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ISOLATION OF NOVEL PARA-PENTYL PHENYL BENZOATE FROM *MONDIA WHITEI.*(HOOK.F.) SKEELS (PERIPLOCACEAE), ITS STRUCTURE, SYNTHESIS AND NEUROPHARMACOLOGICAL EVALUATION.

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Abstract

Background: *Mondia whitei* L. (Hook. F.) Skeels (Periplocaceae) is a medicinal plant used locally in managing pain, fever, loss of appetite and as aphrodiasc in the South-Western states of Nigeria. However, the fruit is consumed habitually in the South-Eastern states of Nigeria, leading to speculation that it may possess some central nervous system effect but which has not been scientifically investigated, hence this study.
Methodology: Fresh fruits of *Mondia whitei* were collected and identified by a taxonomist. They were chopped into small pieces and extracted with absolute ethanol. The crude extract was subjected to various chromatographic techniques to isolate a novel compound whose structure was elucidated from the analysis of the crystal data and by extensive use of spectroscopy. The structure was confirmed by synthesis. The compound was subjected to anxiolytic and sedative activity assay. Computational analysis of the receptor binding event of isolated compound at the gamma amino butyric acid A receptor was also evaluated.
Results: The structure of the compound was elucidated as *para* pentyl phenyl benzoate. The neuropharmacological evaluation of the compound indicated significant ($p<0.05$) depression of the central nervous system. The binding characteristics of the compound to gamma amino butyric acid A receptors appears to be more favorable than those obtained for gamma amino butyric acid, chlorpromazine, benzamidine, and is comparable with the affinity obtained for pentobarbitone and diazepam.
Conclusion These present data provide evidence for the role of *para* pentyl phenyl benzoate in the habitual consumption of the fruit as well as its central nervous system activities.

Keywords: *Mondia whitei,* Periplocaceae, isolation and synthesis, sedative and anxiolytic effect and Para pentylphenyl benzoate.

Introduction

Over the years, the use of medicinal plants in managing variety of diseases has increased, particularly in developing countries (Lahlou, 2007). This increase has been linked to several factors including the report of health beneficial effects of natural products, especially in higher plant-derived extracts and products, with novel bioactive compounds discovered and serving as 'lead' in the discovery and development of useful therapeutic agents (Vuorelaa et al., 2004). In every traditional society, people are faced with several herbal options for food and ailments. The choices of these herbs and the consumption pattern of people evolve over time and are affected by demographic factors (Vabo and Hansen, 2014) as well as societal and cultural development of the people (Drewnowski, 1997; Mela, 1999; Palojoki and Tuomi-Grohn, 2001; Wright et al., 2001; Risvik et al., 2006). A very important consideration about the consumption pattern of herbs in a society is the evaluation of how safe the herbs are and how healthy the consumption pattern is. In essence, there is a need to advance scientific basis to justify the consumption of any herb. *Mondia whitei* L. (Hook. F.) Skeels (Periplocaceae) is a vigorous, woody climber growing to about 3-6 m high. It has an attractive heart shaped leaves and a

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vanilla aroma (Simeon et al., 2007). The dried roots of the plant had been used in traditional medicine for appetite stimulation, pain, indigestion, gastrointestinal disorders, infections, postpartum bleeding *etc.* (Kokawaro, 1976, Neuwinger, 2000, Aremu et al., 2011). The root of the plant is reputed to possess aphrodisiac activity in folkloric medicine, across many African countries. (Watcho et al., 2005, Simeon et al., 2007). Various benzaldehyde derivatives and lignans *e.g* propacin, chlorinated coumarinolignan (Kubo and Kinst-Hori, 1999, Patnam et al., 2005) and isovanillin (Koorbanally et al., 2000) had been reported from the root of the plant. The ripe fruits of this plant are habitually eaten in the Eastern states of Nigeria (Pesonnal communication from Chief Gabriel Ibhanaesebor, former curator at the Department of Botany, Obafemi Awolowo University), especially amongst the restive populace, suggesting it might possess some central nervous activity (CNS) activities. Inspite of the consumption pattern of the ripe fruits, there is scanty report on the phytochemical profile of the fruit and evaluation of its CNS activities which can provide insight into the habitual consumption, hence this study.

Materials and Methods

^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker AV400 (IconNMR) Spectrometer at 400 and 100 MHz respectively while the IR spectrum was acquired on an FT PerkinElmer spectrophotometer and LC-ESI-MS was carried out at the Analytical Centre of the University of Bradford, United Kingdom. Adsorption chromatography (open column) was performed with Kieselgel 60 (ASTM 230–400 mesh, Merck). All Thin Layer Chromatography (TLC) analyses were performed at ambient temperature using analytical silica gel 60 GF_{254} pre-coated aluminum backed plates (Merck, 0.25 mm thick). The resulting spots on TLC plates were visualized under UV light (254 nm) and detected by the use of 10% conc. H_2SO_4 in methanol. Solvents used for extraction and for chromatography were distilled before use. 4-Pentylphenol was obtained from AK Scientific (Union City, CA 94587) United States.

Phytochemical and chemical studies

Plant material collection and identification

Collection of plant material

Fresh fruits of *Mondia whitei* (3.0 kg) were collected from the Obafemi Awolowo University (OAU) Staff School premises, Ile Ife, Nigeria on October 15th, 2011. The vegetative parts were identified by Mr Ogunlowo I.I. of the Department of Pharmacognosy, Obafemi Awolowo University and a voucher specimen with number IFE 17,444 was deposited in IFE Herbarium, Department of Botany, Obafemi Awolowo University. The fruits were chopped into smaller pieces and macerated immediately in absolute ethanol for two days.

