Antidepressant Activity and Mechanism of Aqueous Extract of Vigna Unguiculata ssp. Dekindtiana (L.) Walp Dried Aerial Part in Mice

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Abstract

Objective: Vigna unguiculata ssp. dekindtiana (L.) Walp is used in traditional practice to treat depression-like disorders in some communities of Southwest Nigeria. This study investigated the antidepressant-like effects of the aqueous fraction of the dried aerial parts of V. unguiculata ssp. dekindtiana (AFVU). Methodology: AFVU was evaluated for antidepressant effect on the force-swimming test (FST), tail suspension test (TST) and locomotor activity (LC) in the open field test (OFT) using mice; and its probable neural mechanism(s) investigated using various receptor antagonists. Elemental composition (EC) and phyto-constituents of AFVU were analyzed using standard methods. Results: The AFVU (600 and 800 mg/kg, p.o.) significantly (p<0.05) decreased the immobility time of mice in FST and TST without significant (p<0.05) effect on LC, suggesting that its antidepressant-like effect is specific; anti-immobility effect of AFVU was significantly (p<0.05) blocked by intraperitoneal injection of prazosin (62.5 µg/kg), yohimbine, (1 mg/kg), cyproheptadine (3 mg/kg), sulpiride (50 mg/kg), methylene blue (10 mg/kg) and L-NNA (10 mg/kg) suggesting adrenergic, serotonergic, dopaminergic and nitergic pathways. The EC assured its safety; while phenols and alkaloids were the most abundant phytoconstituents in AFVU. Conclusion: This study concluded that AFVU possessed antidepressant-like effects which may be mediated through multiple receptor pathways.

Keywords: Fabaceae, Atomic Absorption Spectroscopy, Elemental Composition, Phytoconstituents, Forced-swimming Test, Tail Suspension Test, Receptor Antagonists

1. Introduction

Herbal therapy is a type of complementary and alternative therapy that uses plants or herbs to treat various disorders [1] and has been an integral part of many cultures [2]. About 80% of the world population has been reported to depend heavily on medicinal plants for their health care needs [3] and many plant species are known to alleviate human health problems [4]. Furthermore, Medicinal plants have been variously shown to play important roles in drug discovery effort [5]. Several plants commonly used in traditional medicine provide biologically active molecules which could be lead compound(s) for the development of new drugs or modified derivatives with enhanced activity and/or reduced toxicity [5]. It is noted in recent times that synthetic drugs are not proving efficient in the treatment of some diseases, but are also becoming expensive, unavailable and often associated with unwanted effects and adulterations in many developing countries [6]. Hence, there is need to search for new drugs from the medicinal plant since the active components of medicinal plant extracts have greater advantage of being combined with many other substances that may be biologically inactive. These complementary components account for its safety and efficacy as a whole plant hence its superiority over the isolated and pure active components [6].

V. unguiculata ssp. dekindtiana (L.) Walp is a wild subspecies of V. unguiculata (family: Fabaceae) is found in many African countries including Nigeria, Cameroen, Swaziland, Malawi and Tanzania [7]. There is scanty literature on V. unguiculata ssp. dekindtiana (L.) Walp but the cultivated subspecies such as V. unguiculata subspecies unguiculata, (cowpea) is known for its edible beans and the leaves are consumed as vegetables [8]. Ethnomedicinally,
the roasted seeds of the cultivated cowpea has been used for the treatment of insomnia, weakness of memory, indigestion, dyspepsia, periodic palpitation, congestive cardiac failure etc. The plant is also believed to be an excellent medicine for stomatitis, corneal ulcers, colic diseases, kwashiorkor and marasmus. The anti-hyperglycemic and antinociceptive effect of the methanolic extract of boiled and non-boiled seed extracts of *V. unguiculata* spp *unguiculata* has been reported [9]. Ethyl acetate fraction of the leaf of *V. unguiculata* spp *unguiculata* has also been reported to possess antioxidant and anti-atherogenic effect in cholesterol-induced atherosclerosis [9]. The subspecies *dekindtiana* (wild subspecies) is used locally in the South western states of Nigeria where the decoction of its aerial part (leaf and stem) is used to manage pain, fever, convulsion and headache especially migraine (Oral communication). In continuation of our search for centrally acting natural agents, the aqueous fraction of this particular species was investigated for possible antidepressant activity as a follow-up to the preliminary screening result showing central stimulating effect when subjected to novelty-induced behaviours in open field. To further exclude false positive antidepressant effect of this fraction, the selected doses used in this investigation were chosen such that they did not significantly increase locomotion behaviour in OFT since CNS stimulant drugs that cause marked motor stimulation of locomotor activity in OFT can give false positive antidepressant effects [10,11].

