

Antidepressant effects of the hydro-alcoholic extract and fractions of *Anchomanes difformis* (Blume) Engler rhizome (family Araceae) in mice

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ABSTRACT: In ethno-medicinal practice in the South-western part of Nigeria, the rhizome of *Anchomanes difformis* is claimed to be effective in the management of mental illness. This study determined the antidepressant effects of hydro-alcoholic extract and fractions of *Anchomanes difformis* rhizome and the likely mechanisms of action of the extract and the most active fraction in mice. The extract was partitioned into *n*-hexane, ethylacetate, butanol and distilled water to obtain *n*-hexane, ethylacetate, butanol and residual aqueous fractions, respectively. The extract and fractions were administered to mice of both sexes (18 to 25 g) at 60, 125, 250, 500, and 1000 mg/kg, orally. Tail suspension and forced swimming anti-depressant animal models were used. The likely mechanism of anti-depressant action was assessed using yohimbine and cyproheptadine. The data were analyzed using one-way ANOVA followed by Student-Newman-Keul's post hoc test with the level of significance taken as $p < 0.05$. The extract and ethylacetate fraction at 60 to 250 mg/kg significantly ($p < 0.05$) reduced the period of immobility in both the tail suspension and forced swimming tests without significantly impairing the locomotor activity, suggesting an anti-depressant effect. This effect was reversed by yohimbine (1 mg/kg, i.p.), an α_2 -adrenergic receptor antagonist, yohimbine and also by cyproheptadine (0.5 mg/kg, i.p.), a serotonergic receptor antagonist. The study concluded that the extract and ethylacetate fraction of *A. difformis* rhizome possessed an anti-depressant effect in mice which was probably mediated through α_2 -adrenergic and serotonergic pathways.

Keywords: *Anchomanes difformis*, antidepressant, hydro-alcoholic extract, mice.

INTRODUCTION

Depression and related mood disorders rank among the world's greatest public health burdens. Depression is a complex disorder that has been characterized and classified in a variety of ways. The American Psychiatric Association's modified fourth edition (2000) of the *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR)*, described several diagnoses of affective disorders. While major depression and dysthymia (minor) are pure depressive syndromes, bipolar disorder and cyclothymic disorder signify depression in association with mania. A simplified classification on the basis of their presumed origin is as follows: (1) brief reactive or secondary depression (most common), occurring in response to real stimuli such as grief, illness, etc; (2) melancholic and recurrent depression, a genetically

determined biochemical disorder manifested by an inability to experience ordinary pleasure or to cope with ordinary life events; and (3) depression associated with bipolar affective (manic-depressive) disorder (Katzung, 2007).

Since the serendipitous discovery of chlorpromazine in the fifties and consequently the availability of a good number of other antidepressants for the treatment of this disorder, about half of affected individuals do not respond adequately to available medications and psychotherapeutic approaches (Depression Guideline Panel, 1993). This limitation of current antidepressant drugs has (about half of affected individuals do not respond adequately to available medications and psychotherapeutic approaches) warranted ongoing research to identify pharmacological agents and strategies that offer greater therapeutic

efficacy (Santos et al., 2012).

Anchomanes difformis (Blume) Engler is an herbaceous plant in the family Araceae with prickly stem having huge divided leaf and spathe that arise from a horizontal rhizome occurring in the forest of West Africa. It flourishes well in moist shady areas as occurs in southern parts of Nigeria (Akah and Njike, 1990). The Yorubas in the Southwest Nigeria call it 'Ogirisako' or 'Opego' (Soladoye et al., 2005). The rhizome is massively thickened, 6 to 20 cm broad or more, growing horizontally, with distinctive annular leaf-base scars and growing point situated obliquely (Haigh and Boyce, 2012). It is native to some West African countries such as Nigeria, Ghana, Ivory Coast, Senegal, Sierra Leone and Togo.

A. difformis is a plant which has been claimed to have diverse biological activities with many reported folkloric uses which include treatment of mental illness by the herbalists in the Southwestern part of Nigeria (Personal Communication). Apart from the sedative activity of the methanol extract of the rhizome of the plant reported by Eke et al. (2013), there is no information on the potential activities of the plant as antidepressant agent. Therefore, this study was conducted to bridge this gap using forced swimming test and tail suspension behavioral animal models.

MATERIALS AND METHODS

Plant collection and preparation of extract and fractions

Fresh samples of the rhizome of the plant were collected from Ado Ekiti (Latitude 7° 37' 23.84" N; Longitude 5° 13' 15.13" E), Ado Local Government Area, Ekiti State, Southwestern part of Nigeria. The sample was identified as *Anchomanes difformis* (family Araceae) by Mr. Ife Ogunlowo, a botanist at the Department of Pharmacognosy Herbarium, Faculty of Pharmacy, Obafemi Awolowo University, (OAU), Ile-Ife, Nigeria. The specimen was authenticated by Mr. G. Ighanesebhor, Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife. A specimen Voucher numbered IFE 16937, dated 3rd May, 2013 was deposited at the Departmental Herbarium. The collected fresh rhizomes were peeled, washed, cut into small pieces and air-dried for 8 weeks. The dried material was pulverized and 4 portions of 1.00 kg each were soaked in 1 L each of 70% ethanol for 7 days with occasional shaking. The combined extract was passed through a pack of cotton wool and filtered using a Whatman No.1 filter paper. The filtrate was concentrated in a vacuum and dried in the oven at 50°C for 2 days to get a brownish 80.00 g (2.00 % yield) residue. Seventy-five grams of this hydro-alcoholic extract (HAE) was fractionated into n-hexane (HF), ethyl-acetate (EF), butanol (BF), and residual aqueous (AF) fractions. The fractions, weighing 16.44 g (HF), 1.94 g (EF), 4.80 g (BF), and 51.28 g (AF), were kept in the freezer until required.