Isolation of compound 1 (Para pentylphenyl benzoate (pPPB)).

The crude extract from the macerated plant parts was concentrated *in vacuo* to give 71.3 g (2.4% w/w of fresh fruit). The crude extract was dissolved in 500 ml of water and 2.0 L of acetone was added. The acetone soluble portion was concentrated *in-vacuo* to give 25 g of the acetone soluble fraction. 10 g of this was adsorbed **on** 300 g of silica gel mesh 230-400 and eluted on open column with mixtures of the following solvents in increasing polarity; Dichloromethane (DCM) 100% (200 mls), DCM: ethyl acetate (EtOAc) (1:1, 500 mls), EtOAc (100%, 500 mls), EtOAc : methanol (MeOH) (8:2, 300 mls), EtOAc : MeOH (6:4, 600 mls), MeOH (100%, 300 mls). Eluted fractions of about 15 mls collected in test tubes were analyzed on thin layer chromatography (TLC) using the following solvent mixtures, hexane: DCM (6:4, solvent system one), DCM:EtOAc (1:1, solvent system two), EtOAc (100%, solvent system three), EtOAc:MeOH (8:2, solvent system four) and EtOAc:MeOH:H2O:AcOH (10:2:1:0.2, solvent system five). Spots were detected under the ultra-violet light at 254 and 366 nm, and the plates were sprayed with 10% sulfuric acid to give five fractions MW 1-5. MW1 (0.357 g) on a TLC plate gave a spot that did not give any reaction with the sulfuric acid spray but fluoresced under the UV light at 254 and 366 nm. MW1 was purified on silica gel on an open column with 300 mls of DCM (100%), DCM:EtOAc (1:1, 200 mls) and DCM:EtOAc (7.5: 2.5, 300 mls). The eluates were analyzed on TLC plates using solvent system one, leading to two sub-fractions: MW1a (0.034 g) and MW1b (0.285 g). MW1a containing the UV-active spot was purified further on silica gel to give compound **1**, (*p*PPB) (0.0098 g, 0.00033 %w/w, R_f 0.56 (DCM:*n*-Hexane (1:1)). *p*PPB was allowed to undergo slow evaporation from 100% DCM at room temperature to grow the crystal. The crystal was subsequently subjected to X-ray crystallographic analysis.

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Synthesis of *p*PPB

Freshly cut sodium metal (0.032 g, 0.0014 mol.) was slowly added to *n*-butanol (5 mls) in a 250ml round bottom flask and allowed to dissolve. 4-Pentylphenol (7.2 g, 0.0423 mol) was added to the basic solution. Benzoyl chloride (6.1 g, 0.0434 mol) was added to the mixture, stirred and allowed to stand at room temperature overnight. The gel-like product obtained was purified on silica gel using isocratic elution with 100% *n*-hexane to obtain *p*PPB (92% w/w).

Pharmacological studies

Drugs

Diazepam (Valium® Roche, Switzerland), Chlorpromazine HCl (Bavaria Pharma, Vadodara-Gujarat, India), Pentobarbitone sodium (BDH Chemicals Ltd, England), dimethyl sulphoxide (DMSO: Alpha Chemika, Mumbai, India), normal saline (Juhel Pharm., Nigeria) were purchased from the Pharmacy shop.

Experimental animals-

Male Swiss Albino mice (18-25 g) were obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, OAU, Ile-Ife. The EU Directive 2010/63/EU for animal experiment was followed as being implemented by the University Research Committee through the Faculty of Pharmacy Postgraduate Committee, OAU, Ile-Ife, Nigeria. The animals were kept under standard laboratory conditions, allowed free access to standard animal feed and water *ad libitium*. The experiments were carried out between the hours of 8.00 am and 4.00 p.m.

Acute toxicity test

The acute toxicity of the synthesized compound, parapentylphenyl benzoate (*p*PPB) was determined intraperitoneally using Lorke's method (Lorke, 1983) and further described by Oyemitan et al., (2009). The experiment involved two phases. The first phase was an initial dose-finding phase using the dose levels of 10, 100 and 1000 mg/kg (n=3). In the second phase, 4 dose levels of 1000, 1600, 2900 and 5000 mg/kg (n=1) were used. The mice were monitored for 30 min. for general behavioural effect and further for 24 h for mortality. The LD_{50} was calculated using the formula:
$LD_{50} = (L \times H)^{1/2}$,
where L = Lowest dose that kills the animal, H = Highest dose that did not kill the animal.
The working doses were then determined as follows: $< ½\ LD_{50}$

Anxiolytic effect of *p*PPB

Open field test (OFT)

Mice were randomly allotted to control and test groups. The negative control mice were administered with the vehicle 10 % DMSO/normal saline (10 ml/kg, i.p.), while the positive control group received diazepam (1 mg/kg, i.p.). Graded doses, (100, 200 and 300 mg/kg, i.p.) of *p*PPB were administered to the test groups thirty min after treatment each mouse was placed in the center of the open field and behavioural activities such as locomotion, rearing frequency and grooming were counted. Spontaneous motor activity was monitored for 10 min. (Eckeli et al., 2000). Before introducing each animal, the apparatus was carefully cleaned with dilute alcohol to eliminate the possible bias due to the odour that could be left behind by the previous subject.

