm) whose floor was divided into 16 equal squares by black lines. For behavioral analysis, the arena of the open field was designated as (1) corner (one of the four corner squares); (2) periphery (the squares along the walls); or (3) center (the four inner squares). The animals were divided into ten groups of six animals each and received either the extracts (30, 100 or 300 mg kg⁻¹, p.o.), the vehicle or the standard reference drugs diazepam (0.1, 0.3 or 1 mg kg⁻¹, i.p.) or caffeine (10, 30 or 100 mg kg⁻¹, p.o.). Thirty minutes after i.p. and 1 h after oral administration of the test compound, mice were placed individually in the centre of the open field and allowed to explore freely for 5 min. Each session was recorded by a video camera suspended approximately 100 cm above the arena. All animals were regularly handled before individual tests in order to minimize handling-related stress. Videotapes of the arena and the following variables were recorded: number of entries as well as the duration of stay in individual zones, frequency and duration of rearing and stretch attends postures. Thereafter, behavior in the open field was analyzed for 5 min with the aid of the public domain software JWatcher™ Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia. Available at http://www.jwatcher.ucla.edu). Mean values±SEM were calculated for each and compared to vehicle-treated animals.

**Light/Dark test:** The light-dark exploration test is typically used to more directly assess anxiety-related responses. This apparatus is based on the initial model described by Crawley (1981) and as modified by other workers (Belzung and Le Pape, 1994; Belzung *et al.*, 1987). It consists of wooden boxes (45 cm long×30 cm wide×30 cm deep), which are divided into two equal compartments by a wooden board with a 7×7 cm opening located centrally at the floor level, connecting the compartments. One compartment was painted black and covered with a wooden lid. The other box (not covered) was painted white and lit by a 60 W light bulb set 30 cm
above the box. Mice were grouped and treated with drugs as described for the other behavioral tests described above. At the beginning of the experiment, mice were placed individually in the center of the illuminated box, facing the opening away from the dark compartment. Behaviors of the animals were recorded for 5 minutes with a digital camera placed 1 m above the box. Videotapes were scored as mentioned above for the following parameters: 1) frequency of compartment entries; 2) total time spent by mice in each compartment.

**Elevated Plus-Maze test:** The method used was as described for rats (Pellow et al., 1985) with some modifications. The elevated plus maze was made from opaque Plexiglas. It consisted of two opposite open arms (15 × 5 cm) without side walls and two enclosed arms (15 × 5 cm), extending from a central square platform (5 × 5 cm). A rim of Plexiglas (0.5 cm in height) surrounded the perimeter of the open arms to provide additional grip and thus prevent the mice falling off (Rodgers and Johnson, 1995). The maze was elevated to the height of 80 cm from the floor and placed in a lit room (~750 lux). The animals were divided into ten groups of six animals each and received treatments similar to that described for the open field test. Animals were placed individually in the central platform of the EPM for 5 min and their behavior recorded on a videotape with a digital camera placed 100 cm above the maze. Behavioural parameters were scored from the videotapes as follows: 1) number of closed and open arm entries (absolute value and percentage of the total number); 2) time spent in exploring the open and closed arms of the maze (absolute time and percentage of the total time of testing); 3) number of head-dips (absolute value and percentage of the total number) protruding the head over the edge of either an open (unprotected) or closed (protected) arm and down toward the floor; 4) number of stretch-attend postures (absolute value and percentage of the total number) the mouse stretches forward and retracts to original position from a closed (protected) or an open (unprotected) arm. An arm entry was counted only when all four limbs of the mouse were within a given arm.

In a separate experiment, the mice were treated with flumazenil 3 mg kg⁻¹, 15 min before PH (30, 100, 300 mg kg⁻¹) or diazepam (0.3 mg kg⁻¹) treatment.

To compute total distances travelled by the mice in the open field arena and the elevated plus maze, the software Behavior Collect (http://cas.bellarmine.edu/tietjen/Downloads/computer_programs_for_data_collection.htm) was used to obtain raw XY data from the videos. These data were then exported into Microsoft® Office Excel 2007 and further analyzed. Distance between two X-Y coordinate pairs was calculated from the formula:

\[\sqrt{(X_i - X_j)^2 + (Y_i - Y_j)^2}\]

Behavioural parameters for all the tests were scored from videotapes with the aid of the public domain software JWWatcher™ Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia. Available at http://www.jwatcher.ucla.edu/).

**Forced swimming test:** The FST was based on that described by Porsolt et al. (1977, 1978). Mice were divided into ten groups of six animals each and received either the extract (30, 100 or 300 mg kg⁻¹, p.o.), the vehicle or the standard reference drugs imipramine (3, 10 or 30 mg kg⁻¹, p.o.) or fluoxetine (3, 10 or 30 mg kg⁻¹, p.o.). Thirty minutes after i.p. and 1 h after oral administration of the test compounds, mice were gently dropped individually into transparent cylindrical polyethylene tanks (25 cm high, 10 cm internal diameter) containing water (25 to 28°C) up to a level of 20 cm and left there for 6 min. Four identical polyethylene cylinders were prepared and four animals, separated by opaque screens, were exposed simultaneously and videotaped. Each session was recorded by a video camera suspended approximately 100 cm above the cylinders. After each session, animals were removed from the cylinders, dried with absorbent towels, placed in cages near to a heater until they were completely dried and then returned to their home cages. Water was changed for each mouse and tanks were cleaned in between studies. An observer scored the duration of immobility (when it floated upright in the water and made only small movements to keep its head above water), during the last 4 min of the 6 min test, from the videotapes with the aid of the public domain software JWWatcher™ Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia. Available at http://www.jwatcher.ucla.edu/).