2. Materials and Methods

2.1. Plant Identification and Authentication

The aerial parts (leaf and stem) of *Vigna unguiculata* (wild) were collected at the back of Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife in August 2016. The plant was identified by Mr. O.S. Shasanya of the Forestry Research Institute of Nigeria (FRIN), Ibadan and voucher specimen number 109763 was deposited. The species was further authenticated by Professor Illoh of the Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife.

2.2. Preparation of Plant Materials

The plant materials were air-dried at room temperature for two weeks and pulverized with a mechanical grinder. Thereafter, 2.9 kg of the powder was extracted with 8.0 litres of absolute methanol. The extract was concentrated *in vacuo* to yield 96 g crude extract (3.3%). Sixty grams of the crude extract was successively partitioned into n-hexane, ethyl acetate, n-butanol and aqueous fractions. The fractions were again concentrated *in vacuo* to give n-hexane fraction (HF 14.4 g, 24%), ethyl-acetate fraction (EAF 2.2 g, 3.7%), n-butanol fraction (BF 3.6 g, 6%) and aqueous fraction (AF 23.6 g, 39.3%). The extract and fractions were freeze-dried and placed in a desiccator for further use. The aqueous fraction of *V. unguiculata* (AFVU) showed CNS stimulatory effect when subjected to novelty-induced behaviours in open field test (OFT) during the preliminary screening and was therefore chosen for further investigation for the antidepressant tests.

2.3. Equipment and Apparatus

Open-field, Perkin Elmer Analyst 400 Atomic Absorption Spectrometer, Shimadzu spectrophotometer, diamond attenuated total reflectance (ATR) accessory on an Agilent Cary 630 spectrophotometer, swimming cylindrical jar.

2.4. Drugs

Prazosin hydrochloride, (+) sulpiride, yohimbine hydrochloride, atropine sulphate, cyproheptadine hydrochloride, L-arginine, L-NG-Nitroarginine, imipramine hydrochloride, methylene blue and fluoxetine hydrochloride were all from Sigma Aldrich, St. Louis, MO, USA; diazepam (Roche, Basel, Switzerland) and normal saline (Unique Pharmaceutical Limited, Lagos, Nigeria). AFVU and the various drugs were dissolved in normal saline and freshly prepared on each day of the experiment.

2.5. Animals

Adult male albino mice (18–25 g) were obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, OAU, Ile-Ife. The animals were maintained on standard animal pellets and water *ad libitum*. The experiment was carried out in strict compliance with the National Institute of Health, 1985 [12] as being implemented by the Faculty of Pharmacy Postgraduate Committee on behalf of OAU Research Committee. The animals were fasted overnight prior to the experiments which were carried out between 9.00 am to 3.00 pm to avoid changes in circadian rhythm that may affect the outcome of behavioural investigations. The experiment was carried out and data collected in September 2016.

2.6. Spectrophotometric Phytochemical Estimation

Determination of Total Alkaloid

To 1mg/ml of AFVU was added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel and 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 μg/ml) were prepared in the same manner as described for the fraction (AFVU). The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed...
as mg of AE/g of extract [13].

**Total flavonoids Content**

The total flavonoids content was estimated using the procedure earlier described [14]. A total of 1 ml of AFVU was diluted with 200 µl of distilled water separately followed by the addition of 150 µl of sodium nitrite (5%) solution. This mixture was incubated for 5 minutes and then 150 µl of aluminium chloride (10%) solution was added and allowed to stand for 6 minutes. Then 2 ml of sodium hydroxide (4%) solution was added and made up to 5 ml with distilled water. The mixture was shaken well and left it for 15 minutes at room temperature. The absorbance was measured at 510 nm. Appearance of pink colour showed the presence of flavonoids content. The total flavonoids content was expressed as gallic acid equivalent mg GAE/g extract on a dry weight basis using the standard curve.