Elevated plus-maze test (EPM)

The animals were randomly divided into negative control, positive control and test groups (n=6). The negative control mice received vehicle, 10% DMSO/normal saline (10 ml/kg, i.p.). The positive control group received diazepam (1 mg/kg, i.p.). Graded doses (100, 200 and 300 mg/kg, i.p.) of *p*PPB were administered to the test groups. 30 min. after treatment, each mouse was placed at the center of the plus maze and allowed to explore the maze for five min. During the 5min. test period, the following measurements were recorded: the number of open and closed arms entries and the time spent in open and closed arms. Entry into an arm was defined as the point when the animal places all four paws into the arms. The procedure was conducted in a quiet environment and observation was made from an adjacent corner. The results were expressed as mean ratio of percentage of time spent in open arms and mean ratio of percentage of number of open arm entries.

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Hole board test (HB)

Different groups of animals were assigned into negative control, positive control and test groups (n=6). The negative control group was administered with vehicle (DMSO, 10 %v/v in normal saline, 10 ml/kg, i.p.) The positive control group received chlorpromazine hydrochloride 1 mg/kg, i.p. (Wakeel et al., 2004). Graded doses (100, 200 and 300 mg/kg,i.p.) of *p*PPB were administered to the test groups. 30 min after treatment each mouse was placed in the center of the hole-board and allowed to freely explore the apparatus and number of head dips displayed by each mouse was recorded for five min.

Sedative test

Sodium pentobarbitone-induced hypnosis was used to assess the sedative activity of *p*PPB as previously described (Adeyemi et al., 2010). Different groups of mice (n=6) were randomly selected into negative, positive and test groups. The negative group mice were pre-treated with vehicle (DMSO 10% v/v, in normal saline, 10 ml/kg), positive group was pretreated with diazepam 3 mg/kg, i.p., while the test groups were pretreated with (100, 200 and 300 mg/kg, i.p.) of *p*PPB respectively. Thirty min post treatment, each mouse was injected with pentobarbitone sodium 30 mg/kg i.p. (Gupta et al., 2012; Okoli et al., 2010) and observed for onset and duration of sleep. The time duration from induction of sleep to loss of righting reflex was the onset of sleep or sleep latency (SL) while the interval between loss of and recovery of righting reflex was recorded as the duration or total sleeping of sleep (TST).

Statistical analysis

Results were expressed as standard error of the mean (SEM). The results were analyzed using the one-way analysis of variance (ANOVA) followed by post hoc Dunnett's multiple comparison test between the test agent and negative control group. The level of significance was set at p< 0.05% for all treatment carried out compared with the control group.

Results

Chemical Studies

The compound was synthesized as described under the experimental. The scheme is as shown below**.**

Crystallographic data for the isolated compound

Crystal data

$C_{18}H_{20}O_2$	$Z = 2$
$M_r = 268.34$	$F(000) = 288$
Triclinic, $P\bar{1}$	$D_x = 1.215$ Mg m^{-3}
a = 7.984 (4) Å	Mo *K*a radiation, l = 0.71073 Å
b = 9.211 (5) Å	Cell parameters from 1019 reflections
c = 10.910 (6) Å	q = 2.4–22.9°
a = 75.503 (7)°	m = 0.08 mm^{-1}
b = 87.017 (7)°	*T* = 150 K
g = 70.928 (7)°	Block, colourless
V = 733.8 (7) Å^3	0.48 × 0.44 × 0.43 mm

Data collection

Bruker APEX 2 CCD diffractometer	1661 reflections with *I* > 2s(*I*)
Radiation source: fine-focus sealed tube	$R_{int} = 0.061$
w rotation with narrow frames scans	q_{max} = 25.0°, q_{min} = 1.9°
Absorption correction: multi-scan	*h* = -9®9

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SADABS v2012/1, Sheldrick, G.M., (2012)	
T_{min} = 0.964, T_{max} = 0.967	k = -10®10
7482 measured reflections	l = -12®12
2576 independent reflections	

Refinement

Refinement on F^2	Hydrogen site location: difference Fourier map
Least-squares matrix: full	All H-atom parameters refined
$R[F^2 > 2s(F^2)] = 0.064$	$w = 1/[s^2(F_o^2) + (0.0652P)^2 + 0.0226P]$ where $P = (F_o^2 + 2F_c^2)/3$
$wR(F^2) = 0.185$	$(D/s)_{max} < 0.001$
S = 1.05	$Dñ_{max}$ = 0.32 e Å^{-3}
2576 reflections	$Dñ_{min}$ = -0.25 e Å^{-3}
262 parameters	Extinction correction: *SHELXL*, $Fc^* = kFc[1+0.001xFc^2l^3/\sin(2q)]^{-1/4}$
0 restraints	Extinction coefficient: 0.069 (13)
Primary atom site location: structure-invariant direct methods	

Physical and Spectroscopic data on pPPB:

