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Sedative, Anticonvulsant and Analgesic activities of Fresh Leaf Essential Oil of *Plectranthus aegyptiacus* from Southwest Nigeria in Mice

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Abstract

Background: The folkloric use of *Plectranthus aegyptiacus* (Forssk.) C. Chr. in the Southwest Nigeria includes; pain, sensory diseases, cough and fever. This study investigated the anxiolytic, sedative, anticonvulsant and analgesic activities of the essential oil of *P. aegyptiacus* in mice.
Methods: The oil was extracted by hydro-distillation. 5% Tween-80 (0.1 ml/10 g) was used as negative control and different standard drugs were used as positive controls depending on the model. The effect of the oil (50, 100, 150 and 200 mg/kg, *i.p*., n=6) on anxiety, sedation, convulsion, and analgesic activities were assessed on elevated plus maze (EPM), ketamine-induced hypnosis (100 mg/kg, *i.p*.), pentylenetetrazole (PTZ) (85 mg/kg, *i.p*.), strychnine (2 mg/kg, *i.p*.), maximal electroshock (MES), acetic acid-induced(1% v/v) writhings and the hot plate models respectively.
Results: The oil (50, 100 and150 mg/kg) significantly ($p< 0.01$, 0.05 and 0.01) increased the time spent on open arms of the EPM, completely blocked the hind limb tonic extension on the MES, and at 200 mg/kg, protected (100%) the mice against PTZ–induced mortality respectively. The oil at all tested doses significantly ($p<0.01$) shortened sleep latency and at (100 and 150 mg/kg) it significantly ($p<0.05$ and 0.01) prolonged total sleeping time respectively. The oil (150 mg/kg) significantly ($p<0.05$) reduced writhings, and at (50, 100 and 150 mg/kg) significantly ($p<0.05$, 0.01 and 0.05) increased the reaction time on the hot plate respectively.
Conclusion: The study concluded that the oil possessed anxiolytic, sedative, anticonvulsant and analgesic activities in mice.

Keywords: *Plectranthus aegyptiacus*; Sedative; Anticonvulsant; Analgesic; Anxiolytic.

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Background

According to World Health Organization, about 70 percent of the world's population relies on plants for their primary health care and some 35,000 to 70,000 species have been used as medicaments [1]. *Plectranthus*, a large genus containing about 300 species are found in Tropical Africa, Asia and Australia, and of the 300 species of *Plectranthus*, 62 species where reported to be used as medicines, ornamentals, foods, flavours and fodder. The plant, *Plectranthus aegyptiacus* is widely spread in tropical Africa, including Nigeria, where it is commonly known as *Efinrin-Oyinbo* and *Yenenu-Oyibo* among the Yoruba and Epie speaking people of southern Nigeria respectively.

Ethnomedicinally, *P. aegyptiacus* is used for treating pain, sensory diseases [2-3], ear ache, sore throat, respiratory system infections, and abdominal disorders [4]. Several studies have identified diverse chemical compounds in the essential oil of *Plectranthus aegyptiacus* and notable ones include myrcene, limonene, camphor, borneol, terpinen-4-ol, α-terpineol, α-cubebene, β-cubebene, caryophyllene oxide, *β*-caryophyllene among others [5-8], also reported octen-3-ol, *α*-terpinene, terpinene-4-ol, *γ*-terpinene, *α*-terpinyl acetate linalool, carvacrol, *β*-bourbonene, *β*-elemene, *α*-gurjunene, *γ*-elemene, aromadendrene, germacrene D, *γ*-muurolene and *α*-amorphene among others as constituents of the plant leaf. Some biological studies reported that *P. aegyptiacus* methanolic extract showed antiprotozoal activities [9], and free radical scavenging activity [9, 10].We recently determined the chemical composition of this oil and the results showed that 61 compounds were detectedwhile51 were fully identified with the major ones being carvacrol, germacrene-D, *p*-cymene and [1,1'-Bicyclopentyl]-2,2'-diol (Unpublished data). Preliminary bioassay assessment in our laboratory showed that the oil of *Plectranthus aegyptiacus* was moderately toxic intraperitoneally but slightly toxic orally, with LD_{50}values of 2154 and 490 mg/kg estimated for oral and intraperitoneal routes respectively, while its major effect was suggestive of CNS depression (Unpublished data).

Some documented traditional uses of *P. aegyptiacus* were found to be closely associated with the central nervous system [2-3]; hence, it became imperative to further evaluate the essential oil from its fresh leaf for possible central activities. In this present study, the essential oil of *P. aegyptiacus* extracted by hydro-distillation was evaluated for central nervous system activities in experimental animals using standard procedures and possibly validates some of its folkloric uses.

Methods

Plant Collection and Identification

The fresh leaves of *Plectranthus aegyptiacus* were collected between July and September 2016, from the wild on the campus of Obafemi Awolowo University (OAU), Ile-Ife, Southwest, Nigeria. The plant was initially identified and authenticated by Mr. I. I. Ogunlowo, Department of Pharmacognosy Faculty of Pharmacy, Obafemi Awolowo University, and further identified by Mr. G. A. Ademoriyo, Botany Department, Faculty of Science, OAU, Ile-Ife, Osun state, Nigeria, and herbarium specimen number IFE–17624 was issued.