Animals

Male and female Swiss albino mice (3 to 5 months and 18 to 25 g), obtained from the Animal Facility of the Department of Pharmacology, Faculty of Pharmacy, OAU, Ile-Ife, were used. The animals were housed in plastic cages with stainless steel wires covering the open top of the cage. They were maintained under natural daylight/night condition. The males were caged separately from the females to prevent mating. They were provided food and water *ad libitum* except during the testing period. All experiments were carried out at approximately the same time (between 9.00 a.m. and 4.00 p.m.) each day to reduce any variation in circadian rhythm (Carney et al., 2002). Each animal was used only once. Animals were habituated to laboratory conditions for at least 2 hours prior to experimental protocol to minimize, if any, nonspecific stress. They were maintained in accordance with the recommendations in the Guide for Care and Use of Laboratory Animals (DHH, NIH Publication No. 85-23, 1985). All the experiments were conducted in strict compliance with ethical principles and guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs used

Cyproheptadine, imipramine and yohimbine (Sigma Chemicals Co, St. Louis, Missouri, U.S.A.) procured from Lagos, Nigeria; sertraline (Pfizer, Nigeria) and normal saline (Ashima Ltd., Ibadan, Nigeria) procured from Kikelomo Pharmacy Stores Limited, Ilesa, Nigeria.

Constitution and administration of the extract, fractions and drugs

The extract, fractions and drugs were weighed on Mettler balance and freshly prepared by dissolving each of them in the vehicle (Normal saline/2.5 % Tween 80 in normal saline) on the day of testing. The extract, fractions and sertraline were given orally in a maximum volume of 10 µl/g body weight to each mouse (Akanmu et al., 2011). Cyproheptadine and yohimbine were administered intraperitoneally (i.p.) also in a maximum volume of 10 µl/g body weight to each mouse.

Determination of the median lethal dose (LD₅₀)

LD₅₀ by oral route of administration was determined using the Lorke's (1983) method for the extract and fractions in an acute toxicity study to establish the safety/toxicity limit in the test animals. Briefly, the test was carried out in two phases:

Phase I

1. Mice in the first group (n = 3) were given 10 mg/kg extract/fraction orally and were observed for mortality within 24 hours. The number of mice that died within the period was recorded over the total number of mice in the group (e.g. 0/3 means no death out of 3 mice).
2. Mice in the second group (n = 3) were given 100 mg/kg extract/fraction orally and similarly observed as above.
3. Mice in the third group (n = 3) were given 1000 mg/kg extract/fraction orally and were observed as above. The number that died was recorded as described above.

Phase II

Three groups of animals (n = 1) were administered 1,600, 2,900, and 5,000 mg/kg, respectively.

The treated animals were monitored for signs of toxicity and mortality within 24 hours. The LD₅₀ was supposed to be calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death (The calculation was not done since no mortality was recorded within 24 hours).

Behavioural assays

Tail suspension test (TST)

The TST was performed by following the method of Steru et al. (1985), with some modifications. Briefly, mice were suspended on a TST apparatus, 58 cm above the floor, by placing adhesive tape approximately 1 to 2 cm from the tip of the tail. Immobility time as an indication of a state of depression was recorded manually using a stop-watch by watching the animal during the 6 minutes experimental period (Akanmu et al., 2011). Mice were considered immobile when they did not show any body movement and hanged passively. The changes in immobility duration were studied for the various treatment groups. In this experiment, 25 mice were divided into five groups (n = 5) and administered orally with different doses of the extract (60, 125, 250, 500, and 1000 mg/kg) or vehicle (10 ml/kg normal saline/2.5% Tween 80 in saline). The extract/fraction or the vehicle was administered orally 60 minutes before TST while the standard drug group, imipramine (25 mg/kg, i.p.)/sertraline (20 mg/kg, p.o.), was administered 30/60 minutes before TST (Dhingra and Goyal, 2008). Another group of 5 mice was pre-treated with yohimbine (1 mg/kg, i.p.)/cyproheptadine (0.5 mg/kg, i.p.) 15 minutes prior to administration of extract/fraction/imipramine (25 mg/kg, p.o.)/sertraline (20 mg/kg, p.o.) before being tested. Each animal was used only once.

Forced swimming test (FST)

The FST method described by Porsolt et al. (1977) was

adopted with some modification. Mice were individually forced to swim in an open transparent cylindrical container (diameter 12 cm, height 15 cm) containing 8 cm of water at 23 ± 1°C. Animals were considered immobile when they remained floating in the water, making only small movements to keep their heads above water (Akanmu et al., 2011). The total duration of immobility was observed and recorded manually by using a stop-watch during the last 4 minutes of the total 6 minutes test period. The changes in immobility duration were studied for the various treatment groups. In this experiment, 25 mice were divided into five groups (n = 5) and administered orally with different doses of the extract (60, 125, 250, 500, and 1000 mg/kg) or vehicle (10 ml/kg normal saline/2.5 % Tween 80 in saline). The extract/fraction or the vehicle was administered orally 60 minutes before FST while the standard drug group, imipramine (25 mg/kg, i.p.)/sertraline (20 mg/kg, p.o.), was administered 30/60 minutes before FST (Dunn and Swiergiel, 2005). Another group of 5 mice was pre-treated with yohimbine (1 mg/kg, i.p.)/cyproheptadine (0.5 mg/kg, i.p.) 15 minutes prior to administration of extract/fraction/imipramine (25 mg/kg, i.p.)/sertraline (20 mg/kg, p.o.). Each animal was used only once.