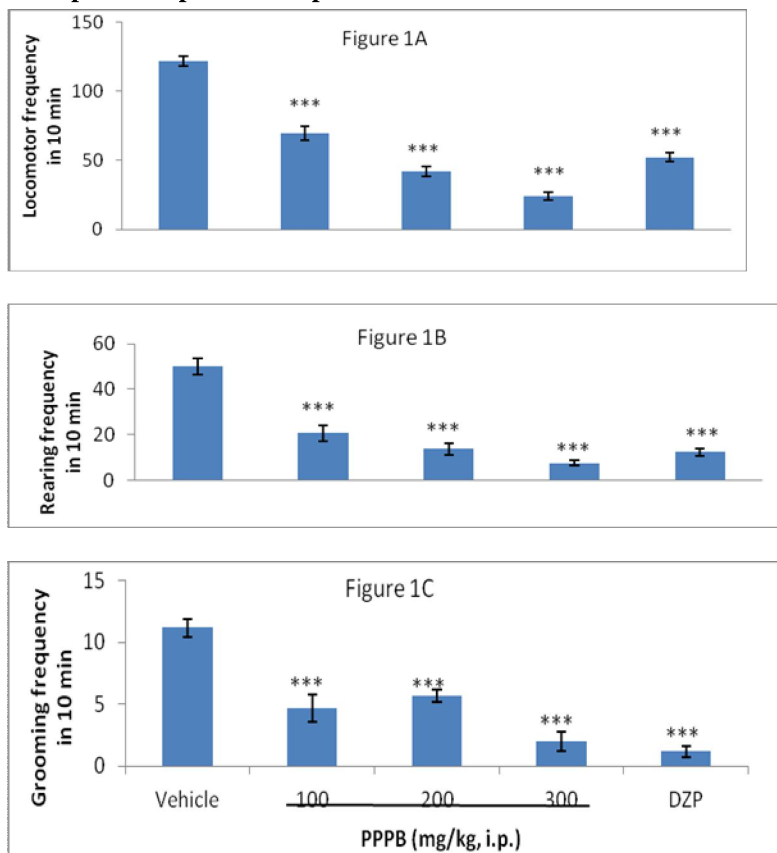

Figure 1A-C: Effect of *p*PPB on locomotor, rearing and grooming activities in mice.
OFT; Open field test, Vehicle; 10% DMSO/Normal saline (10 ml/kg), DZP; Diazepam (1 mg/kg i.p.), *p*PPB; 4-pentyl phenyl benzoate. Each bar represents mean ± SEM, n=6. ***p < 0.001 compared with control (ANOVA, Dunnett's test).

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Physical and Spectroscopic data on pPPB:

Parapentyl phenyl benzoate (*p*PPB), crystal, **m.p**. 50.3 ^{0}C (uncorrected), **FTIR υ_{max} cm^{-1}**: 2953, 2926, 2856, 1731, 1600. **^{1}H NMR** (400 MHz, DMSO-d_6) δ_H: 0.95 (3H, t, H-11′), 1.38 (2H, m, H-10′), 1.39 (2H, m, H-9′), 1.70 (2H, m, H-8′), 2.67 (2H, t, H-7′), 7.16 (2H, d, J = 8.6 Hz, H-3′, 5′), 7.27 (2H, d, J = 8.6 Hz, H-2′, 6′), 7.45 (1H, t, H-4), 7.68 (2H, t, H-3, 5), 8.25 (2H, d, J = 8.4 Hz, H-2, 6). **^{13}C NMR** (100 MHz, DMSO-d_6) δ_c: 165.4 (Cq, C-7), 148.9 (Cq, C-1′), 140.6 (Cq, C-1), 129.8 (Cq, C-4′), 133.5 (C-4), 130.2 (CH, C-2, 6), 129.4 (CH, C-2′, 6′), 128.6 (CH, C-3′,5′), 121.4 (CH, C-3, 5), 35.4 (CH_2, C-7′), 31.5 (CH_2, C-9′), 31.2 (CH_2, C-10′), 22.6 (CH_2, C-8′), 14.1 (CH_3, C-11′), **ESI MS** m/z, (rel. int.), 291.1 $(M+Na)^+$, (100).

Pharmacological Results

Acute Toxicity Test

The acute toxicity test (median lethal dose LD_{50} value) of *p*PPB was estimated to be 1265 mg/kg, i.p., after which three working doses of 100, 200 and 300 mg/kg were selected for further study.

Anxiolytic Effect

Effects of pPPB on Locomotor, Rearing and Grooming activities in OFT in mice.

The *p*PPB at all the three doses (100, 200 and 300 mg/kg, i.p.) significantly ($p < 0.05$) decreased locomotor, rearing and grooming activities compared with the control group in novelty-induced behavioural study in mice. There was also significant ($p < 0.05$) decrease in all the activities of the positive control group (diazepam 1 mg/kg, i.p.) compared to negative control. The effect of the test groups at 200 and 300 mg/kg, i.p. was greater than that of the standard drug for rearing and locomotion (Figure 1A-C).

Effect of pPPB on the elevated plus maze test in mice.

The *p*PPB at 100, 200 and 300 mg/kg, i.p. caused 44.73, 45.95 and 35.63 % respectively caused increase in the total number of entries into the open arms which were not significantly different from the control group which was 42.67 %. There was no significant increase in percentage duration in open arms at 100 and 200 mg/kg, compared with the control group, but at 300 mg/kg, *p*PPB significantly ($p< 0.001$) decreased duration in open arms. However, the standard drug diazepam 1 mg/kg, i.p. significantly ($p < 0.05$) increased the percentage number of open arm entries and the percentage duration ($p<0.01$) in the open arms compared with the control group (Figure 2A and B).