Extraction of essential oil

The volatile fraction of *P. aegyptiacus* fresh leaf was obtained by hydrodistillation for about 4 h using Clevenger-like apparatus, at the Postgraduate Toxicology Laboratory, Department of Biochemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria. Pale yellow oil was obtained and dried over anhydrous sodium sulfate and stored in the refrigerator (4°C) until use. The density of the oil was determined and calculated to be 1.03 g/ml.

Equipment and Reagents

Weighing balance (Mettler Toledo, Fisher Scientific, Waltham (HQ), MA, US), animal cages
(Medwise, Mumbai, India), plexiglas cage, electroconvulsiometer (ECT Unit, Ugo Basile, Comerio (VA), Italy), Clevenger-type distillatory apparatus (The Scientific Instrument Company, Ambala, Haryana, India).

Drugs

Diazepam (Valium®, Roche, Switzerland), pentylenetetrazole (PTZ), strychnine (Sigma, Switzerland, MSDS), phenytoin sodium (Epanutin® Pharma- Deko Pharm. Co. Ltd, Lagos, Nigeria), Glacialacetic acid(BDH, England).

Experimental Animals

Adult male mice (18-25 g) obtained from the animal house, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria were used for the study. The mice were kept under standard laboratory conditions and fed with animal feed prior and throughout the period of experimentation. The ethical clearance for this research was obtained through the Faculty Postgraduate Committee and all animal experiment was carried out in strict compliance with the National Institute of Health (NIH, 1985) guideline on the use of laboratory animal [11] as being implemented by the Obafemi Awolowo University (OAU) Research Committee.

General experimental design

Animals were randomly selected and divided into 5 groups (n=6). Group I served as the negative control and received the vehicle (5% Tween 80, 10 ml/kg) only. Test groups II–IV were treated with the oil at doses of 50,100, 150 and 200 mg/kg respectively, while the positive control group received the appropriate reference drugs; diazepam (1 mg/kg, i.p., and phenytoin sodium (25 mg/kg, i.p.).

Effect of essential oil of *P. aegyptiacus* on elevated plus-maze (EPM) test

Thirty mice were randomly allotted into five groups (n=6). Group I was administered with 5% Tween – 80 (negative control, 0.1 ml/g, i.p.), groups II – IV were injected with various doses (50, 100, and 150 mg/kg, i.p.) of *essential oil of Plectranthus aegyptiacus* (EOPA) respectively. While group V was injected with 1 mg/kg, i.p. of diazepam (positive control). After 30 min pretreatment of the mice with all test materials, each mouse was tested singly on the EPM. The index of open arm avoidance was calculated.

Index of open arm avoidance (IOAA):

IOAA = 100 - [(**%** time spent in the open arms **+%** entries into open arms)***/2***] [12].

Effect of essential oil of P. aegyptiacus on ketamine-induced sleeping time

The test was carried out using the methods described earlier [13, 14], to determine the effects of essential

oil of *P. aegyptiacus* (EOPA) on sleep latency and prolongation of total sleeping time induced by the ketamine. Thirty mice were randomly allotted into five groups (n=6). Group I was administered with 5% Tween – 80 (negative control, 0.1 ml/10g, i.p.), II – IV were injected with various doses (50, 100 and 150 mg/kg, i.p.) of EOPA respectively. Group V was injected with 1 mg/kg, i.p. of diazepam (positive control). After 30 min pretreatment of the mice, ketamine (100 mg/kg, i.p.) was administered. The time interval between ketamine administration and loss of righting reflex was considered as latency to sleep (SL), while the time from the loss to the regaining of righting reflex was the duration or total sleeping time (TST).

Effect of essential oil of P. aegyptiacus on chemo-convulsion

Effect of essential oil of P. aegyptiacus on pentylenetetrazole (PTZ)-induced convulsion

The PTZ-induced convulsion model as described by [15, 16]was used. Thirty six (36) mice were randomly allocated into six groups (n=6). Group I was administered with vehicle (negative control), groups II-V were treated with 50, 100, 150 and 200 mg/kg i.p. of essential oil, while group VI was administered with diazepam (1 mg/kg, i.p.) as positive control. All the animals were pre-treated 30 min prior to administration of PTZ (85 mg/kg, i.p.). Each treated mouse was assessed for onset of convulsion or convulsion latency (CL) and time of death (TD) for 30 min. Percentage protection were calculated for each treated group and compared to controls (vehicle and reference drug). Animals that survived beyond 30 min post PTZ injection were assumed in this model as protected [12], and the time of death assumed to be 30 min for animals that survive beyond 30 min for the purpose of statistical analysis. The CL and TD were expressed in Mean ± SEM and statistically compared to vehicle group. Percentage protections were calculated as the ratio of animal that survive to the number of animal in each group.