Statistical analysis

Statistical analysis of data collected from observations was performed using the software Primer of Biostatistics (Primer of Biostatistics, Version 4, Stanton A. Glantz). Results were expressed as mean ± SEM (n = 5). Significant differences between groups were determined by analysis of variance followed by Student-Newman-Keuls *post hoc* test. Differences between data sets were considered as significant when p value was less than 0.05.

RESULTS

Results of acute toxicity testing (LD₅₀ determination)

The results for acute toxicity test using Lorke's Method for oral route of administration are as shown in Tables 1 and 2. The results showed that there was no mortality up to 5000 mg/kg body weight of the extracts and fractions via oral route of administration. Therefore, the LD₅₀ of each of the extracts and fractions via oral route in mice was taken to be greater than 5000 mg/kg, body weight.

Effects of orally administered Hydro-alcoholic extract (HAE) on period of immobility of mice in TST and FST models

Results from TST (Figure 1, Panel A) showed that HAE reduced immobility period at 125 and 250 mg/kg where the magnitude of the immobility recorded was inversely dose-

Table 1. Acute toxicity test (Lorke's (1983) Method) for hydro-alcoholic extract and fractions of *A. difformis* rhizome in mice (oral route): Phase I.

Dose Level	Mortality within 24 hours (n=3)					% Mortality within 24 hours				
	HAE	HF	EF	BF	AF	HAE	HF	EF	BF	AF
10 mg/kg	0/3*	0/3	0/3	0/3	0/3	0.00	0.00	0.00	0.00	0.00
100 mg/kg	0/3	0/3	0/3	0/3	0/3	0.00	0.00	0.00	0.00	0.00
1000 mg/kg	0/3	0/3	0/3	0/3	0/3	0.00	0.00	0.00	0.00	0.00

*0/3 indicates no death occurred out of 3 mice tested. HAE = Hydro-alcoholic extract of *A. difformis*; HF = n-Hexane fraction; EF = Ethylacetate fraction; BF = Butanol fraction; AF = Residual aqueous fraction.

Table 2. Acute toxicity test (Lorke's (1983) Method) for hydro-alcoholic extract and fractions of *A. difformis* rhizome in mice (oral route): Phase II.

Dose level	Mortality within 24 hours (n=3)					% Mortality within 24 hours				
	HAE	HF	EF	BF	AF	HAE	HF	EF	BF	AF
1000 mg/kg	0/1*	0/1	0/1	0/1	0/1	0.00	0.00	0.00	0.00	0.00
1600 mg/kg	0/1	0/1	0/1	0/1	0/1	0.00	0.00	0.00	0.00	0.00
2900 mg/kg	0/1	0/1	0/1	0/1	0/1	0.00	0.00	0.00	0.00	0.00
5000 mg/kg	0/1	0/1	0/1	0/1	0/1	0.00	0.00	0.00	0.00	0.00

*0/1 indicates that the mouse did not die. HAE = Hydro-alcoholic extract of *A. difformis*; HF = n-Hexane fraction; EF = Ethylacetate fraction; BF = Butanol fraction; AF = Residual aqueous fraction of hydro - alcoholic extract of *A. difformis* rhizome.

dependent. The effects at these doses were significant [F (6, 28) = 14.306; $p < 0.0001$] when compared to vehicle-treated control group. The response at 125 mg/kg was comparable with the effect induced by imipramine (25 mg/kg, i.p.).

Panel B of Figure 1 shows the result of effect of HAE on immobility period in FST. HAE reduced immobility period in this model at 60, and 125 mg/kg significantly [F (6, 28) = 24.023; $p < 0.0001$] when compared to vehicle-treated control. Again, as in TST, only the effect at 125 mg/kg was comparable to the response induced by the standard reference drug, imipramine (25 mg/kg, i.p.).

Effects of orally administered n-Hexane fraction (HF), Ethyl-acetate fraction (EF), Butanol fraction (BF), and Residual aqueous fraction (AF) on period of immobility of mice in TST and FST models

Results from TST and FST showed that HF did not have any significant effect on immobility period at all dose levels compared to vehicle-treated control (Figure 2, Panels A and B).

Analysis of TST results revealed that EF reduced immobility period at 60, 125 and 250 mg/kg (Figure 2, Panel A). These effects were significant [F (6, 28) = 9.350; $p < 0.0001$] when compared to control. These effects were, however, not comparable to the response induced by imipramine (25 mg/kg, i.p.). On FST, the results showed that EF produced the same effects on immobility period as in TST, reducing it at 60, 125 and 250 mg/kg compared to

control (Figure 3, Panel B). These effects, though not dose-dependent, were significantly [F (6, 28) = 17.164; $p < 0.0001$] different from that produced by control. The standard antidepressant drug, imipramine (25 mg/kg, i.p.) significantly reduced immobility period on the two models.

Analysis of results from TST revealed that BF did not have any significant effect on immobility period at all tested doses when compared to vehicle-treated control group (Figure 4, Panels A). However, analysis of FST results indicated that BF increased immobility period at all doses tested with the responses induced at 60 and 125 mg/kg being significant [F (6, 28) = 51.594; $p < 0.0001$] when compared to control (Figure 4, Panel B).