**Determination of tannin Content**

The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the AFVU sample was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent was added to 1 ml of 35 % Na₂CO₃ solution and diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm. A calibration curve of gallic acid was constructed and linearity was obtained in the range of 10-50 µg/ml. The total phenolics content in AFVU was expressed as mg of GAE/g of extract [13].

**Total Phenolics Content**

The total phenolics content of AFVU was estimated using Folin-Ciocalteau reagent [16]. About 20 µg of AFVU was taken separately and it was made up to 1 ml with distilled water. Then 500 µl of diluted Folin-phenol reagent (1:1 ratio with water) and 2.5 ml of sodium carbonate Na₂CO₃ (20%) were added. The mixture was shaken well and incubated in the dark for 40 minutes for the development of colour. After incubation, the absorbance was measured at 725 nm. A calibration curve of gallic acid was constructed and linearity was obtained in the range of 10-50 µg/ml. The total phenolics content in AFVU was expressed as mg of gallic acid equivalent (mg GAE/g extract) by using the standard curve.

**2.7. Atomic Absorption Spectrometer (AAS)**

**Measurement**

AFVU (0.5 g) was weighed using analytical weighing balance into digesting flask, 5 ml of the mixture of nitric and perchloric acid in ratio (4:1) was added, it was heated at 100°C until the solution got discoloured. On cooling, it was made up to the mark of 50 ml in volumetric flask with distilled water. The dilute filtrate solution was used for analysis of elements of interest (K, Na, Mg, Ca, Cu, Fe, Mn, Zn, Ni, Cd, Cr and Pb) by AAS (Perkin Elmer Analyst 400 Atomic Absorption Spectrometer) using suitable hollow cathode lamps. The concentration of various elements was determined by relative method using A.R. grade solutions of elements of interest.

**2.8. Spectroscopic Analysis**

Fourier Transform Infrared (FTIR), spectra were obtained with the aid of a diamond attenuated total reflectance (ATR) accessory on an Agilent Cary 630 spectrophotometer within scanning range of 4000 to 650 cm⁻¹ at a resolution of 4 cm⁻¹ and 16 scans [17].

**2.9. Pharmacological Experiments**

**2.9.1. Acute Toxicity Study**

The toxicity study was carried out in two phases. In the first phase, AFVU was administered to three groups of mice (n=3) at the oral doses of 10, 100 and 1000 mg/kg respectively. In the second phase, another three groups of mice (n=1) were administered 1500, 2900 and 5000 mg/kg of AFVU. After treatment, each mouse was monitored for signs of toxicity for 1 h and mortality after 24 h. From the data obtained, LD₅₀ was determined [18].

**2.9.2. General Experimental Design**

Mice were randomized into 5 groups (n=6). Groups I (negative control received normal saline 10 ml/kg), groups II-IV (AFVU-400, 600 and 800 mg/kg) and group V was positive (standard drug). Each treatment was through the oral route (p.o.) and pretreatment was for 1 h prior to test.

**2.9.3. Antidepressant Experiments**

**2.9.2.1. Effect of AFVU on Forced-Swimming Test (FST) in Mice**

The method used was as described previously [19] which fundamentally assess behavioral despair as a model of antidepressant test. The swimming chamber consists of a cylindrical jar (diameter 20 cm, height 30 cm) filled to 20 cm with water maintained at 25 °C. Time of immobility (when the animal remains passive or inactive) was estimated for the last 4 min out of total time of 6 min. The parameters observed were struggling which consists of swimming (when the animal is active with its limbs) or climbing (when the mouse tries to jump out of the water or uses its forelimbs to climb the wall of the container), and immobility (when the mouse just remains floating without apparent movement). After each experiment, the mouse was cleaned with a hand towel before returning to the home cage. Imipramine (20 mg/kg, p.o.) was used as the reference drug.