Effect of essential oil of *P. aegyptiacus* on strychnine-induced convulsion

Strychnine has been shown to act by directly antagonizing glycine at spinal cord and brain stem. The procedure described in the previous section above was repeated using strychnine (STR) 3 mg/kg, i.p. as the convulsant agent [15, 16]. All the treatments were administered after pretreated 30 min prior to the injection of strychnine [12]. Each treated mouse was observed for onset of convulsion - convulsion latency (CL) and time of death (TD) for a total period of 30min period. Percentage protections were calculated for each treated group and compared to controls (vehicle and reference drug). The mean ±SEM were calculated for the onset of convulsion and time of death. Animals that survived beyond 30 min post STR injection were regarded in this model as protected [12]. The CL and TD were expressed as mean ± SEM and statistically compared using ANOVA, followed by Dunnett's post hoc. The ratio of animal that survive to the number of animals in each group was also estimated and expressed in percentage.

Effect of essential oil of P. aegyptiacus on electroshock-induced convulsion

The effect of essential oil of *P. aegyptiacus* (EOPA) on generalized seizures was evaluated by the maximal electroshock (MES) method as described [15]. Generalized seizures were induced with electroshock through the ear lobes by electrode clamp, which delivered an alternating current of constant frequency at 50Hz and 50mA for 0.2sec to elicit hind-limb tonic extension (HLTE) in the mice. Mice18 – 25g were randomly divided into five groups (n=6). Group I was administered with 5% Tween – 80 (negative control, 10ml/kg, i.p.), II – IV were treated with 50, 100 and 150 mg/kg, i.p. respectively of essential oil of *Plectranthus aegyptiacus* (EOPA), while group V was administered with phenytoin (25 mg/kg, i.p.). After 30 min of pre-treatment with all test materials, each mouse was subjected to the MES test. Protection against HLTE was taken as positive result.

Effect of essential oil of P. aegyptiacus on Thermo- and Chemo-induced pain

Effect of essential oil of P. aegyptiacus on acetic acid–induced writhings

The acetic acid writhing test in mice was conducted as described [17-18]. Thirty mice were randomly allotted into 5 groups (n=6). Food was withdrawn for 2 h before the experiment. Group I was administered with 5% Tween – 80, (0.1ml/10g i.p.) control, II–IV groups were treated with 50, 100, 150 mg/kg of essential oil of *P. aegyptiacus* (EOPA) intraperitoneally (i.p.) respectively, While group V was administered with diclofenac 100 mg/kg, i.p. (positive control). Thirty min after treatment with all test materials (5% Tween–80, EOPA and diclofenac), 1% acetic acid (10 ml/kg, i.p.) was administered to all groups I-V, and delayed for 5 min before assessment of each mouse for 20 min in the plexiglas cage. The number of writhings or abdominal constrictions displayed by each mouse was recorded and suppression of writhings was considered as a positive result [17-18].

Effect of essential oil of P. aegyptiacus on the hot plate

The hot-plate test was carried out using the method described [18]. In this test, thirty mice were randomly allocated into 5 group (n=6). Group I was administered with 5% Tween – 80, (0.1 ml/10 g, i.p. (control),II– IV groups were treated with 50, 100, 150 mg/kg of essential oil of *P. aegyptiacus* (EOPA) intraperitoneally (i.p.) respectively, while group V was injected with morphine 10 mg/kg i.p. as control. Thirty minutes after treatment with all test materials (5% tween – 80, EOPA and Morphine). Each mouse was dropped gently on the hot plate maintained at 55°Cand the time taken for the mouse to lick the fore or hind paw on the hot plate was taken as the reaction time. The test was carried out after treatment at intervals of 30 min for a total period of 120 min (i.e. 30, 60, 90 and 120 min). The cut off time was set at 30 s to avert damage to the animal's paw.

Statistical Analysis

The results obtained were expressed as Mean plus or minus Standard Error of the Mean (Mean ± SEM) and analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's comparison between the treated groups and controls. The level of significance was set at 95% confidence interval at $p<0.05$ for all treatment carried out compared to control groups. GraphPad Instat was used for the analysis of all results followed by Dunnett's post hoc test, while GraphPad Prism, version 5.01 (UK) was used for plotting the Graph. The results of the influence of antagonists on the effect of essential oil were analyzed with two-way ANOVA, followed by Student–Newman-Keuls post hoc test for comparison among the various groups.

Results

Effect of the essential oil of P. aegyptiacus on percentage time spent in open and closed arms/5 min on the EPM

The essential oil of *P. aegyptiacus* (EOPA) (50-150 mg/kg) and diazepam (1 mg/kg) showed a significant [p<0.05-0.01; $F_{(4, 25)}$ = 5.8] increase in the time spent in the open arms of the EPM by the mice compared to the vehicle. The various mean percentage times spent in the open arm of the EPM were found to be 71.5, 62.4, 67.4 and 67.27 for the EOPA (50, 100, 150 mg/kg) and diazepam (1mg/kg) respectively. The Index of open arm avoidance (IOAA) was also found to be 37.46, 43.11, 40.73 and 41.37 for EOPA (50, 100 and 150 mg/kg) and diazepam 1 mg/kg respectively (Table 1).