Results obtained from TST showed that AF did not have any significant effect on immobility period at all doses tested when compared to control (Figure 5, Panel A). Analysis of obtained results from FST showed that AF significantly [F (6, 28) = 19.455; $p < 0.0001$] increased immobility period at all doses tested when compared to control (Figure 5, Panel B).

Likely mechanisms of action of antidepressant effects of the extract and ethylacetate fraction of *A. difformis* rhizome in mice

The results of possible mechanisms of action of antidepressant effects of the extract of *A. difformis* and the most active fraction (EF) with antidepressant activity comprising adrenergic and serotonergic pathways tested on TST and FST models are presented in Tables 3 to 6.

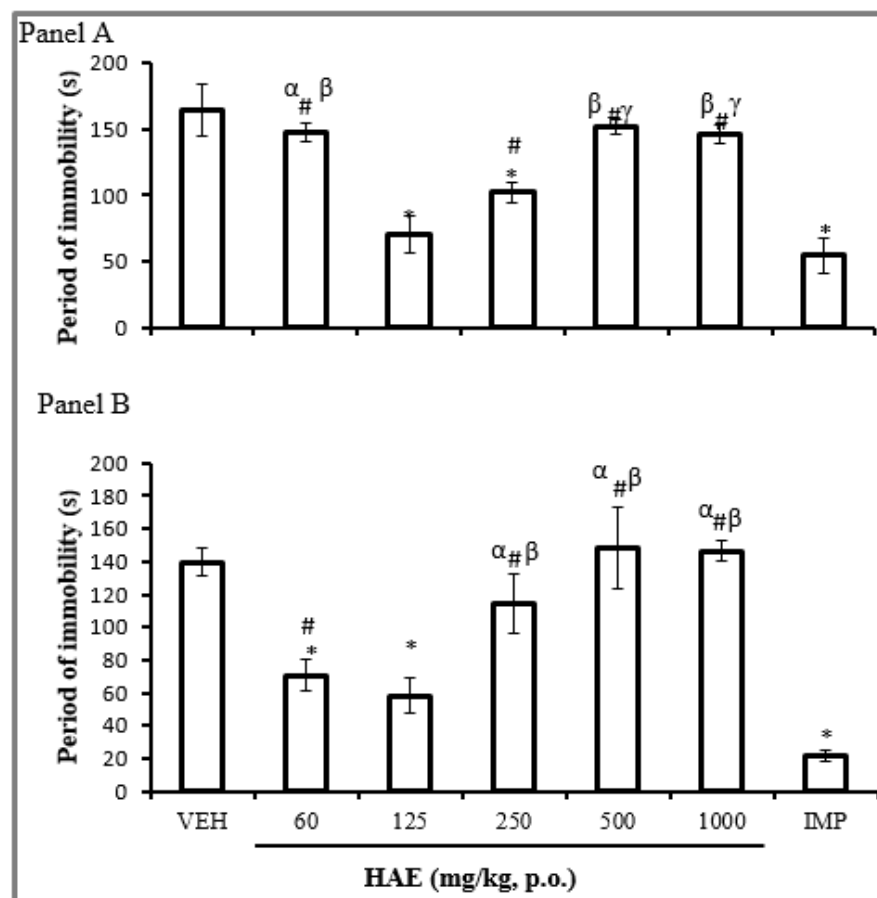


Figure 1. Effects of orally administered HAE on period of immobility of mice in TST (Panel A) and FST (Panel B). HAE: Hydro-alcoholic extract of *A. difformis* rhizome; IMP: Imipramine (25 mg/kg, i.p.); VEH: Vehicle (2.5 % Tween 80 in saline, 10 ml/kg, p.o.); *significant $p < 0.05$ compared to vehicle-treated control; #significant $p < 0.05$ compared to standard drug, imipramine; $^{\alpha}$ significant $p < 0.05$ compared to 60 mg/kg HAE; $^{\beta}$ significant $p < 0.05$ compared to 125 mg/kg HAE; $^{\gamma}$ significant $p < 0.05$ compared to 250 mg/kg HAE.

Adrenergic pathway involvement

When tested on TST model, HAE (125 mg/kg, p.o.) as well as imipramine (25 mg/kg, i.p.) significantly reduced immobility period compared to control. This effect was significantly [F (5, 24) = 17.428; $p < 0.0001$] blocked by pretreatment of mice with yohimbine (1 mg/kg, i.p.) 15 minutes prior to administration of HAE (125 mg/kg, p.o.)/imipramine (25 mg/kg, i.p.). However, the antagonist administered alone to mice did not produce any significantly different response compared to control (Table 3). The result from the FST model followed the same trend in that HAE (125 mg/kg, p.o.) as well as the reference antidepressant drug, imipramine (25 mg/kg, i.p.) significantly reduced immobility period when compared to control. This reduction was significantly [F (5, 24) = 43.370; $p < 0.0001$] blocked by pretreating mice with yohimbine (1 mg/kg, i.p.) 15 minutes prior to administration of HAE (125 mg/kg, p.o.)/imipramine (25 mg/kg, i.p.). The antagonist

administered alone did not produce any significant effect compared to control (Table 3).