**2.9.2.1.1. Effect of Various Antagonists on the Antidepressant Activity of AFVU on the FST in Mice**

In order to delineate the probable mechanism of action of
AFVU in the FST, mice (n=6) were intraperitoneally pretreated with prazosin (62.5 μg/kg, i.p., an α1-adrenoceptor antagonist), yohimbine (1 mg/kg, i.p., an α2-adrenoceptor antagonist), cyproheptadine (3 mg/kg, i.p., a 5-HT2 receptor antagonist), sulpiride (50 mg/kg, i.p., a dopamine D2 receptor antagonist) and atropine (1 mg/kg, i.p., a muscarinic receptor antagonist) [20]; L-Arginine [750 mg/kg, i.p], methylene blue (10 mg/kg, i.p.) [21]; and L-NNA (10 mg/kg, i.p.) [22]. All treatments were 15 min prior to AFVU (800 mg/kg, p.o.) treatment. Sixty min post-AFVU, mice were subjected to FST. The antagonists were used at doses that did not modify locomotor behaviors of mice in FST according to standard procedures [20-22].

2.9.2.2. Effect of AFVU on Tail Suspension Test (TST)

The TST is also based on behavioural despair model for screening antidepressant-like drugs and the method described by Potdar and Kibile [23] was used. Each animal was suspended with the tail attached to a bar 30 cm high on the laboratory table for 5 min and time of immobility assessed. The total time the animal remains inactive or motionless out of the 5 min session was estimated as the immobility time. Depressed animals (negative controls) normally exhibit shorter period of struggling compared to mice pretreated with antidepressant-like drugs. Fluoxetine (20 mg/kg, p.o.) was used as the reference drug.

2.9.2.3. Effect of AFVU on Open Field Test (OFT) in Mice

The OFT was carried out according to Rogoz et al. [24] in order to determine the effect of the extract on spontaneous locomotor activity. Mice were treated as previously described with vehicle, AFVU (400, 600 and 800 mg/kg), and diazepam (1 mg/kg) as the standard drug. Parameters assessed include rearing or vertical locomotion (when the animal places its forelimbs in the air or against the wall of the cage), horizontal locomotion (number of squares crossed with all the four limbs) and total locomotor activity (addition of vertical and horizontal locomotion).

2.9.2.4. Statistical Analysis

Results were expressed as mean ± S.E.M. The significance of difference between treated groups and negative group were analysed using one way analysis of variance (ANOVA), followed by Dunnett’s or Student-Newman-Keuls post hoc analysis. GraphPad InStat® Biostatistics software (GraphPad Software, Inc., La Jolla, USA) was employed. The level of significance for all tests was set at p < 0.05 compared to the negative control group.

3. Results

3.1. Spectrophotometric Quantitative Phytochemical Estimations of AFVU

Phytochemical estimation of AFVU (Table 1) showed that it contained several phytoconstituents including total alkaloid, 87.58 ± 1.02 mg atropine equivalent/g AFVU by reference to standard curve (y = 0.0051x + 0.078, R² = 0.9765), total phenols 86.32 ± 2.34 mg gallic acid equivalent/g AFVU by reference to standard curve (y = 0.0042x + 0.0221, R² = 0.9947), tannin (28.65 ± 5.09 mg gallic acid equivalent/g AFVU) by reference to standard curve (y = 0.0049x + 0.0596, R² = 0.999) and total flavonoids (18.56 ± 0.69 mg gallic acid equivalent/g AFVU) by reference to standard curve (y = 0.003x + 0.125, R² = 0.845).

Table 1. Spectrophotometric quantitative phytochemical estimations of AFVU

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Alkaloid content</td>
<td>87.58 ± 1.02 mg of AE/g of extract</td>
</tr>
<tr>
<td>Total phenol</td>
<td>86.32 ± 2.34 mg of GAE/g of extract</td>
</tr>
<tr>
<td>Tannin content</td>
<td>28.65 ± 5.09 mg of GAE/g of extract</td>
</tr>
<tr>
<td>Total flavonoid content</td>
<td>18.56 ± 6.92 mg QE/g extract</td>
</tr>
</tbody>
</table>

*Values are means of triplicate determination ± Standard deviation; where AE is atropine equivalent, GAE is gallic acid equivalent, and QE is quercetin equivalent.
3.2. Atomic Absorption Spectroscopy (AAS) of AFVU

The AAS analysis of AFVU (Table 2) showed the presence of seven elements in a descending order of magnitude: K, Mg, Fe, Na, Ca, Zn and Mn; while Cd, Pb, Ni, Cu and Cr were not detected. Potassium was more abundant while Nickel was the least.