Effect of the essential oil of P. aegyptiacus on percentage entries into open and closed arm /5 min on EPM

The EOPA (50, 100 and 150 mg/kg, i.p.), and diazepam (1 mg/kg) showed no significant increase in number of entries into the open arms of the EPM by the mice compared to the vehicle (5% Tween-80). The various mean percentage number of entries into the open arm of the EPM were found to be 50.51, 53.59, 51.38, 51.15 and 50.00 for the vehicle, EOPA (50, 100, and 150 mg/kg) and diazepam (1 mg/kg) respectively (Table 2).

Index of open arm avoidance (IOAA)

The index of open arm avoidance was calculated for the EOPA and diazepam and the results summarized in Table 3.The Index of open arm avoidance (IOAA) was also found to be 37.46, 43.11, 40.73 and 41.37 for EOPA (50, 100 and 150 mg/kg) and diazepam 1 mg/kg respectively.

Effects of the essential oil on ketamine-induced sleep latency (SL) in mice

The EOPA (50-150 mg/kg, i.p.) dose-dependently and diazepam (1 mg/kg, i.p.) caused significant ([p<0.01, $F_{(4, 25)}$ = 12.6] reduction in the sleep latency (SL) induced by ketamine (100 mg/kg, i.p.) compared to the vehicle (Figure1A)

Effects of the essential oil on ketamine-induced total sleeping time (TST) in mice

The EOPA (50-150 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) caused significant [p<0.01; $F_{(4, 25)}$ = 18.2] increase in the total sleeping time (TST) induced by ketamine (100 mg/kg, i.p.) compared to the vehicle (Figure 1B).

Effects of the essential oil on pentylenetetrazole (PTZ)-induced convulsion test

The results of the effect of the oil on PTZ-induced convulsion are presented in Table 4.The EOPA (50, 100, 150 and 200 mg/kg) caused no significant increase in the convulsion latency (CL) compared to the vehicle respectively. While diazepam (1 mg/kg, i.p.) caused a significant [p<0.01; $F_{(5, 30)}$ = 12.3] increase in CL compared to the vehicle. The EOPA (100, 150 and 200 mg/kg) and diazepam (1 mg/kg) caused significant [p<0.01; $F_{(5, 30)}$ = 31.5] increase in the time of death (TD) compared to the vehicle. The EOPA (50 mg/kg) showed no significant prolongation of the time of death (TD) compared to the vehicle. Furthermore, it was found that, the oil at 100, 150 and 200 mg/kg offered 16.7%, 16.7% and 100 % protection for the mice respectively. Diazepam 1 mg/kg also offered 100% protections for the animals.

Effects of the essential oil on strychnine-induced convulsion test

The EOPA (50, 100 and 150 mg/kg, i.p.) caused insignificant prolongation in the convulsion latency (CL) and time of death compared to the vehicle (5% Tween-80). However, diazepam (5mg/kg) caused significant [p<0.01, $F_{(4, 25)}$ = 12.0] and [p<0.01, $F_{(4, 25)}$ = 6.4] prolongation of convulsion latency and time of death in mice respectively compared to the vehicle. Diazepam (5 mg/kg, i.p.) also offered 33.33% protections against strychnine-induced mortality (Table5).

Effects of the essential oil on maximal electroshock (MES) – induced convulsion test

The results obtained on the MES test showed that all the mice in the vehicle (5% Tween-80) group displayed spontaneous hind limb tonic extension (HLTE). However, all the animals pretreated with the oil (50, 100 and 150 mg/kg, i.p.) and phenytoin (25 mg/kg, i.p.) blocked the HLTE in the MES test and there was no mortality in all the treatment groups (Table 6).

Effects of the essential oil on acetic acid-induced writhings in mice

The essential oil (50, 100 and 100 mg/kg, i.p.) and diclofenac(50 mg/kg, i.p.) similarly, caused significant [p<0.01, $F_{(4,25)=}$10.6] reduction in the number of acetic acid–induced abdominal writhings compared to the vehicle (Figure 2). The percentage of analgesia produced by the EOPA (50, 100 and 150 mg/kg) and the diclofenac (50 mg/kg) were 41.41, 48.95, 49.22 and 56.52 respectively.

Effects of the essential oil on hot-plate nociceptive test in mice

The reaction time for all doses of the essential oil of *P. aegyptiacus* (50, 100 and 150 mg/kg) showed significant increase [p<0.05-0.01, $F_{(4,25)}$ = 7.0] at 90 min post treatment compared to the vehicle, while EOPA (100 and 150 mg/kg) also significantly [p<0.05, F (4, 25) = 5.0] increased reaction time compared to the vehicle at 120 min. However, the control (morphine, 10 mg/kg) also increased the reaction time significantly [p<0.01, $F_{(4, 25)}$ = 9.9] and [p<0.01, $F_{(4, 25)}$ = 4.5] at 30 and 60 min compared to the vehicle respectively (Figure 3).