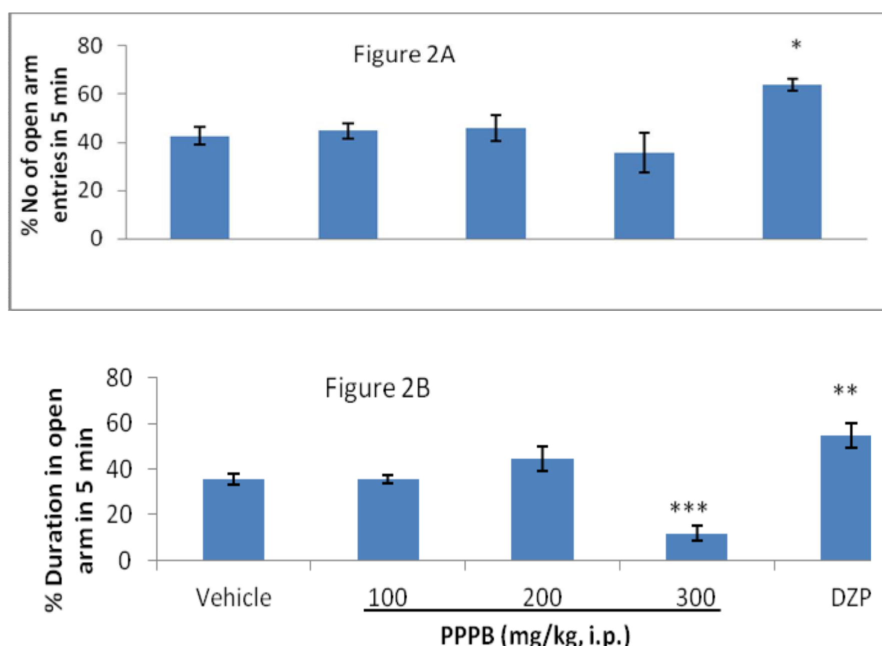

Figure 2A-B: Effect of *p*PPB on. the elevated plus maze test in mice

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EPM: Elevated plus maze, Vehicle: 10% DMSO/Normal saline (10 ml/kg), DZP:Diazepam (1 mg/kg i.p.), *p*PPB: 4-pentylphenyl benzoate. Each bar represents mean ± SEM, n=6. *p < 0.05, **p < 0.01, ***p < 0.001 compared with control (ANOVA, Dunnett's test).

Effect of pPPB on the hole board test in mice.

The *p*PPB at 300 mg/kg, i.p. caused a significant (p < 0.001)) decrease in exploratory activity by reduction in the number of head dips by the mice compared with control group similarly to the standard drug, chlorpromazine at 1 mg/kg, i.p. (Figure 3).

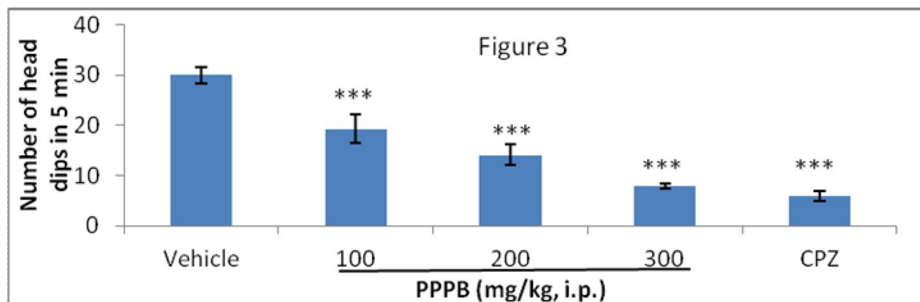

Figure 3: Effect of *p*PPB on the hole board test in mice
HB: Hole board, Vehicle: 10% DMSO/normal saline (10 ml/kg), CPZ: chlorpromazine HCl (1 mg/kg i.p.), *p*PPB; 4-pentylphenyl benzoate. Each bar represents mean ± SEM, n=6.

***p < 0.001 compared to control (ANOVA, Dunnett's test) .

Sedative Activity
Effect of pPPB on sleep latency and duration of sleep in sodium pentobarbitone - induced sleeping time in mice.

The pPPB(100 mg/kg) significantly (p < 0.05) shortened sleep latency but the at 200 and 300 mg/kg, i.p. significantly (p < 0.01) shortened the sleep latency (Fig. 4A. However, at all the doses used, pPPB and diazepam significantly (p < 0.001) prolonged total sleeping time compared with the vehicle (Fig. 4B).

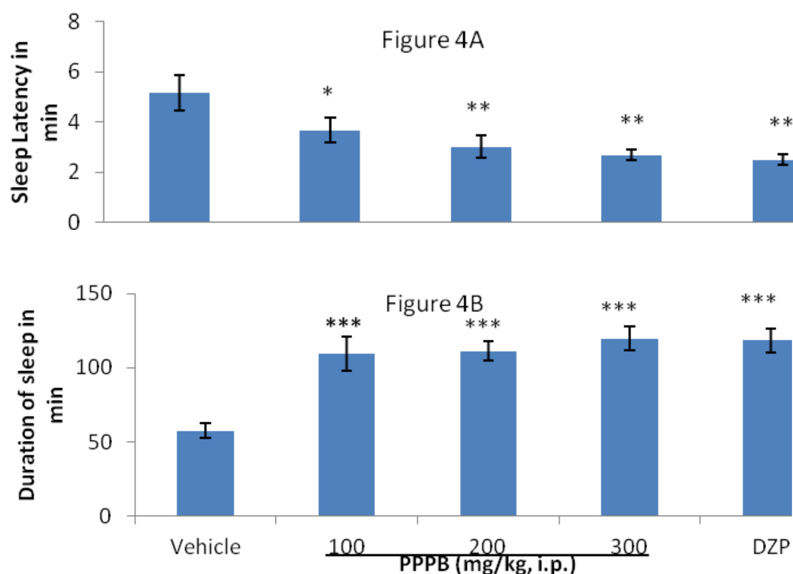

Figure 4A-B: Effect of *p*PPB on sodium pentobarbitone-induced sleeping time in mice

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Vehicle: 10% DMSO/normal saline (10 ml/kg), DZP: Diazepam (3 mg/kg, i.p.). *p*PPB: parapentylphenyl benzoate. Each bar represents mean ± SEM, n=6. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared with control (ANOVA, Dunnett's test).