<table>
<thead>
<tr>
<th>Metals</th>
<th>mg per 100 g of AFVU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium (K)</td>
<td>11,427.500 ± 0.097</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>231.8000 ± 0.004</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>225.000 ± 0.048</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>23.710 ± 0.021</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>7.250 ± 0.054</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>3.700 ± 0.003</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>1.740 ± 0.005</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>ND</td>
</tr>
<tr>
<td>Copper</td>
<td>ND</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>ND</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>0.000 ± 0.000</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>0.030 ±0.006</td>
</tr>
</tbody>
</table>

The result of each of the parameter is in triplicate and presented in mean ± standard deviation.
ND means not detected

3.3. Results of Spectroscopic Analysis

3.3.1. Result of FT-IR Spectroscopic Analysis

AFVU gave absorption bands at 3315.5 which can be attributed to the –OH stretching vibration of phenols, alcohols or polyhydroxy compounds; the peak at 1638 cm\(^{-1}\) can be due to a –C=\(\equiv\)C- in ketone compounds; the peak observed at 1389.6 can be attributed to phenols or tertiary alcohol; while the absorption band observed at 1054.8 cm\(^{-1}\) could be assigned to phosphate ion of phosphate compounds. The results are presented in Table 3.

<table>
<thead>
<tr>
<th>No</th>
<th>Wave number cm(^{-1}) [Test sample]</th>
<th>Wave number cm(^{-1}) [Reference article]</th>
<th>Functional group assignment</th>
<th>Phytoconstituents identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3315.5</td>
<td>3600 - 3100</td>
<td>O-H stretch, Hydroxy group, H-bonded</td>
<td>Phenols, alcohol, Polyhydroxy compounds</td>
</tr>
<tr>
<td>2</td>
<td>1638.2</td>
<td>1650 – 1600</td>
<td>C=O stretching, vibration, Ketone group</td>
<td>Ketone compounds</td>
</tr>
<tr>
<td>3</td>
<td>1389.6</td>
<td>1410 -1310</td>
<td>O-H bend, alcoholic group</td>
<td>Phenol or tertiary alcohol</td>
</tr>
<tr>
<td>4</td>
<td>1054.8</td>
<td>1100 - 1000</td>
<td>Phosphate ion</td>
<td>Phosphate compounds</td>
</tr>
</tbody>
</table>

Figure 1. FT-IR Spectrum of AFVU
3.4. Oral Acute Toxicity Profile of AFVU in Mice

There was no mortality among the animals in the two phases of the model used; hence the LD₅₀ of the aqueous fraction of the aerial part of AFVU was estimated to be ≥5000 mg/kg, p.o. in mice.

3.5. Effect of AFVU on Forced Swimming Test (FST) in Mice

The effect of AFVU (400, 600 and 800 mg/kg) in the forced swim test is shown in Table 4. AFVU at 600 and 800 mg/kg significantly \[F(4, 25) = 25.481, \ p<0.05\] reduced the immobility time when compared to the vehicle (normal saline) group. The standard reference drug (imipramine 20mg/kg, p.o.) also caused significant (p<0.05) reduction in immobility but its effect was lesser than AFVU at 600 and 800 mg/kg.

3.6. Effect of AFVU on Tail Suspension Test (TST) in Mice

The effect of AFVU (400-800 mg/kg, i.p.) in the tail suspension test is shown in Table 4. The AFVU at 600 and 800 mg/kg significantly \[F(4, 25) = 10.194, \ p<0.05\] reduced the immobility time when compared to the vehicle group. Similarly, the standard antidepressant drug, fluoxetine (20 mg/kg, p.o.) also caused significant (p<0.05) reduction in the immobility time on the TST but its effect was lower than AFVU at 600 mg/kg.

3.7. Effects of Various Antagonists on the Antidepressant Activity of AFVU in Mice

The results obtained for the effects of various antagonists on the antidepressant-like activity of AFVU are presented in Figure 1A-H. Pretreatment with prazosin (62.5 µg/kg), yohimbine, (1 mg/kg), cyproheptadine (3 mg/kg), sulpiride (50 mg/kg), methylene blue (10 mg/kg) and L-NNA (10 mg/kg) significantly \[p<0.05, F(4, 25)=27.943, 24.073, 26.224, 37.143, 41.725\] and 34.421) reversed the antidepressant-like effect of AFVU (800 mg/kg, p.o.) respectively. Atropine (1 mg/kg) had no effect, while L-Arginine (750 mg/kg) significantly \[p<0.05, F(4, 25) = 57.844,\] potentiated the antidepressant-like effect of AFVU in the FST.