The results from TST model showed that EF (250 mg/kg, p.o.) as well as imipramine (25 mg/kg, i.p.) significantly reduced immobility period compared to control; an effect which was significantly [F (5, 24) = 21.273; $p < 0.0001$] blocked by pretreating mice with α_2 -adrenergic receptor antagonist, yohimbine (1 mg/kg, i.p.) 15 minutes prior to administration of EF (250 mg/kg, p.o.)/imipramine (25 mg/kg, i.p.). The antagonist administered alone produced a response which was not significantly different from control (Table 4). Also, results from FST model revealed that EF (250 mg/kg, p.o.) like imipramine (25 mg/kg, i.p.) significantly reduced immobility period when compared to control. However, pretreatment of mice with yohimbine (1 mg/kg, i.p.) 15 minutes prior to administration of EF (250 mg/kg, p.o.)/imipramine (25 mg/kg, i.p.) significantly [F (5, 24) = 93.749; $p < 0.0001$] reversed this effect. The antagonist administered alone produced a response which

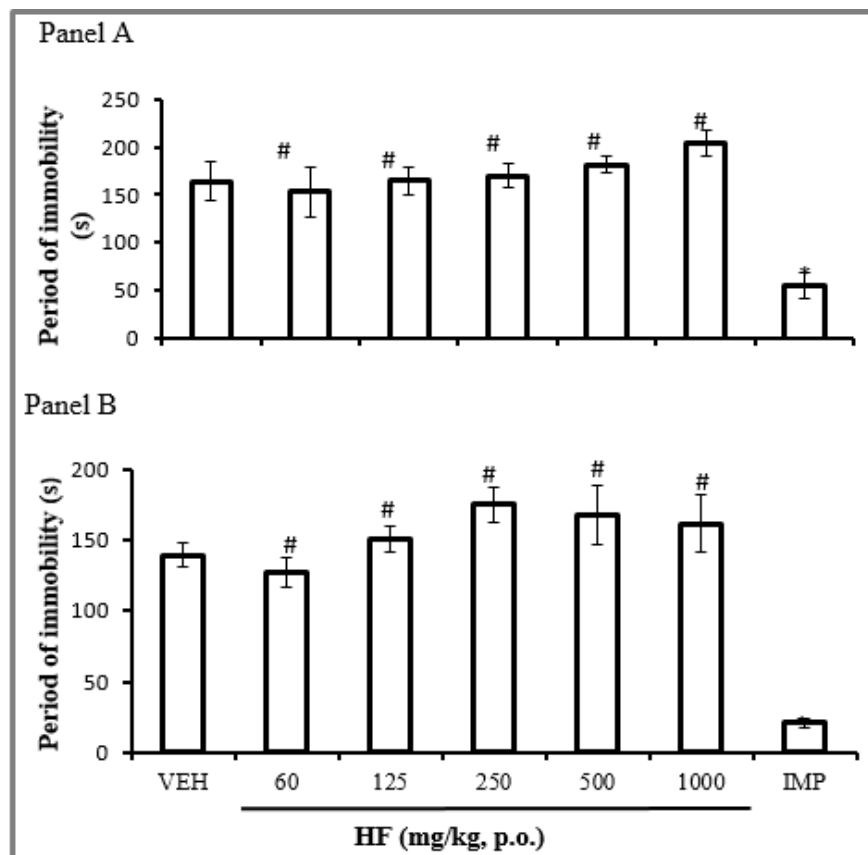


Figure 2. Effects of orally administered HF on period of immobility of mice in TST (Panel A) and FST (Panel B): *significant $p < 0.05$ compared to vehicle-treated control; #significant $p < 0.05$ compared to standard drug, imipramine. HF: N-hexane fraction of *A. difformis* rhizome; IMP: Imipramine (25 mg/kg, i.p.); VEH: Vehicle (2.5 % Tween 80 in saline, 10 ml/kg, p.o.).

Table 3. Influence of pretreatment of mice with yohimbine (1 mg/kg, i.p.) 15 min prior to administration of HAE (125 mg/kg, p.o.)/imipramine (25 mg/kg, i.p.) on effects of HAE/imipramine on immobility period using TST and FST models.

Treatment	Immobility period (min): TST	Immobility period (min): FST
VEH (10 ml/kg, p.o.)	168.60 ± 21.34	139.60 ± 8.77
VEH + YHB	175.40 ± 17.47	130.60 ± 5.64
VEH + HAE	70.20 ± 13.58 [#]	58.40 ± 11.16 [#]
YHB+ HAE	192.20 ± 2.13	134.40 ± 5.73
VEH + IMP	55.40 ± 12.36 [#]	21.20 ± 3.44 [#]
YHB + IMP	126.60 ± 6.18	74.60 ± 2.73

[#]significant $p < 0.05$: Treatment in the presence of antagonist versus treatment alone. HAE: Hydro-alcoholic extract of *A. difformis* rhizome (125 mg/kg, p.o.); VEH: Vehicle (2.5 % Tween 80 in saline, 10 ml/kg, p.o.); IMP: Imipramine (25 mg/kg, i.p.); YHB: Yohimbine (1 mg/kg, i.p.).

was not significantly different from control (Table 4).

Serotonergic pathway involvement

Analysis of result from TST revealed that HAE (125 mg/kg,

p.o.) as well as sertraline (20 mg/kg, p.o.) significantly reduced immobility period when compared to control. This effect was significantly [F (5, 24) = 24.001; $p < 0.0001$] reversed following pretreatment of mice with cyproheptadine (0.5 mg/kg, i.p.) 15 minutes prior to oral administration of HAE (125 mg/kg)/sertraline (20 mg/kg).