Discussion

This study evaluated the effects of essential oil of *P. aegyptiacus* (EOPA) on anxiolytic, sedation, convulsion and nociception in mice. The results obtained from this research work, showed that the oil exhibited significant effect on the central nervous system. Anxiety is a known state of intense apprehension, uncertainty and fear resulting from the anticipation of a threatening event or situation, often to a degree that normal physical and psychological functioning is disrupted. Studies have demonstrated that anxiety disorders have the highest lifetime prevalence estimates (13.6–28.8%) and the earliest age of onset (11 years) of psychiatric disorders [19-20]. Therefore, the search for new therapeutic agents continues, and medicinal plants have emerged as a crucial source for the development of drugs to treat neurological disorders and play an important role for patients who respond poorly to conventional treatments [21]. Previous studies have revealed that

the conventional plus-maze is highly sensitive to the influence of benzodiazepine/ $GABA_A$ receptor-related manipulations[12, 22-23].

The oil (50-150 mg/kg) caused a significant ($p<0.05$-0.01) increase in the time spent on the open arms of the EPM compared to the vehicle. There was however no significant difference in the number of entries into the open arms of the EPM for the oil or diazepam compared to the vehicle. On analysis of index of open arms avoidance (IOAA), the values obtained were 37.46 – 43.11 for the oil and 41.37 for diazepam (1 mg/kg). Based on these results, it can be suggested that the oil of *P. aegyptiacus* demonstrated a significant anxiolytic activity in this model.

Sedatives are known for their inhibitory effect on the CNS, which is caused by either augmentation of GABA inhibitory effect by binding to $GABA_A$ receptor like benzodiazepines, or antagonizing the effect of glutamate by blocking glutamate receptors such as N-methyl-D-aspartate (NMDA) AMPA, kainate, glycine or metabotropic receptors [24-26]. The oil (50 - 150 mg/kg) significantly ($p<0.001$) reduced sleep latency (SL), but at 100 and 150 mg/kg, it caused significant ($p<0.05$-0.01) prolongation of total sleeping time (TST) in the mice induced by ketamine (100 mg/kg, i.p.). The decrease in SL and prolongation of TST are indications of sedative activity of the oil [13-14], thus further confirming exhibition of inhibitory effect as earlier shown on our novelty-induced behavioural (NIB) studies.

The pentylenetetrazole (PTZ) is an antagonist at $GABA_A$ receptor complex and produces seizures by inhibiting the GABA pathway in the CNS resulting in an imbalance between the ionic concentrations of the membrane [27-29]. Drug protections against the tonic-clonic seizures induced by PTZ are considered to be useful to control myoclonic and absence seizures (most common in childhood) in human [27]. Studies have shown that activation of N-methyl-D-aspartate (NMDA) receptor is involved in the initiation and generalization of PTZ-induced seizures [28]. The effect of the oil on the PTZ- induced convulsion showed no significant prolongation in convulsion latency (CL) compared to the vehicle (5% Tween - 80). Diazepam (1 mg/kg), caused a significant ($p<0.01$) increase in convulsion latency (CL) compared to the vehicle. The EOPA at 100, 150 and 200 mg/kg significantly ($p<0.01$) increased the time of death (TD) in mice compared to the vehicle, while at 50 mg/kg of the EOPA, there was an insignificant prolongation in TD compared to the vehicle. Furthermore, it was found that, the oil at 100, 150 and 200 mg/kg offered 16.7%, 16.7% and 100% protection in the mice respectively, while diazepam (1 mg/kg) also offered 100% protection against PTZ-induced convulsion in the animals. It can therefore be suggested that, the oil at high concentration possessed significant activity in this model.

Strychnine act by directly antagonizing glycine at spinal cord and brain stem, thus increasing spinal reflexes [15]. The EOPA at all test doses (50, 100 and 150 mg/kg, i.p.) did not significantly alter the strychnine-induced convulsion, as there was no significant increase in CL and TD compared to the vehicle. However, diazepam (5 mg/kg, i.p.) significantly ($p<0.01$) increase the CL and TD compared to the vehicle. Diazepam at 5 mg/kg offered 33.33% protection for mice in the study, compared to 0.00% protection offered by the EOPA and vehicle.