ED₅₀ (dose responsible for 50% of the maximal effect) for each drug was determined by using an iterative computer least squares method, with the following nonlinear regression (three-parameter logistic) equation

\[Y = \frac{a + (b - a)}{1 + 10^{(\log_{10}X - X_0)}}\]

where, \(X\) is the logarithm of dose and \(Y\) is the response. \(Y\) starts at \(a\) (the bottom) and goes to \(b\) (the top) with a sigmoid shape.
Tail suspension test: The TST was carried out as previously described (Steru et al., 1985). Mice were allowed to acclimatize to the room for 3.5–4 h before the test. Groups of six mice were treated with PHE (30, 100 or 300 mg kg\(^{-1}\), p.o.), imipramine (3, 10 or 30 mg kg\(^{-1}\), p.o.), fluoxetine (3, 10 or 30 mg kg\(^{-1}\), p.o.) or vehicle. One hour after oral administration of the test compounds, mice were individually suspended by the tail from a horizontal bar (distance from floor = 30 cm) using adhesive tape (distance from tip of tail = 1 cm). Duration of immobility, defined as the absence of all movement except for those required for respiration, was recorded by an observer for 6 min from video recordings of the test as described above for forced swimming test. Mice that climbed up on their tails during the test session were gently pulled down and testing continued. Mice that continued to climb their tails were excluded from the study.

Effect of catecholamine depletion by reserpine and \(\alpha\)-methyldopa pretreatment in the tail suspension test: To investigate the possible role of noradrenergic system in the actions of PHE, a separate experiment in which catecholamines were depleted by treatment with \(\alpha\)-methyldopa (\(\alpha\)-MD) and/or reserpine was carried out as previously described by (O’Leary et al., 2007). Because the TST presents some advantages over the FST in allowing an objective measure of immobility and does not induce hypothermia by immersion in water (Ripoll et al., 2003), it was chosen for this study. Two different strategies were employed to deplete catecholamines. The doses of \(\alpha\)-MD and reserpine were chosen on the basis of work done by others (O’Leary et al., 2007; Van Giersbergen et al., 1990). To deplete newly synthesized pools of noradrenaline (NE) and dopamine (DA), mice were treated with a single dose of \(\alpha\)-MD (400 mg kg\(^{-1}\), i.p.) 3.5 h before behavioral testing. To deplete vesicular pools of NE and DA, mice were treated with a single dose of reserpine (1 mg kg\(^{-1}\), s.c.) 24 h before behavioral testing. In an effort to deplete both the vesicular and cytoplasmic pools of NE and DA, mice were pretreated with a combination of reserpine (1 mg kg\(^{-1}\), s.c., 24 h before behavioral testing) and \(\alpha\)-MD (200 mg kg\(^{-1}\), i.p., 3.5 h before behavioral testing), respectively. All control animals received 0.9% saline on the same schedule as the treated groups.

Motor co-ordination-rotarod test

Rotarod test: The effect on motor co-ordination was assessed using rotarod apparatus (Model 7600, Ugo Basile, Comerio, Italy) rotating at a speed of 12 rpm. This apparatus consists of a base platform and a rotating rod of 3 cm diameter with a non-slip surface. The rod, 50 cm in length, is divided into five equal sections by six disks. Five mice were tested simultaneously. The mice were placed individually on the cylinder. Before the start of the experiment, animals were trained to stay on the rotarod for 300 sec. Mice that failed to learn the test or did not reach the criterion (300 sec endurance) were excluded from the study. On the test day, latencies to fall from the rod were measured after administration of the test compounds or vehicle.

Statistical analyses: All data are presented as Means±SEM (n = 6). To compare differences between groups, one-way ANOVA was performed with Newman-Keuls’s test as post hoc. Also, two-way Analysis of Variance (ANOVA) with groups as a between-subject factor and compartment as a within-subject factor followed Bonferroni’s as post hoc were performed.

Fitted midpoints (ED\(_{50}\)) of the dose-response curves in the FST and TST were compared statistically using F-test (Miller, 2003; Motulsky and Christopoulos, 2003). GraphPad Prism software was used for all statistical analyses and ED\(_{50}\) determinations. p < 0.05 was considered statistically significant.

RESULTS

Phytochemical analysis: The phytochemical analysis of \(P. \) hirsuta showed it contains alkaloids, flavonoids, tannins and terpenoids with tannins and flavonoids being the most dominant chemical constituents.