Table 4. Effects of AFVU on FST and TST behavioural models in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>FST (Mean ± S.E.M)</th>
<th>TST (Mean ± S.E.M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(10 ml/kg)</td>
<td>158.2 ± 7.0</td>
<td>164.5 ± 13.4</td>
</tr>
<tr>
<td>AFVU</td>
<td>400</td>
<td>154.5 ± 6.1</td>
<td>147.5 ± 4.4</td>
</tr>
<tr>
<td>AFVU</td>
<td>600</td>
<td>94.2 ± 7.6*</td>
<td>105.5 ± 4.2*</td>
</tr>
<tr>
<td>AFVU</td>
<td>800</td>
<td>99.0 ± 4.9*</td>
<td>128.0 ± 4.4*</td>
</tr>
<tr>
<td>Imipramine</td>
<td>20</td>
<td>125.3 ± 2.9*</td>
<td>-</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>20</td>
<td>-</td>
<td>125.5 ± 3.7*</td>
</tr>
</tbody>
</table>

Each group represents Mean ± SEM (n=6). AFVU, FST and TST represent aqueous extract of *V. Unguiculata*, forced-swimming test and tail suspension test respectively. *p<0.05 compared to the vehicle \[F(4, 25) = 25.481, \ p<0.05\] and \[F(4, 25) = 10.194, \ p<0.05\] for FST and TST respectively (ANOVA, Dunnett’s).
Figure 2. A–H: The effects of various antagonists: A (yohimbine), B (prazosin), C (cyproheptadine), D (sulpiride), E (atropine), F (methylene blue) and H (L-arginine) on the anti-immobility activity of AFVU in FST.
3.8. Effect of AFVU on Locomotor Activity in the Open Field Test (OFT)

The AFVU at 400, 600 and 800 mg/kg did not show any significant effects on locomotor activity in mice, also, there was no dose dependent increase in locomotor activity by AFVU as assessed by the OFT (Table 5). However, the standard sedative drug, diazepam (1 mg/kg) significantly [F(4, 25)=7.257, p = 0.05] reduced rearing behaviour in mice compared to the vehicle.

4. Discussion

This study evaluated the antidepressant effect of aqueous fraction of the aerial parts of *Vigna Unguiculata* ssp. *dekindtiana* (L.) Walp (AFVU) using force swimming test (FST) and tail suspension test (TST) behavioural models in mice; the neural mechanism(s) of its antidepressant-like effect was determined in the FST model using various antagonists. Furthermore, in order to fully characterize the aqueous fraction used in this study, its elemental composition was determined and its phytoconstituents assayed. The results obtained showed that AFVU possessed antidepressant-like activity and contained various phytoconstituents.

Acute toxicity test carried out on the extract showed that at doses up to 5000 mg/kg, p.o., there was no mortality; hence its LD₅₀ was estimated to be 5000 mg/kg, p.o., and according to Lorke (16) it is non-toxic. Consequently, the entire test doses (400, 600 and 800 mg/kg, p.o.) used were non-toxic to the animals.

Forced swimming and tail suspension behavioural models are the two most widely used animal models for screening antidepressant agents [25]. These two models have the mutual advantages of ease, fast, simple inexpensive equipment and sensitive to detect antidepressant-like drugs [26]. Other shortcomings of these models are that they are sensitive to only acute treatments and their validation for non-monoamine antidepressants is unclear [27]. Poor face values and construct validities are also limitations to FST and TST [28]. Conversely, TST is also disadvantaged in being restricted to mice or to strains that do not climb their tails. Furthermore, Cryan et al. [29] reported that TST can be used to distinguish between antidepressants and other psychotropic drugs such as antipsychotic and anxiolytics.