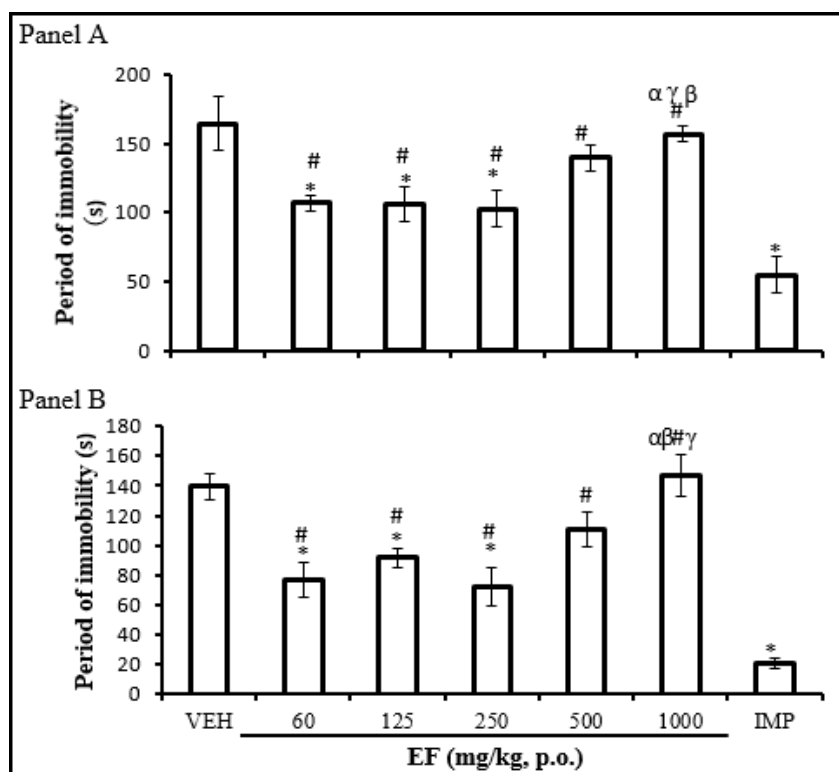


Figure 3. Effects of orally administered EF on period of immobility of mice in TST (Panel A) and FST (Panel B). #significant $p < 0.05$ compared to standard drug, imipramine; α significant $p < 0.05$ compared to 250 mg/kg EF; β significant $p < 0.05$ compared to 60 mg/kg EF; γ significant $p < 0.05$ compared to 500mg/kg EF; $\beta\gamma$ significant $p < 0.05$ compared to 125 mg/kg EF. EF: Ethyl-acetate fraction of *A. difformis* rhizome; IMP: Imipramine (25 mg/kg, i.p.); VEH: Vehicle (2.5 % Tween 80 in saline, 10 ml/kg, p.o.); *significant $p < 0.05$ compared to vehicle-treated control.

Table 4. Influence of pretreatment of mice with yohimbine (1 mg/kg, i.p.) 15 min prior to administration of EF (250 mg/kg, p.o.)/imipramine (25 mg/kg, i.p.) on effects of EF/imipramine on immobility period using TST and FST models.

Treatment	Immobility period (min): TST	Immobility period (min): FST
VEH (10 ml/kg, p.o.)	168.60 ± 21.34	139.60 ± 8.77
VEH + YHB	175.40 ± 17.47	130.60 ± 5.64
VEH + EF	69.40 ± 9.73 [#]	60.20 ± 3.65 [#]
YHB+ EF	211.40 ± 6.55	134.20 ± 3.22
VEH + IMP	55.40 ± 12.36 [#]	21.20 ± 3.44 [#]
YHB + IMP	126.60 ± 6.18	74.60 ± 2.73

[#]significant $p < 0.05$: Treatment in the presence of antagonist versus treatment alone. EF: Ethyl-acetate fraction of *A. difformis* rhizome (250 mg/kg, p.o.); VEH: Vehicle (2.5 % Tween 80 in saline, 10 ml/kg, p.o.); IMP: Imipramine (25 mg/kg, i.p.); YHB: Yohimbine (1 mg/kg, i.p.).

Administration of the antagonist alone did not produce a significant effect when compared to control (Table 5). Also, the same results as from TST model were obtained from FST model on the effect of HAE/sertraline on immobility period in mice in the presence of 5-HT receptor antagonist. The blockade of the reduction of immobility period induced

by HAE (125 mg/kg, p.o.)/sertraline (20 mg/kg, p.o.) by the antagonist, cyproheptadine (0.5 mg/kg, i.p.) being statistically significant [$F(5, 24) = 18.634$; $p < 0.0001$]. The results are presented in Table 5.

Results from TST model showed that EF (250 mg/kg, p.o.) like sertraline (20 mg/kg, p.o.) significantly reduced