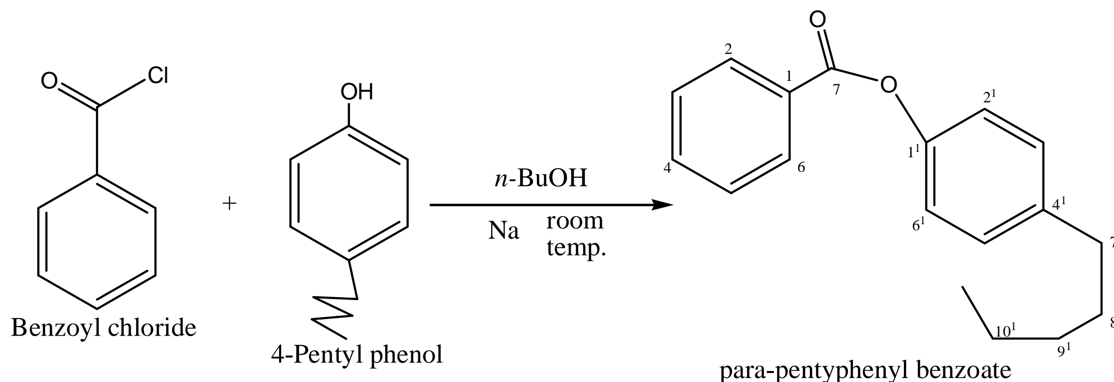
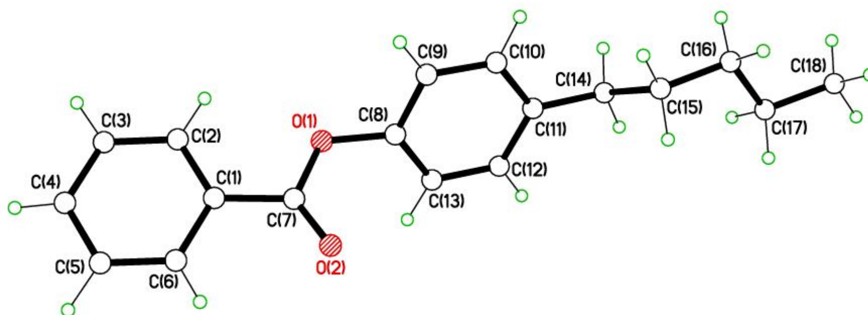


Figure 5: Showing the synthesis of *p*PPB.

Discussion

Structural elucidation

The yield of the compound was 0.00033% w/w of the fresh fruit. The crystals readily grew from a slow evaporation from dichloromethane chloroform compound readily formed crystals form. The structure (Figure 6) was solved through X-ray crystallography by direct methods (Sheldrick, 2001). All non-H atoms were refined anisotropically while H atoms were freely refined with isotropic displacement parameters (Sheldrick, 2015). CCDC 1443327 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Neither the bond lengths and angles, nor the packing of the molecules in the crystal lattice of *p*PPB are unremarkable. The twist angle between aromatic ring C(1) > C(6) and the C(7)/O(1)/O(2) group is 9.65(16)°, that between the ester group and aromatic ring C(8) > C(13) is 63.8(2)°, and that between the two aromatic rings is 55.51(10)° (Figure 6).



.**ORTEP diagram of *p*PPB**

Figure 6: Molecular structure of *p*PPB determined by X-ray crystallography

A single crystal X-ray diffraction study was conducted on a long, lath-shaped crystal. Due to the thin habit of the crystal, the diffraction was fairly weak, but the structure was well determined. A molecular formula of $C_{18}H_{20}O_2$ was indicated with one molecule in the asymmetric unit. A search of the Cambridge Structural Database revealed the structure was novel, as it had not been previously determined (Allen, 2002). To confirm the structure, a simple one-pot synthesis of *p*PPB was carried out from the benzoylation of readily available para-pentyl phenol (from AK Scientific CA, USA) with benzoyl chloride. The ^{1}H NMR spectrum of the synthesized compound gave nine signals while the ^{13}C NMR spectrum gave 14 signals in all at δc: 14.1 (CH_3), 22.6, 31.2 31.5, 35.4 (CH_2), 121.4, 128.6, 129.4, 130.2 and 133.5 (aromatic CH), 140.6, 148.9 (quaternary carbons) and 165.4 (ester carbonyl carbon). The ESI MS gave a signal at m/z of 291.1 $(M+Na)^+$ and 269.2 $(M + 1)^+$ in the positive mode for $C_{18}H_{20}O_2$. For the biosynthetic origin of *p*PPB in the plant species, we propose that the pentyl phenyl precursor might be a product of the polyketide pathway, formed through cyclization of a triketoacid, followed by decarboxylation.