The maximal electroshock (MES) is a widely used tool to screen drugs for generalized tonic-clonic seizures. MES basically causes disruption of signals (impulse) transduction in the neurons, and damage cell due to facilitation of Ca^{2+} influx into the cell, thus resulting to prolongation of duration of convulsion [30-31]. Apart from Ca^{2+} ion, MES may also facilitate the influx of other positive ions like Na^{+} which blockade by drugs can prevent MES- induced tonic extension [31]. Currently available anticonvulsant drugs like phenytoin and valproate act by modulation of these ion channels [32-33]. The results obtained for the MES test indicate that the mice in the vehicle group demonstrated spontaneous hind limb tonic extension (HLTE) induced by the maximal electroshock (MES). The EOPA at all the doses (50, 100 and 150 mg/kg) used in this study completely blocked the HLTE caused by the MES. Similarly, phenytoin (25 mg/kg) also blocked HLTE in this model. Therefore, the results obtained from the PTZ and MES- induced convulsion tests strongly indicate that the oil demonstrated significant anticonvulsant activity possibly through facilitation of GABAergic neurotransmission. Chemical agents that protect against PTZ-induced tonic–clonic are considered to be useful in managing myoclonic and absence seizures in humans [34].

Ethno-medicinally, *P.aegyptiacus* is used for treating sensory diseases [2, 3]. Hence, this present finding validates the ethno-medicinal application of this plant.

The acetic acid induced test was used to evaluate the peripheral analgesic activity of the EOPA in mice. The EOPA (50 and 100 mg/kg, i.p.) doses caused insignificant decrease in the acetic acid-induced writhings compared to the vehicle. However, at 150 mg/kg, the oil and diclofenac (50 mg/kg) caused a significant ($p<0.05$) decrease in the acetic acid-induced writhings compared to vehicle. The EOPA also at all doses (50, 100 and 150 mg/kg, i.p.) offered 32.03 %, 35.16% and 43.23% analgesia respectively, while diclofenac (50 mg/kg, i.p.) offered 48.70 % analgesia, signifying peripheral analgesic activity [35]. Prostaglandins are known chemical mediator that induced abdominal contraction by activating and sensitizing the peripheral chemo-sensitive nociceptors which are mostly responsible for causing inflammatory pain [36]. Non-steroidal anti-inflammatory drugs like diclofenac, blocks prostaglandins synthesis, reduce nociception [37]. The result obtained here showed that the EOPA possessed significant peripheral analgesic activity similarly to essential oils of other medicinal plants [38-39].

The hot-plate test is commonly used to investigate nociception and analgesia in rodents; especially central analgesic effects of morphine and other narcotic analgesics [17-18]. The presynaptic central axonal terminal is a major site of the action of opioids, cannabinoids, γ-aminobutyric acid (GABA) receptor ligands, greater success is achieved by pharmacologic manipulation of inhibitory circuits, in particular ligands for the μ-opioid receptor, which mediate all of the actions of morphine [40]. The EOPA (100 and 150 mg/kg) significantly ($p<0.05$) prolonged the reaction time on the hot plate nociceptive test at 90 and 120 min respectively compared to the vehicle signifying central analgesic activity [40]. There was no significant increase on the reaction time at 50 mg/kg compared to the vehicle, whereas the standard drug (morphine at 10 mg/kg, i.p.) significantly ($p<0.01$) prolonged the reaction time at 30 and 60 min respectively, post administration compared to the vehicle. While morphine peak effect was observed to be at 60 min after which its effect became negligible, the oil showed a peak effect at 120 min post administration. The results obtained from the hotplate test and acetic acid – induced writhings strongly suggested that the oil possesses strong analgesic activity which may be mediated centrally and peripherally [37, 40]. Thus, these findings justified the ethno-medicinal uses of the plant in the treatment of pains and related conditions. The results obtained here corroborated previous study in which *P. amboinicus*, the same genera of the plant was reported to possess analgesic and anti-inflammatory activity. Hence, this result can be used to validate the folkloric use of the plant.

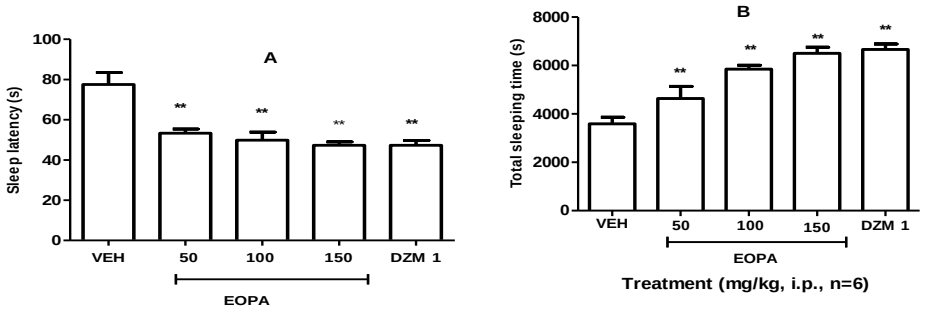

Figure 1. Effects of the essential oil on ketamine-induced sleep latency (Panel A) and total sleeping time (Panel B) in mice
Bars represent mean values with standard error of mean ±SEM, (n=6). VEH, EOPA and DZM represent vehicle (5% Tween 80, 0.1 ml/10 g), essential oil of *P. aegyptiacus* and diazepam respectively. **p<0.01, statistically significant compared to vehicle (B) (ANOVA, Dunnett's test)