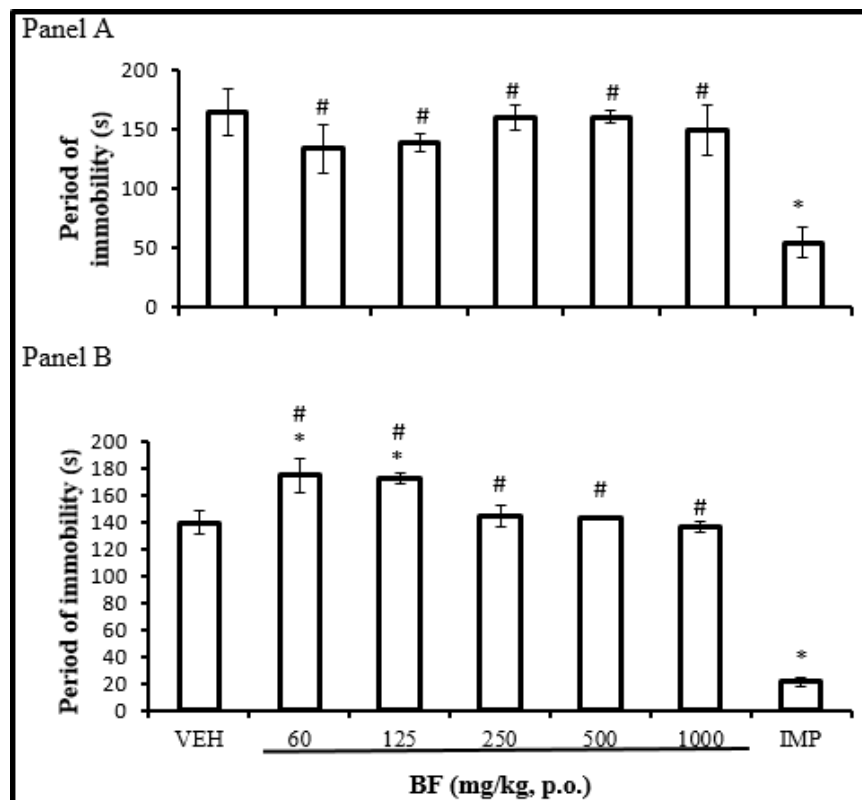


Figure 4. Effects of orally administered BF on period of immobility of mice in TST (Panel A) and FST (Panel B). #significant $p < 0.05$ compared to standard drug, imipramine. BF: Butanol fraction of *A. difformis* rhizome; IMP: Imipramine (25 mg/kg, i.p.); VEH: Vehicle (Normal saline, 10 ml/kg, p.o.). *significant $p < 0.05$ compared to vehicle (control).

Table 5. Influence of pretreatment of mice with cyproheptadine (0.5 mg/kg, i.p.) 15 min prior to administration of HAE (125 mg/kg, p.o.)/sertraline (20 mg/kg, p.o.) on effects of HAE/sertraline on immobility period using TST and FST models.

Treatment	Immobility period (min): TST	Immobility period (min): FST
VEH (10 ml/kg, p.o.)	164.40 ± 19.76	139.60 ± 8.77
VEH + CYP	189.80 ± 13.95	141.00 ± 7.03
VEH + HAE	70.20 ± 13.58 [#]	67.00 ± 13.24 [#]
CYP+ HAE	160.20 ± 14.13	151.00 ± 23.44
VEH + STR	19.00 ± 2.86 [#]	19.00 ± 2.86 [#]
CYP + STR	132.60 ± 9.26	115.20 ± 3.71

[#]significant $p < 0.05$: Treatment in the presence of antagonist versus treatment alone. HAE: Hydro-alcoholic extract of *A. difformis* rhizome (125 mg/kg, p.o.); VEH: Vehicle (2.5 % Tween 80 in saline, 10 ml/kg, p.o.); STR: Sertraline (20 mg/kg, p.o.); CYP: Cyproheptadine (0.5 mg/kg, i.p.).

immobility period by mice when compared to vehicle treated control group. Pretreatment of mice with 5-HT antagonist, cyproheptadine (0.5 mg/kg, i.p.) 15 min prior to oral administration of EF (250 mg/kg)/sertraline (20 mg/kg) significantly [$F(5, 24) = 29.797$; $p < 0.0001$] reversed the reduction of immobility period produced by these treatments (Table 6). The antagonist administered to mice

alone did not produce any significant effect when compared to control. Also, analysis of result from FST model revealed that EF (250 mg/kg, p.o.)/sertraline (20 mg/kg, i.p.) significantly reduced immobility period compared to control. This effect was significantly [$F(5, 24) = 79.386$; $p < 0.0001$] blocked by pretreatment of mice with cyproheptadine (0.5 mg/kg, i.p.) 15 minutes prior to oral

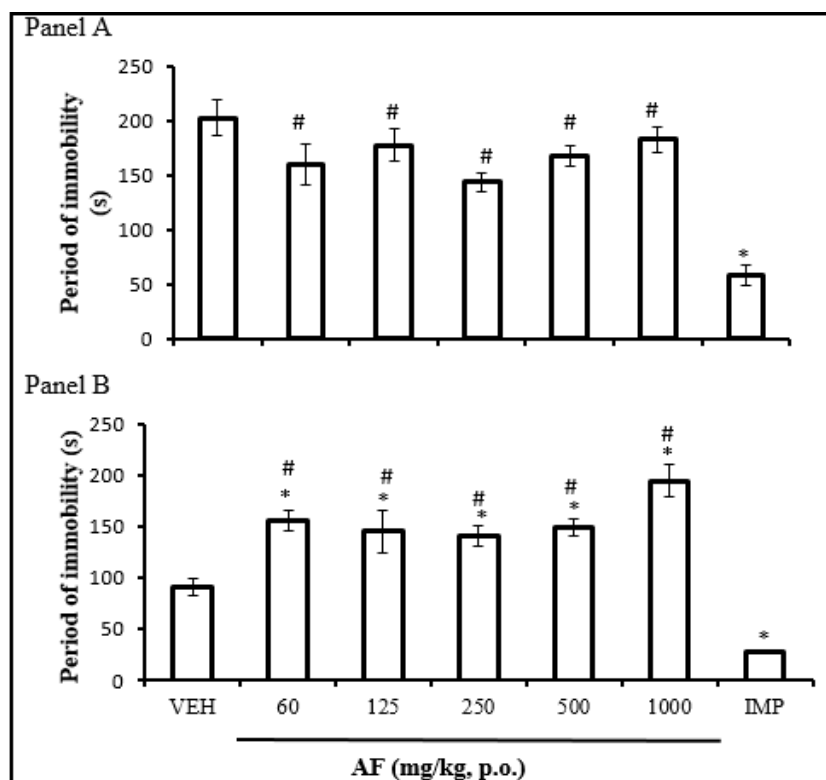


Figure 5. Effects of orally administered AF on period of immobility of mice in TST (Panel A) and FST (Panel B). *significant $p < 0.05$ compared to vehicle-treated control; #significant $p < 0.05$ compared to standard drug, imipramine. AF: Residual Aqueous fraction of *A. difformis* rhizome; IMP: Imipramine (25 mg/kg, i.p.); VEH: Vehicle (Normal saline, 10 ml/kg, p.o.).