Open field: In the open field test, all drug treated mice showed significant differences in both the number of entries into the various fields as well as the time spent in the various zones (Fig. 1 and 2). \(Palisota \) hirsuta treated mice exhibited anxiolytic-like activity similar to diazepam by significantly increasing the percentage number of center entries (\(F_{1,14} = 6.08, p = 0.0058\)) (Fig. 1d) and the percentage time spent in the center of the open field (\(F_{1,16} = 7.95, p = 0.0018\)) (Fig. 2d). Two-way ANOVA also revealed a significant difference in both the number (\(F_{2,30} = 36.54, p < 0.0001\)) (Fig. 1a) and duration (\(F_{2,34} = 201.44, p < 0.0001\)) (Fig. 2a) of entries into the various zones. Concentration also showed little effect on the number of zonal entries (\(F_{1,14} = 2.76, p = 0.0560\)) but did not affect the time spent in the zones (\(F_{1,16} = 0.18, p = 0.9071\)) significantly. Concentration also induced significantly (\(F_{6,38} = 4.66, p = 0.0013\)) the same effect at all values of entries duration.

Caffeine treatment did not have much effect on both the percentage number (\(F_{1,16} = 1.78, p = 0.1918\)) (Fig. 1f) and duration of entries (\(F_{1,16} = 0.33, p = 0.8021\)) (Fig. 2f) into the center of the field. Two-way ANOVA revealed
Fig. 1: Effects of acute PHE (30, 100 and 300 mg kg$^{-1}$), diazepam (0.1, 0.3, 1.0 mg kg$^{-1}$) and caffeine (10, 30 and 100 mg kg$^{-1}$) treatment on the number of zonal entries for (a) PHE, (b) diazepam, (c) caffeine and (d) % entries into central zone for PHE, (e) diazepam and (f) caffeine in the open field test. Data are presented as group Mean±SEM. Significantly different from control: *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test and significant difference when the zonal entries where compared to each other: 'p<0.05, ''p<0.01, '''p<0.001 (two-way repeated measures ANOVA followed by Bonferroni’s post hoc).

Fig. 2: Effects of acute PHE (30, 100 and 300 mg kg$^{-1}$), diazepam (0.1, 0.3, 1.0 mg kg$^{-1}$) and caffeine (10, 30 and 100 mg kg$^{-1}$) treatment on the total time spent in zones for (a) PHE, (b) diazepam, (c) caffeine and (d) % time spent in central zone for PHE, (e) diazepam and (f) caffeine in the open field test. Data are presented as group Mean±SEM. Significantly different from control: *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test and significant difference when the zonal entries where compared to each other: 'p<0.05, ''p<0.01, '''p<0.001 (two-way repeated measures ANOVA followed by Bonferroni’s post hoc).
that there was a significant difference in both the number ($F_{2,36} = 247.60; p<0.0001$) (Fig. 1c) and duration ($F_{2,36} = 370.31; p<0.0001$) (Fig. 2c) of entries into the various zones but concentration did not affect both parameters ($F_{3,36} = 1.61; p = 0.2042$) ($F_{3,36} = 1.10; p = 0.3624$).

Consistent with the anxiolytic nature of diazepam, it was able to significantly increase both the percentage number of entries ($F_{3,36} = 5.62; p = 0.0079$) (Fig. 1e) and the percentage time spent in the more exposed center of the arena ($F_{3,36} = 5.37; p = 0.0094$) (Fig. 2e). Two-way ANOVA revealed a significant difference in both the number ($F_{2,36} = 42.28; p<0.0001$) (Fig. 1b) and duration ($F_{2,36} = 88.37; p<0.0001$) (Fig. 2b) of entries into the various zones. Concentration also affected the number of entries into the zones ($F_{3,36} = 4.88; p = 0.0060$) but did not have much effect on the duration ($F_{3,36} = 0.49; p = 0.6893$).

All treatment groups did not show any significant difference compared to the vehicle treated group in the total distance travelled in the arena [PHE ($F_{3,36} = 0.17; p = 0.9136$), caffeine ($F_{3,36} = 3.27; p = 0.0487$), diazepam ($F_{3,36} = 9.27; p = 0.0452$)] (Fig. 3). However, comparing the 3D line plots generated from the time and XY data, the PHE and diazepam treated animals made much more visits to the center of the arena indicating decrease in thigmotactic behavior compared to the vehicle treated animals. Exposure to caffeine however made the mice highly thigmotactic, spending most of the time along the walls of the open field arena (Fig. 3 lower panel).

**Light dark box:** In the light/dark test, acute administration of PHE (30-300 mg kg$^{-1}$ p.o.) induced anxiolytic-related effects like diazepam by significantly increasing the time spent in the lit box ($F_{3,30} = 12.200; p<0.0001$) and decreased the time spent in the dark box ($F_{3,30} = 13.551; p<0.0001$) (Fig. 4d). There were no significant changes in the frequency of entries into the light ($F_{3,30} = 1.337; p = 0.2905$) as well as the dark compartment ($F_{3,30} = 1.234; p = 0.3249$) of the box (Fig. 4a). Two-way ANOVA revealed that there was a significant difference ($F_{3,30} = 33.66; p = 0.0002$) in the duration of entries into the light and dark box but concentration does not affect the result ($F_{3,30} = 0.01; p = 0.9983$). Concentration also induced significantly ($F_{3,30} = 33.97; p<0.0001$) the same effect at all values of entries duration.