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Pharmacological studies

The results of the acute toxicity profile of *p*PPB indicated the LD_{50} to be 1265 mg/kg, i.p. indicating moderate toxicity level (Lorke, 1983). Hence, the working doses (100-300 mg/kg,i.p.) used in this study were non-toxic as they were well below half of the LD_{50} obtained forthe compound. This result is preliminary as it will be worthwhile to explore the oral route for the acute toxicological profile. The intraperitoneal route has been variously supported for use in evaluating new compounds with central activities (de Carvalho *et al*., 1995). Furthermore, in order to prevent biodegradation and or inactivation of newly discovered compounds through the gastrointestinal route, due partly to enzymatic action or first-pass effect (Gavhane & Yadav, 2012) the oral route may be by-passed in preliminary screening protocol. In the course of the determination of the acute toxicity level of *p*PPB, it was observed to have an inhibitory effect on general behaviors; hence further neuropharmacological activities were carried out. The open field test (OFT) is often used for the screening of anxiolytic and anxiogenic substances (Eckeli *et al*., 2000). Evaluation of the effect of *p*PPB in the OFT showed that *p*PPB-treated mice exhibited depression of all activities compared with control with significant ($p < 0.01$) decrease in locomotor, rearing and grooming activities, suggesting possible suppression of the central nervous system by the compound. The results in Figure 1B indicate that mice treated with *p*PPB showed reduced frequency in rearing activity compared with control in the OFT, suggesting central depressant effect (Okoli et al., 2010) which became more pronounced at 300 mg/kg, i.p. and greater than that of diazepam (1 mg/kg i.p). Grooming in animal behavioural model is a response to stress and reduction in grooming behavior after the administration of any substance suggests possible alleviation of anxiety or sedation (Oyemitan et al., 2009). Figure 1C showed that *p*PPB decreased the frequency of grooming activity significantly ($p<0.05$), suggesting either sedative or anxiolytic effects (Oyemitan et al., 2013). The elevated plus maze (EPM) is one of the animal behavioural models used for anxiety research. The model involves spontaneous or natural dislike for height, unprotected openings and novel environment. Several chemicals used to reduce anxiety have been reported to increase the exploration of the open arms in the EPM test. The preference of mice to spend much time in the closed arms is a reflection of the aversion towards the open arms which is as a result of fear for open spaces. Drugs that increase open arms entries and durations in open arms compared with control are considered as anxiolytics and vice versa for anxiogenics (Yadav et al., 2008; Trullas and Skolnick, 1993). Decrease in exploratory behavior indicated by decrease in number of head dips in mice using the hole -board test is an indication that the test compound possessed central nervous system depressant activity (Adeyemi et al., 2010; Wakeel et al., 2004). In the present study *p*PPB significantly ($p < 0.05$) reduced the number of head dips by the mice. The effect of *p*PPB at 300 mg /kg, i.p. was comparable with the standard drug chlorpromazine (1 mg/kg, i.p.). The *p*PPB significant ($p < 0.05$) decrease in sleep latency (Figure 4A) and significant ($p < 0.05$) potentiation of pentobarbital-induced sleeping time (Figure 4B) suggesting sedative effect (Oyemitan et al., 2013; Adeyemi et al., 2010). This central depressant activity gave an insight into the habitual consumption of the fruit especially among the restive Nigerian population. The hypnotic effect of drugs have been evaluated by assessing behavioural motor activity and or potentiation of pentobarbital-induced sleeping time in laboratory animal model (Gonzalez-Trujano et al., 2006) and enhancement of barbital hypnosis indicate CNS depressant activity (Argal and Pathak, 2006).

Computational analysis of receptor binding event

The crystal structure of the human $GABA_A$ receptor composed of a homopentameric assembly in complexation with four benzamidine molecules solved at 2.97 Å was retrieved from the RCSB database (www.rcsb.org), PDB code 4COF (Miller and Aricescu, 2014). The $GABA_A$ receptor is a ligand-mediated chloride channel that has been observed to mediate the actions of a number of CNS depressants and drugs such as diazepam, zolpidem, pentobarbitone, chlorpromazine and so on (Gielen et al., 2012; Goldschen-Ohm et al., 2014; Ziemba AM and Forman, 2016). Agonistic binding to the $GABA_A$ receptor facilitates channel opening, chloride ion influx and synaptoneuronal hyperpolarization that impedes the transmission of action potential. The homopentamer features five binding sites occurring at interfaces between every pair of the five interacting subunits in extracellular portion of the transmembrane molecule. The binding site of gamma amino butyric acid (GABA) is located between the alpha and beta subunits while benzodiazepine (BDZ) binding site exists between alpha and gamma subunits. Two 15.0 x 15.0 x 15.0 docking grids were built using AutoDock Tool (Santos-Martins et al., 2014; Goodsell et al., 1996) and centered at 21.283, 1.799, 113.559 around the GABA binding site, and at 0.794, 26.012, 120.41 around BDZ binding sites, respectively.

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Table 1: Autodock Vina binding free energies and computed physicochemical properties.

	Binding energy (kcal/mol)GABA site	Binding energy (kcal/mol) BDZ site	Log P (o/w)	LogS	Weight	ASA	ASA_P
GABA	-4.5	-4.6	-0.6	0.7	104.1	274.7	152.1
Diazepam	-8.4	-8.0	3.8	-4.8	284.7	498.9	80.8
Pentobarbitone	-7.6	-7.1	2.1	-3.4	226.3	418.9	151.7
Benzamidine	-6.4	-6.3	1.1	-1.1	121.2	313.4	125.5
Chlorpromazine	-6.7	-6.8	4.6	-4.5	319.9	569.7	97.2
*p*PPB	-7.9	-7.9	5.6	-6.0	268.4	544.7	32.3
Correlation GABA site	1.00	0.97	-0.31	0.63	-0.45	-0.32	0.30
Correlation (BDZ site)	0.97	1.00	-0.20	-0.04	-0.41	-0.64	-0.08