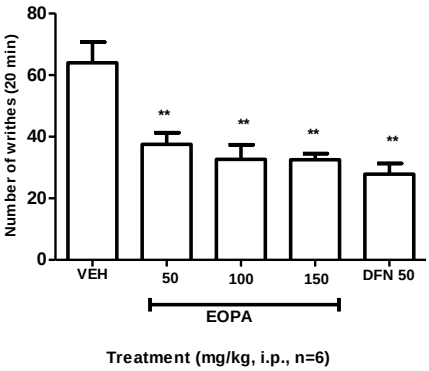

Figure 2. Effect of EOPA on the acetic acid-induced abdominal writhings in mice
VEH, EOPA and DFN represent vehicle (5% Tween 80), essential oil of *P. aegyptiacus* and diclofenac respectively.**p<0.01 statistically significant compared to the vehicle (ANOVA, Dunnett's post hoc test)

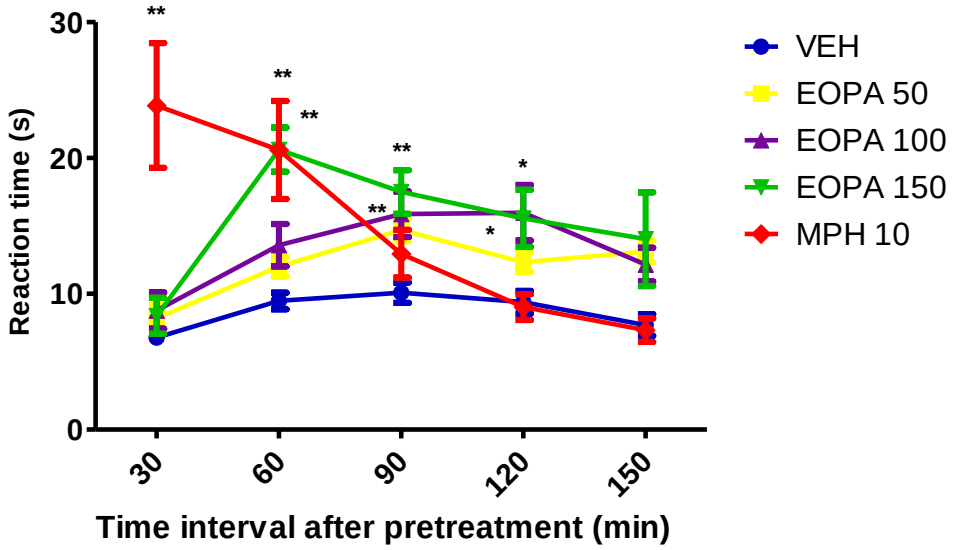

Figure 3. Effect of EOPA on the reaction time of mice on the hot plate
VEH, EOPA AND MPH represent vehicle, essential oil of *P. aegyptiacus* (50, 100 and 150 mg/kg, i.p.)and morphine (10 mg/kg, *i.p.*) respectively.n=6. *p<0.05, **p<0.01; compare to vehicle (ANOVA, Dunnett's test)

Table 1. Percentage time spent in open and closed arms/300 seconds on the EPM

	Treatment groups, *i.p.* (n=6)									
	VEH (0.1 mg/10g)		**EOPA (50 mg/kg)**		**EOPA (100 mg/kg)**		**EOPA (150mg/kg)**		**DZM (1 mg/kg)**	
	OA	**CA**	**OA**	**CA**	**OA**	**CA**	**OA**	**CA**	**OA**	**CA**
M±SE M	121.7±10.50	178.±10.64	214.5±26.95**	85.50±26.95	187.2±7.02*	112.±7.02	202.00±13.49**	98.00±13.49	201.80±10.06**	98.17±10.06
%	**40.57**	**59.43**	**71.5**	**28.5**	**62.4**	**37.6**	**67.4**	**32.6**	**67.27**	**32.73**

VEH = vehicle (5% Tween 80); EOPA= essential oil of *Plectranthus aegyptiacus*; DZM = diazepam, OA and CA = (open and closed arms)
*p<0.05- 0.01 statistically significant compared to the vehicle (ANOVA, Dunnett's test).

Table 2. Percentage number of entries into open and closed arm/5 min on EPM

	Treatment groups, *i.p.* (n=6)									
	VEH (0.1 mg/10 g)		**EOPA (50 mg/kg)**		**EOPA (100 mg/kg)**		**EOPA (150 mg/kg)**		**DZM (1 mg/kg)**	
	OA	**CA**	**OA**	**CA**	**OA**	**CA**	**OA**	**CA**	**OA**	**CA**
M±SE M	8.50 ± 1.26	8.33 ± 1.31	5.00 ± 1.18	4.33 ± 0.99	9. 33 ±1.52	8. 83 ±1.40	7.33 ±1.93	7.00 ± 1.89	6.00 ± 0.58	6.00 ± 0.58
%	**50.51**	**49.49**	**53.59**	**46.41**	**51.38**	**48.62**	**51.15**	**48.85**	**50**	**50**