Caffeine (10-100 mg kg$^{-1}$ i.p.) significantly decreased ($F_{3,30} = 4.839; p = 0.0108$) the time spent by mice in the lit box and increased significantly ($F_{3,30} = 4.839; p = 0.0108$) the time spent in the dark box (Fig. 4f). The frequency of entry into the light and dark compartments also increased significantly ($F_{3,30} = 6.310; p = 0.0035$, $F_{3,30} = 4.373; p = 0.0160$, respectively) (Fig. 4c). Two-way ANOVA revealed that there was significant difference ($F_{3,30} = 250.99; p<0.0001$) in the duration of entries into the light and dark areas but this was not dependent on the concentration ($F_{3,30} = 0.00; p = 1.000$). Concentration also induced significantly ($F_{3,30} = 8.84; p = 0.0002$) the same effect at all values of entries duration.

![Fig. 3: Effects of acute PHE (30, 100 and 300 mg kg$^{-1}$), diazepam (0.1, 0.3 and 1.0 mg kg$^{-1}$) and caffeine (10, 30 and 100 mg kg$^{-1}$) on total distance travelled in the open field test. Data are presented as group Means±SEM.](image-url)

Fig. 3: Effects of acute PHE (30, 100 and 300 mg kg$^{-1}$), diazepam (0.1, 0.3 and 1.0 mg kg$^{-1}$) and caffeine (10, 30 and 100 mg kg$^{-1}$) on total distance travelled in the open field test. Data are presented as group Means±SEM. Significantly different from control (Ctrl): *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test. Line plots (lower panels) 3D plots were generated from the time and XY data obtained (see Materials and Methods) using SigmaPlot Version 10 (Systat Software Inc., Point Richmond, CA, USA)
Fig. 4. Effects of acute PHE (30, 100 and 300 mg kg\(^{-1}\)), diazepam (0.1, 0.3, 1.0 mg kg\(^{-1}\)) and caffeine (10, 30 and 100 mg kg\(^{-1}\)) treatment on number of compartmental entries for (a) PHE, (b) diazepam, (c) caffeine and (d) on the time spent in compartment for PHE, (e) diazepam and (f) caffeine in the light/dark test. Data are presented as group Means±SEM. Significantly different from control (Ctrl): *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test and significant difference when open compartment and closed compartment where compared: ¹p<0.05, ²p<0.01, ³p<0.001 (two-way repeated measures ANOVA followed by Bonferroni’s post hoc).

By contrast, diazepam (0.1-1.0 mg kg\(^{-1}\) i.p.) induced anxiolytic-related measures in the light/dark test (Fig. 4b and 4e). Diazepam significantly increased the duration of time spent in the lit box (F\(_{3,30} = 26.73; p<0.0001\)) and decreased the time spent in the dark box (F\(_{3,30} = 26.73; p<0.0001\)) (Fig. 4e). Frequencies of entries into the light and dark compartments were however not significantly different from each other (F\(_{3,30} = 2.420; p = 0.0961\)) (F\(_{3,30} = 2.079; p = 0.1352\)) (Fig. 4b). Two-way ANOVA revealed that there was a significant difference (F\(_{3,30} = 65.13; p<0.0001\)) in the duration of entries into the light and dark compartments but concentration does not affect the result (F\(_{3,30} = 0.00; p = 1.000\)).

**Effects on elevated plus maze:** The effects of *P. hirsuta*, diazepam and caffeine on conventional elevated plus-maze parameters are shown in Fig. 5-7.

Administration of *P. hirsuta* (30-300 mg kg\(^{-1}\)) significantly increased open arm activity by increasing the percentage number of entries (F\(_{1.16} = 1.33; p = 0.2987\)) (Fig. 5d) as well as the percentage time spent in the open arm of the elevated plus maze (F\(_{2,16} = 4.41; p = 0.0192\)) (Fig. 6d). The PHE also significantly reduced risk assessment by decreasing both the percentage protected stretch attend postures (F\(_{3,16} = 2.49; p = 0.0976\)) (Fig. 7a) and percentage protected head dips (F\(_{3,16} = 4.10; p = 0.0245\)) (Fig. 7d) measured from the closed arm. Diazepam (0.1-1 mg kg\(^{-1}\)) also increased the percentage entries and time spent in the open arm of the EPM (F\(_{3,16} = 2.04; p = 0.1486\)) and (F\(_{3,16} = 0.76; p = 0.5334\)), respectively (Fig. 5e and 6e). Percentage protected stretch attend postures (F\(_{3,16} = 2.35; p = 0.1113\)) (Fig. 7b) and percentage protected head dips (F\(_{3,16} = 5.74; p = 0.0073\)) (Fig. 7e) were also significantly reduced, a confirmation of its anxiolytic activity. Caffeine (10-100 mg kg\(^{-1}\)), an agent that induces anxiety, significantly increased open arm avoidance by decreasing the percentage entries (F\(_{3,16} = 1.28; p = 0.3152\)) (Fig. 5e) and time spent (F\(_{3,16} = 1.99; p = 0.1564\)) (Fig. 6e) respectively in the open arm of the EPM test. Caffeine treatment however increased the percentage protected stretch attend postures (F\(_{3,16} = 1.22; p = 0.3354\)) (Fig. 7e) and percentage protected head dips (F\(_{3,16} = 5.48; p = 0.0087\)) (Fig. 7f) indicated an increase in risk assessment behavior.