The coordinates of benzamidine present in the PDB file were stripped. Using AutoDock Vina (Trott et al., 2010) with an exhaustiveness of 8, energy minimized molecules of GABA, diazepam, pentobarbitone, chlorpromazine, benzamidine, and *pPPB* were each independently docked into the two binding sites. The top pose in each case was saved for analysis. To better capture the structural basis of interaction of the six molecules within the employed binding sites of $GABA_A$ receptor, five physicochemical descriptors including logP(o/w), logS, molecular weight, total accessible surface area (ASA), and polar accessible surface area (ASA_P) were calculated (Table 1). Each of the descriptor was then correlated with the binding free energies using Pearson correlation coefficient, where the numerator captures the sample variance, and the two denominators the binding energy (e) and physicochemical descriptors' (p) standard deviations. $\bar{e}$ and $\bar{p}$ are the means of the binding energies and descriptor, respectively.

$$r_{xy} = \frac{\left(\frac{1}{N}\sum_{i=1}^{N}(e_i - \bar{e})(p_i - \bar{p})\right)}{\left(\sqrt{\left(\frac{1}{N}\sum_{i=1}^{N}(e_i - e)^2\right)} \times \sqrt{\left(\frac{1}{N}\sum_{i=1}^{N}(p_i - p)^2\right)}\right)}$$

With a -7.9 kcal/mol binding energy, the interaction of *p*PPB at both $GABA_A$ receptor binding sites is expected to be thermodynamically stable. While none of the studied ligands feature non-negative binding energies, the energetics of *p*PPB appears to be more favorable than those obtained for GABA, chlorpromazine, benzamidine, and comparable with the affinity obtained for pentobarbitone and diazepam. With the exemption of GABA and benzamidine, all ligands are relatively lipophilic with logP values ranging from 2.1 for pentobarbitone to 5.6 for *p*PPB. With a logP value of 5.6, *p*PPB is highly lipophilic, and is expected to easily to cross the blood brain barrier and subsequently spontaneously bind within both GABA and benzodiazepine binding sites on $GABA_A$ receptor. A correlation of the free energy of binding with molecular descriptors shows logS and the accessible surface area as the two most important properties influencing receptor interaction. Decreasing logS values and increasing ASA values do directly contribute to improved binding at $GABA_A$ receptor binding sites.

At atomistic resolution, the strong binding demonstrated by *p*PPB at $GABA_A$ receptor binding sites was found to derive largely from its ability to enlist both electrostatic and hydrophobic forces in interacting with the binding site residues (Figure 7).

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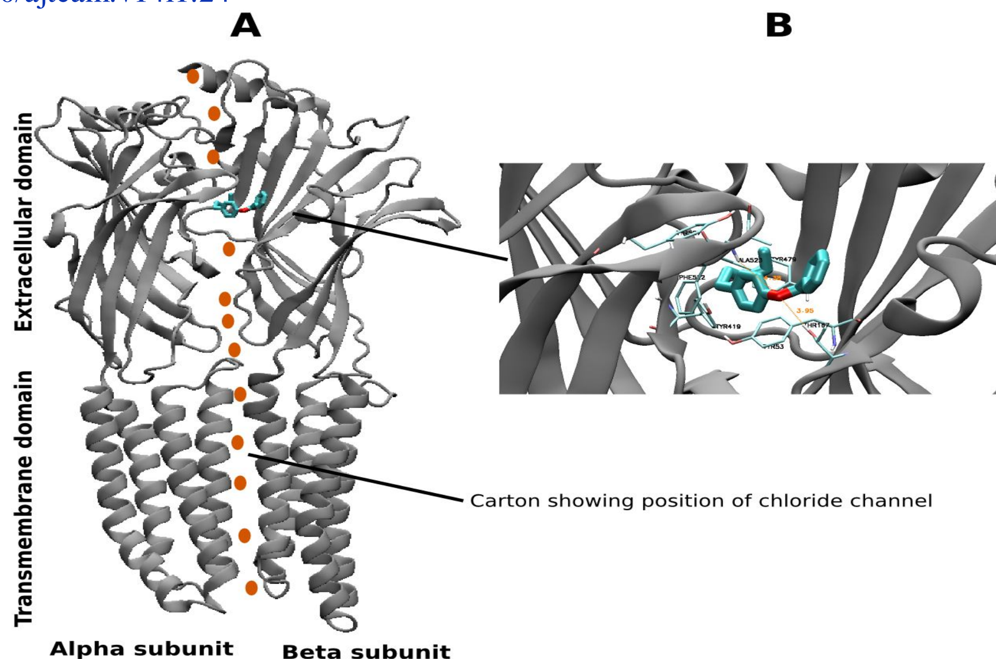

Figure 7: Showing the binding of *p*PPB with the $GABA_A$ binding site.

The esteratic oxygen of *p*PPB forms hydrogen bonds with the backbone atoms of Thr167 (beta subunit) and Ala523 (alpha subunit), while the *p*-pentylphenyl tail was buried inside a hydrophobic cavity at the binding site formed by five tyrosine residues-Tyr522, Tyr479, Tyr527, Tyr419 and Tyr53 (beta subunit). The *p*-pentylphenyl tail was additionally held down by a pi-stacking arrangement with Tyr522.

Conclusion

This study reported the isolation of *p*-pentylphenyl benzoate from the ethanolic fruit extract of *M. whitei* for the first time. Its acute toxicity study confirmed that the fruit extract is safe for the mice used in the study while the neuropharmacological evaluation of the compound indicated that it possessed significant CNS depression in mice. Although, the compound *p*PPB was found to be present in the fresh fruit studied at a concentration of 0.00033% (w/w), the favourable binding energy potential of the compound with $GABA_A$ receptors coupled with its high lipophilicity that enables crossing of the blood brain barrier makes the compound potent in its CNS depression activity. These neuropharmacological activities reported here lend credence to the habitual consumption of the fruit especially among the restive Nigerian population.

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