VEH = vehicle (5% Tween 80); EOPA= essential oil of *P. aegyptiacus*; DZM = diazepam, OA and CA = (open and closed arms)

Table 3. Index of open arms avoidance (IOAA) on the EPM

	Treatment groups, *i.p.* (n=6)			
	EOPA (50 mg/kg)	**EOPA (100mg/kg)**	**EOPA (150mg/kg)**	**DZM (1 mg/kg)**
%TSOA	**71.5**	**62.4**	**67.4**	**67.27**
%EOA	**53.59**	**51.38**	**51.15**	**50**
IOAA	**37.46**	**43.11**	**40.73**	**41.37**

IOAA=100 - [(% time spent in open arms + % entries into open arms)/2]
IOAA, EOPA, %TSOA, %EOA and DZM represent, index of open arms avoidance, essential oil of *Plectranthus aegyptiacus*, % time spent in open arms, % entries into open arms and diazepam respectively.

Table 4. Effects of essential oil of *P. aegyptiacus* on pentylenetetrazole-induced convulsion test in mice

Treatment (*i.p.*) n=6	**Convulsion Latency (Mean±SEM) (S)**	**Time of Death (Mean±SEM) (S)**	**% Protection**
Vehicle 0.1 ml/10 g	52.69±3.18	4.83±0.60	0.00
EOPA 50 mg/kg	98.17±5.21	8.50±0.76	0.00
EOPA 100 mg/kg	153.00±29.95	14.17±3.34**	16.7
EOPA 150 mg/kg	280.67±10.17	15.00±3.10**	16.7
EOPA 200 mg/kg	312±12.29	30.00±0.00**	100
DZM 1 mg/kg	1288.2 ±56.01**	30.00±0.00**	100

Vehicle, EOPA and DZM represent vehicle (5% Tween 80), essential oil of *Plectranthus aegyptiacus* and diazepam respectively.
*p<0.05 – 0.01, statistically significant compared to the vehicle (ANOVA, Dunnett's test)

Table 5. Effects of essential oil of *P. aegyptiacus* on strychnine-induced convulsion test in mice

Treatment (*i.p.*) n=6	**Convulsion Latency (Mean±SEM) (s)**	**Time of Death (Mean±SEM) (s)**	**% Protection**
Vehicle 0.1 ml/10 g	252.50 ± 20.28	5.50 ± 0.72	0.00
EOPA 50 mg/kg	266.67 ± 16.22	11.17 ± 1.54	0.00
EOPA 100 mg/kg	164.00 ± 23.06	4.83 ± 0.48	0.00
EOPA 150 mg/kg	151.00 ± 22.29	3.50 ± 0.34	0.00
DZM 5 mg/kg	396.17 ± 47.46**	17.00 ± 3.08**	33. 33

Vehicle, EOPA and DZM represent vehicle (5% Tween 80), essential oil of *P. aegyptiacus* and diazepam respectively.
**p<0.01 statistically significant compared to the vehicle (ANOVA, Dunnett's test)

Table 6. Effect of essential oil of *P. aegyptiacus* on maximal electroshock (MES)

Treatment *i.p.* (N=6)	MES induced HLTE	Duration of HLTE (s) Mean±SEM	Protection against HLTE (%)	Mortality (%)
Vehicle 0.1 ml/ 10 g	6/6	79.33±15.92	0	0
EOPA 50 mg/kg	0/6	0±0.00**	100	0
EOPA 100 mg /kg	0/6	0±0.00**	100	0
EOPA 150 mg/ kg	0/6	0±0.00**	100	0
PHNY 25 mg/ kg	0/6	0±0.00**	100	0

VEH, EOPA and PHNY represent vehicle (5% Tween 80), essential oil of *P. aegyptiacus* and phenytoin respectively.
**p<0.01 statistically significant compared to the vehicle (ANOVA, Dunnett's test)

Conclusions

It is hereby concluded that the essential oil of *P. aegyptiacus* displayed significant depressant activity on the central nervous system, exhibited significant analgesic, sedative and anticonvulsant activities in mice. Therefore, the various CNS effects of the oil that have been shown in this research inferentially established the pharmacological basis for the use of the plant traditionally in the treatment of pain, and central nervous system diseases.

Authors' Contribution

Author EDA was the Masters' student that carried out the studies for his research work under the direct supervision of author IAO and carried out all the pharmacological animal experiments in collaboration with author IAO. Author IAO was the supervisor of author EDA. He conceived and initiated the work, designed the project with author AOO. He coordinated the preparation and submission of the manuscript for publication. Author IJO was a PhD student in the Department of Biochemistry. He participated in the initial design of the work, personally supervised the hydrodistillation of the plant materials to obtain the essential oil used in the study. Author AOO was the postdoc-supervisor of author AIO. She participated in the selection of the plant, design of the work, carried out the GC/MS analysis and identified the components of the essential oil.

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Conflict of interest

The authors declare that they have no competing interests.

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