Exposure to both the extract (Fig. 8a) (F\(_{3,16} = 2.44; p = 0.1018\)) and diazepam (Fig. 8b) (F\(_{1,16} = 1.36; p = 0.2903\))
Fig. 5: Effects of acute PHE (30, 100 and 300 mg kg$^{-1}$), diazepam (0.1, 0.3, 1.0 mg kg$^{-1}$) and caffeine (10, 30 and 100 mg kg$^{-1}$) treatment on the number of arm entries for (a) PHE, (b) diazepam, (c) caffeine, (d) % number of open arm entries for PHE, (e) diazepam and (f) caffeine in the elevated plus maze test. Data are presented as group Means±SEM. Significantly different from control: *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test and significant difference when the zonal entries where compared to each other: †p<0.05, ‡p<0.01, ‡‡p<0.001 (two-way repeated measures ANOVA followed by Bonferroni’s post hoc).

Fig. 6: Effects of acute PHE (30, 100 and 300 mg kg$^{-1}$), diazepam (0.1, 0.3, 1.0 mg kg$^{-1}$) and caffeine (10, 30 and 100 mg kg$^{-1}$) treatment on the time spent in various arms for (a) PHE, (b) diazepam, (c) caffeine and (d) % time spent in the open arm for PHE, (e) diazepam and (f) caffeine in elevated plus maze test. Data are presented as group Means±SEM. significantly different from control: *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test and significant difference when the zonal entries where compared to each other: †p<0.05, ‡p<0.01, ‡‡p<0.001 (two-way repeated measures ANOVA followed by Bonferroni’s post hoc).
Fig. 7: Effects of acute PHE (30, 100 and 300 mg kg⁻¹), diazepam (0.1, 0.3, 1.0 mg kg⁻¹) and caffeine (10, 30 and 100 mg kg⁻¹) treatment on the % protected stretch attend postures for (a) PHE, (b) diazepam, (c) caffeine, (d) on the % protected head dips for PHE, (e) diazepam and (f) caffeine in elevated plus maze test. Data are presented as group Mean±SEM. Significantly different from control: *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test.

Fig. 8: Effects of acute PHE (30, 100 and 300 mg kg⁻¹), diazepam (0.1, 0.3 and 1.0 mg kg⁻¹) and caffeine (10, 30 and 100 mg kg⁻¹) on total distance travelled in the open field test. Data are presented as group Mean±SEM. Significantly different from control (Ctrl): *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test. Line plots (lower panels) 3D plots were generated from the time and XY data obtained (see Materials and Methods) using SigmaPlot Version 10 (Systat Software Inc., Point Richmond, CA, USA).

did not have any significant effect on the total distance travelled in the EPM compared to the vehicle treated animals. However, treatment with caffeine caused a significant (F₁₅,₇₅ = 5.80, p = 0.0070) decrease in the total distance travelled confirming its anxiogenic nature (Fig. 8c). Comparing the 3D line plots (Fig. 8), PHE and diazepam treated animals seemed to have made a greater number of visits into the open arms than the closed arms of the EPM which is indicative of their anxiolytic properties. By contrast, caffeine treated animals however made more closed arms entries than open arm entries.

Effect of flumazenil on anxiolytic properties of PHE and diazepam: Statistical analysis showed that PHE
Fig. 9: Effects of acute PHE (30, 100 and 300 mg kg\(^{-1}\)) and diazepam (0.3 mg kg\(^{-1}\)) treatment 15 min after pretreatment with flumazenil (3 mg kg\(^{-1}\), i.p.) on the (a) % number of open arm entries, (b) % time spent in the open arm, (c) % protected stretch attend postures and (d) on the % protected head dips in the elevated plus maze test. Data are presented as group Mean±SEM. Significantly different from control: *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls test and significant difference when the zonal entries where compared to each other: †p<0.05, ‡p<0.01, ‡‡p<0.001 (two-way repeated measures ANOVA followed by Bonferroni’s post hoc)