Table 6. Influence of pretreatment of mice with cyproheptadine (0.5 mg/kg, i.p.) 15 min prior to administration of EF (250 mg/kg, p.o.)/sertraline (20 mg/kg, p.o.) on effects of EF/sertraline on immobility period using TST and FST models.

Treatment	Immobility period (min): TST	Immobility period (min): FST
VEH (10 ml/kg, p.o.)	164.40 ± 19.76	139.60 ± 8.77
VEH + CYP	189.80 ± 13.95	141.00 ± 7.03
VEH + EF	69.40 ± 9.73 [#]	60.20 ± 3.65 [#]
CYP+ EF	157.40 ± 8.81	153.40 ± 7.43
VEH + STR	19.00 ± 2.86 [#]	19.00 ± 2.86 [#]
CYP + STR	132.60 ± 9.26	115.20 ± 3.71

[#]significant $p < 0.05$: Treatment in the presence of antagonist versus treatment alone. EF: Ethyl-acetate fraction of *A. difformis* rhizome (250 mg/kg, p.o.); VEH: Vehicle (2.5 % Tween 80 in saline, 10 ml/kg, p.o.); STR: Sertraline (20 mg/kg, p.o.); CYP: Cyproheptadine (0.5 mg/kg, i.p.).

administration of EF (250 mg/kg)/sertraline (20 mg/kg). The antagonist administered alone did not produce any significant response compared to control. The results are presented in Table 6.

DISCUSSION

LD₅₀ (24 hours) for each of these plant materials was

greater than 5000 mg/kg since none of the tested mice died up to this dose level at the second phase of the test (Tables 1 and 2). This suggests that the extract and fractions were non-toxic to mice via the oral route when acutely administered (Siqueira et al., 1998). The oral route was chosen for this study not only for its low toxicity profile by this route but also for the fact that oral mode of administration by cold infusion is the method employed by

herbalists in the use of this plant for the treatment of mental disorders.

The FST and TST models used in this study are proven behavioural models for screening biological agents for anti-depressant activity in mice (Steru et al., 1985; De Vry, 1995; Cryan et al., 2002). The effect induced at 125 mg/kg in both models was comparable to the effect elicited by the standard reference drug, imipramine (25 mg/kg, i.p.), suggesting an antidepressant effect of the extract in mice. Of all the four fractions of HAE tested in this study, only EF showed consistent significant reduction of immobility period, hence adjudged to have demonstrated an antidepressant activity in the two models used. The fraction (EF) significantly reduced immobility period at 60, 125, and 250 mg/kg in TST as well as in FST models. This also suggests that the antidepressant-like activity which occurred at low doses of the extracts as well as the most active fraction of the extract resided in the moderately non-polar phyto-constituents of the rhizome. Incidentally, this fraction was the most active of all the fractions in reducing anxiety-like behaviour in mice in a related study (Agboola, 2017). This is in line with the findings of past researchers who had reported the combined anxiolytic and antidepressant activities in one therapeutic agent (Onasanwo et al., 2010; Foyet et al., 2012). For instance, Sertraline, an SSRI, which was used as standard reference drug in this study, was approved for the treatment of depression, as well as some types of anxiety disorders such as panic disorder, obsessive-compulsive disorder, social phobia, and post-traumatic stress disorder (Katzelnick et al., 1995; Rapaport et al., 2001).

The anti-immobility effects of HAE (125 mg/kg, p.o.) and EF (250 mg/kg, p.o.) were significantly blocked by pretreatment with yohimbine (1 mg/kg, i.p.), an α_2 -adrenoceptor antagonist and cyproheptadine (CYP) (0.5 mg/kg, i.p.), a serotonergic receptor (5-HT₂) antagonist on both TST and FST models, suggesting a dual mechanism of action of the plant extract and its EF fraction involving the adrenergic as well as the serotonergic pathways (Tables 3 to 6). Many researchers have reported the involvement of noradrenergic and serotonergic (Delgado et al., 1999) pathways in the mechanism of actions of some classes of antidepressant drugs, such as the Monoamine Oxidase Inhibitors, Selective Serotonin Reuptake Inhibitors and the Tricyclic Antidepressants, based on the monoamine hypothesis of depression (Adongo et al., 2015). The hypothesis states that depression is caused by a functional deficit of monoamine transmitters at certain sites in the brain, while mania results from a functional excess (Rang et al., 2007; Kiss, 2008). In FST and TST, false-positive results can be obtained with certain drugs, in particular, psychomotor stimulants, which decrease immobility time by stimulating locomotor activity (Zomkowski et al., 2006). The results of novelty-induced horizontal locomotor activity at the doses where anti-depressant activity was recorded in this study showed no significant excitatory effect on the CNS

(Agboola, 2017), thus limiting the probability of a false-positive inference.

Conclusion

The hydro-alcoholic extract and ethyl-acetate fraction of the rhizome of *Anchomanes difformis* demonstrated an anti-depressant-like effect which was probably exerted through the adrenergic and serotonergic pathways in mice.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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