(30-300 mg kg\(^{-1}\)) as well as diazepam (0.3 mg kg\(^{-1}\)) increased both the percentage number of open arm entries (PHE F\(_{1,4}\) = 4.116; p = 0.0242) (diazepam F\(_{4,4}\) = 3.142; p = 0.0419) (Fig. 9a) and the percentage time spent on open arms (PHE F\(_{2,4}\) = 6.792; p = 0.0036) (diazepam F\(_{4,4}\) = 1.713; p = 0.3169) (Fig. 9b) significantly compared to control. The effect of benzodiazepine antagonist flumazenil alone was not significantly different from the control animals in all experiments (Fig. 9). The percentage number of open arm entries (F\(_{4,4}\) = 4.021; p = 0.0283) (Fig. 9a) as well as the percentage time spent on open arms (F\(_{4,4}\) = 3.752; p = 0.0117) (Fig. 9b) were significantly decreased when flumazenil was injected intraperitoneally 15 min before administration of diazepam. However, i.p. injection of flumazenil could not affect significantly the percentage number of entries (F\(_{3,3}\) = 2.597; p = 0.0883) induced by PHE administration (Fig. 9a) but it was able to decrease significantly the percentage time spent on the open arms (F\(_{4,4}\) = 4.0395; p = 0.0195) (Fig. 9b).

Similar effects were observed for the percentage numbers of protected stretch attend postures and head dips (Fig. 9c and d). The percentage numbers of protected stretch attend postures and head dips for flumazenil-treated animals alone was not significantly different from control. After administration of flumazenil, the numbers of protected stretch attend postures (F\(_{1,4}\) = 19.11; p = 0.0024) (Fig. 9c) as well as head dips (F\(_{1,4}\) = 43.92; p = 0.0002) (Fig. 9d) were significantly increased for diazepam in the presence of flumazenil compared to diazepam alone.

Just like diazepam, concomitant administration of flumazenil and PHE caused a significant increase in both the percentage numbers of protected stretch attend postures (F\(_{1,4}\) = 24.04; p = 0.0012) (Fig. 9c) and the protected number of head dips (F\(_{1,4}\) = 24.04; p = 0.0012) (Fig. 9d).

Effects on motor coordination in rota-rod: The result indicate that there is no significant difference in the duration on the rotating drum when the PHE treated mice (F\(_{5,5}\) = 1.668; p = 0.2074), imipramine treated mice (F\(_{3,3}\) = 0.9428; p = 0.4396) or fluoxetine treated mice were compared to the control group (F\(_{3,3}\) = 0.9711; p = 0.4256) (Fig. 10).
Effect on immobility periods in FST and TST:
*Palisota hirsuta* extract (30, 100 and 300 mg kg⁻¹ p.o.), fluoxetine (3, 10 and 30 mg kg⁻¹ p.o.) and imipramine (3, 10 and 30 mg kg⁻¹ p.o.) all administered 60 min before the test period, significantly decreased the immobility periods of mice in a dose dependent manner in both the FST and the TST when compared to control group, indicating significant antidepressant activity. Comparing the dose-response curves obtained in the FST (Fig. 11a), the curve for PHE (ED₅₀ 114.55±72.69) was found to be significantly different from that of both fluoxetine (F₁,₁₈ = 24.24, p<0.0001) and imipramine (F₁,₁₈ = 12.43, p=0.0013). The curves for fluoxetine (ED₅₀ 6.12±4.87) and imipramine (ED₅₀ 13.21±1.11) were also found to be significantly (F₁,₁₈ = 10.28, p=0.0005) different from each other. The order of the test drugs in terms of potency was fluoxetine > Imipramine > PHE.

From the dose-response curves obtained for the TST (Fig. 11b), all the drugs tested caused a reduction in the periods of immobility compared to the control. The curve for PHE was found to be significantly different from that of fluoxetine (F₁,₁₈ = 33.60, p<0.0001) and imipramine (F₁,₁₈ = 34.83, p<0.0001). However, the curves for imipramine and fluoxetine were not significantly different from each other (F₁,₁₈ = 0.202, p = 0.209) indicating similar antidepressant effect in the TST.

From ED₅₀ values (in mg kg⁻¹, Table 1) the potency of the drugs in the TST was in this order: fluoxetine (12.15±11.03) > imipramine (12.96±0.01) > PHE (70.42±0.06). The standard drugs decreased the immobility periods more than the *P. hirsuta* in both the FST and the TST, indicating that these drugs at the dose used in this study has greater efficacy as an antidepressant than the plant extract.

**Pretreatment with α-methyldopa:** Figure 12 shows the effects of α-methyldopa (MeDOPA) pretreatment on the behavioral effects of antidepressants in the TST. PHE (F₅,₁₈ = 1.10, p = 0.3776) and imipramine (F₅,₁₈ = 1.95, p = 0.1618) were not able to significantly attenuate or reverse the immobility induced by MeDOPA. Fluoxetine on the other hand, was able to significantly (F₅,₁₈ = 12.61, p = 0.0002) reverse the immobility in a dose-related manner. Pretreatment with MeDOPA significantly inhibited catecholamine synthesis as revealed by the inability of imipramine to reduce or reverse this immobility without producing significant effect on serotonin content as revealed by the ability of fluoxetine to reverse the immobility induced by MeDOPA.

**Pretreatment with reserpine:** From the effects of pretreatment with reserpine, fluoxetine (Fig. 12) was able...
DISCUSSION

Ethnomedical and pharmacological knowledge about this plant would allow us to presume that it has a depressant activity on CNS, which could be used to decrease anxiety or depression states in patients. The present work has shown anxiolytic activity by the ethanolic leaf extract of *Paliota hirsuta*, as assessed by the open field, light/dark box and elevated plus maze tests. The effect of the plant extract was qualitatively similar to that of diazepam, an anti-anxiety agent. Behavioral models used in the study are based on unconditioned responses to stimuli which are thought to be indicative of generalized anxiety symptoms in humans (Crawley, 1999).

The open field test is utilized to evaluate the animal emotional state. PHE increased the percentage of corner entries, an index of anxiety, as well as the percentage time spent in the central portion of the arena just like diazepam. Both the extract and diazepam caused a reduction in peripheral movement or thigmotaxis without having much effect on the total distance covered. The open-field model examines anxiety-related behavior characterized by the normal aversion of the animal to an open, brightly lit area (Choleris *et al.*, 2001; Mehan *et al.*, 2002). Thus, animals removed from their acclimatized cage and placed in a novel environment express anxiety and fear, by showing alteration in all or some parameters, such as decreases in ambulation and exploration time in the center of the open field with increased peripheral movement or thigmotaxis (Bhattacharya and Mitra, 1991). These parameters are attenuated by classical anxiolytics and potentiated by anxiogenic agents. Open field activity, therefore, represents a valid measure of "anxiety-like" behavior in drug-treated and genetically manipulated animals (Choleris *et al.*, 2001; Prut and Belzung, 2003).

The light/dark test is an ethologically-based approach-avoidance conflict test and it is sensitive to drugs that affect anxiety (Chauolff *et al.*, 1997; Costall *et al.*, 1989; Crawley *et al.*, 1997). In this experiment, PHE treated mice, just like diazepam, spent a significantly more time in the lit chamber of the box than control animals. However, both drugs failed to have any significant effect on the frequency of lit chamber entries. According to (Young and Johnson, 1991), the time spent in the illuminated compartment, rather than the number of transitions, is the most consistent and usual parameter for evaluating anxiolytic activity, while (Lepicard *et al.*, 2000) affirmed that the number of transitions reflected both anxiety and exploration, whereas the time spent in the light area was a stronger indication in the study of anxiety emphasizing that the latter is the most robust indicator in the anxiety study and that the first is also a sign of exploratory activity.
The elevated plus maze is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli (fear of a novel, brightly lit open space and fear of balancing on a relatively narrow, raised platform) that can induce anxiety in humans (Dawson and Tricklebank, 1995; Inaizumi and Onodera, 2000; Jung et al., 2000). This test has been demonstrated to be bi-directionally sensitive to both anxiolytic drugs; in particular benzodiazepines (Handler and Mithani, 1984; Lister, 1987; Pellow et al., 1985), as well as compounds which induce anxiety in man (Lister, 1987; Pellow et al., 1985; Pellow and File, 1986). Generally, an anxiolytic agent increases the number of entries into and the time spent in the open arms of the EPM. In agreement with previously published reports, diazepam increased the percentage time spent in open arms and open arm entries (Helton et al., 1998; Moser, 1989). In the present study, oral administration of an extract prepared from the leaves of *Psalisota hirsuta* induced an anxiolytic-like effect in mice, since it increased the percentage number of entries and the percentage time spent on open arms of the EPM test.

Etiological measures of risk assessment, such as stretched-attend postures and head-dipping, which have been validated and shown by factor analysis to be a more predictive determinant of anxiety were also used in addition to using the spatio-temporal indicators of anxiety in the EPM (Rodgers et al., 1997; Rodgers and Johnson, 1995). Both PHE and diazepam were able to markedly decrease the percentage protected forms of both stretch attend postures and head dipping indicating reduced anxiety/fear related behaviours. Exposure of animals to the extract did not have much effect on the total distance travelled in the EPM. However, comparing the total distance travelled in the EPM to that in the open field, the animals seem to move more in the open field than in the EMP which can be indicative of the aversiveness of the EPM. Treatment with flumazenil, a specific antagonist of the benzodiazepine site in the GABA<sub>A</sub> benzodiazepine receptor complex, was able to reverse the anxiolytic effect induced by both diazepam and the extract indicating that the effects are mainly mediated via the GABAergic system (Rowlett et al., 2001).

Data presented here also indicate that the ethanolic extract of *P. hirsuta* has an antidepressant-like effect in two widely-used animal models of depression. The forced swimming test has been used in preclinical tests to evaluate behavioral despair- a measure of failure to seek escape from an aversive stimulus (Crawley et al., 1997). FST has a high degree of predictive validity as shown by its sensitivity to major classes of antidepressants, tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), atypical antidepressants, selective serotonin reuptake inhibitors (SSRIs) and electroconvulsive therapy (Borsini and Meli, 1988; Dalvi and Lucki, 1995). In the tail suspension test, mice immediately engage in several agitation- or escape-like behaviors, followed temporally by increasing bouts of immobility. Like the forced swimming test, immobility is reduced by a broad range of pharmacological and somatic treatments (Cryan et al., 2004, 2005; Ferrault et al., 1992, Teate et al., 1990).

Though statistical analyses did not show any significant difference, the ED<sub>50</sub> for PHE and imipramine were lower in the TST thus confirming the superior sensitivity of the TST. However, fluoxetine was more effective in the FST in contrast to a report indicating that TST shows a greater sensitivity to antidepressant effects of 5-HT uptake inhibitors (Steru et al., 1987). Difference in experimental conditions may explain the differences in observations since factors such as strain and temperature affect such results (Porsolt et al., 2001).

To eliminate the involvement of compromised motor activity and coordination, the rotarod test was used to show that PHE at the doses used did not have such effects. In this study, an attempt was made to investigate the mechanism of the antidepressant action of PHE. The effect of pretreatment of mice with α-methyltyrosine and reserpine, are known to alter the monoaminergic systems. Consistent with views from the aminergic theories of depression, an earlier and lengthier literature has employed monoamine depletion strategies in animals to show that acute and/or adaptive changes in either serotonergic or noradrenergic transmission mediate the attainment by antidepressants of many depressive-like behaviors in animals. The monoamines, dopamine, serotonin (5-HT), noradrenaline and adrenaline in the frontal cortex play crucial roles in processes involved in the control of mood, cognition and motor behaviour functions that are compromised in depression (Millan et al., 2000). The α-Methyltyrosine, an L-aromatic amino acid decarboxylase inhibitor is known to inhibit the biosynthesis of catecholamines and 5-HT (DeMuth and Ackerman, 1983; Schiønnell et al., 1993). It was therefore, hypothesized that pretreatment with α-methyltyrosine will have more effect on catecholaminergic than the serotoninergic pathways. This is confirmed by our results which showed that the antidepressant effect of imipramine (a tricyclic antidepressant) was abolished by pretreatment with α-methyltyrosine whilst the SSRI fluoxetine reversed the effects of α-methyltyrosine. It must however be pointed out that imipramine is a non-selective inhibitor of monoamine transporters; inhibiting both NET and SERT (Iversen, 2006). In comparison, pretreatment with α-methyltyrosine similar to imipramine, though not quantitatively, abolished the antidepressant effects of the extract.

Pretreatment with reserpine increased baseline immobility and attenuated the effects of imipramine and
PHE in the TST but did not affect that of fluoxetine. The results obtained for imipramine in reserpine-pretreated mice is consistent with the effects of reserpine. Reserpine is an irreversible inhibitor of the vesicular monoamine transporter 2 (VMAT-2) which is located primarily within the CNS and is responsible for transporting monoamines from the cytoplasm into secretory vesicles (Ji et al., 2007; Metzger et al., 2002). Treatment with reserpine therefore leads to depletion of vesicular monoamine stores - both serotonin and noradrenaline (Fukui et al., 2007) suggesting both serotonin and noradrenaline might be important in the antidepressant effects of PHE as well as imipramine. The inability of reserpine pretreatment to affect the actions of fluoxetine seem to suggest that reserpine does not affect vesicular storage of 5-HT to the same extent as that of noradrenaline. At the dose level used in this experiment (O'Leary et al., 2007) showed that reserpine depleted tissue in the frontal cortex by 78%, 93 and 95%, respectively for 5-HT, noradrenaline and dopamine.

Putting these results together it may be inferred that actions of PHE were similar to the TCA, imipramine. However, the involvement of noradrenergic and dopaminergic systems seems to be greater in PHE than imipramine. Behavioral and biochemical data indicate the contribution of the dopamine (DA) neurotransmitter system in the pathophysiology of anxiety and depression (Rogoz et al., 2002a, b). It is also well established that stress activates the mesocorticolimbic DA system and increases extracellular DA in the nucleus accumbens septi and medial prefrontal cortex, inducing anxiolytic-like behavioral effects (Salamone, 1994). There is also evidence that stress induced increases in DA metabolism can be attenuated by anti anxiety drugs, such as diazepam (Decker and Moaugh, 1991). Animal studies (Costall et al., 1987; Pich and Samarin, 1986) showed that D2 receptor antagonists such as haloperidol present anxiolytic-like effects. Recent data (Rogoz et al., 2004) suggest that preferential receptor agonists may play a role in the therapy of anxiety and/or depression. In addition, continual administration of antidepressants, including imipramine, significantly augmented D1 receptor mRNA expression, in the nucleus accumbens and improved the activity of central mesolimbic D2 and D3 receptors (Maj et al., 1998). Based on these, the probable involvement of dopaminergic mechanisms in the CNS effects exhibited by the extract in this study cannot be overlooked.

It must however be pointed out that, PHE contains several secondary metabolites and therefore apart from the speculated GABAergic and dopaminergic involvement in the anxiolytic and anti depressant effects, other mechanisms and neurotransmitters may be involved such as, serotonergic and glutamatergic neurotransmissions. Further experiments, may be necessary therefore to confirm the exact mechanism/s involved in these CNS effects.

ACKNOWLEDGMENTS

The authors are grateful for the technical assistance offered by Messrs Thomas Ansah, Gordon Darku and George Ofei of the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi.

REFERENCES