In these models, immobility reflects a state of despair in animals which is claimed to reproduce a condition similar to or mimic human depression [30,31]. In this study, AFVU reduced the immobility time in FST and TST consistent with antidepressant-like effect signifying potential antidepressant activity. Numerous research findings have shown that agents that shortened the immobility time in FST and TST signifies antidepressant-like effects [23,24,32,33]. For example, it was reported that the methanolic extract of *Passiflora foetida* leaves shortened the immobility time in FST and TST in mice [32], the effect of which was ascribed to its antidepressant-like effect. Likewise, *Withania somnifera* fat extract was demonstrated to reduce the immobility time in FST and TST [33] and the effect was attributed to its antidepressant-like effect. Since AFVU reduced the immobility time in FST and TST, it is therefore suggestive that AFVU may possess antidepressant-like effect.

Previous studies implicated dopaminergic [34], α₁-adrenoceptors [35], α₂-adrenoceptor [36], serotonergic [37], nitric oxide signaling pathway [21] and cholinergic [38] neural mechanisms in the expression of antidepressant-like effect in the behavioural despair models of depression. In this study, pretreatment of mice with prazosin, yohimbine, cyproheptadine, sulpiride, methylene blue, L-Nitroarginine but not atropine abolished the antidepressant-like effect of AFVU indicating that its antidepressant-like effect may be mediated through α₁-, α₂-adrenoceptors, 5-HT₃, dopaminergic and nitric oxide neurotransmissions, while muscarinic cholinergic mechanism may not be involved.
The results of the neural mechanism obtained here are similar to earlier one reported for amentoflavone isolated from Cnestis ferruginea in which prazosin and yohimbine showed similar effect [20]. The present and previous results strongly suggest that the reversal of this antidepressant effect might involve α1- and α2-adrenoceptors, while dopaminergic, serotonergic and muscarinic cholinergic mechanism may play insignificant roles. Likewise, Dhir and Kulkarni [39] reported that the antidepressant effect of MK-801 (dizocilpine; N-methyl-d-aspartate receptor antagonist) was reversed by L-arginine and potentiated by nitric oxide synthase inhibitor (methylene blue) thereby supporting the notion that NO signaling pathway is involved in the antidepressant effects of antidepressant agents [39]. Interestingly and in contrary to earlier works [39] in this finding, L-arginine [a precursor for nitric oxide synthase (NOS)] potentiated the antidepressant-like effect of AFVU while methylene blue (nitric oxide synthase inhibitor) and L-NNA inhibited its antidepressant-like effect, thus, also suggesting the involvement of NO signaling pathway in the antidepressant effects of AFVU. The potentiation of AFVU by L-arginine may be due to additive effects of AFVU and L-arginine since the antidepressant-like effect of L-arginine has been reported [40].

Earlier research findings have demonstrated that drugs that alter general motor activity may give false-positive/negative results in FST and TST [32]. Therefore, the effect of the extract on general locomotor activity was assessed dose dependently in mice. The OFT results (Table 3) indicated that AFVU at all the 3 doses tested (400, 600 and 800 mg/kg, p.o.) did not alter significantly (p>0.05) rearing or locomotor activity when compared to the vehicle, implying that the doses of AFVU used in the FST and TST were unlikely to give false positive effect, hence the antidepressant-like effect of AFVU demonstrated here could not be due to the stimulation of general motor activity.

The quantitative phytochemical estimation of AFVU revealed the presence of alkaloids, phenols flavonoids and tannins. Plant secondary metabolites have been demonstrated to possess diverse biological and therapeutic effects [41]. Therefore, the observed antidepressant effect of AFVU may be due to the synergistic or additive effects of these phytoconstituents or their bio-enhancement may be due to the presence of other chemical substances in AFVU, since it has been observed that no single chemical component is responsible for the efficacy of herbal medicines [42]. However, effort should be geared towards isolating the major secondary metabolites found in the plant such as alkaloids, phenolic compounds, flavonoids and tannins, in order to determine the contributions of each to the antidepressant-like activity of this fraction.

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation [43]. Hence, AFVU was subjected to FTIR analysis for the identification of chemical constituents therein. FT-IR result further confirmed the presence of phenolic compounds in addition to other functional groups (Table 3). The high abundance of phenols and alkaloids may additively or synergistically interact with other phytoconstituents to elicit the observed antidepressant effects of AFVU. Several reports have shown that alkaloids present in medicinal plant extracts are responsible for their antidepressant effects [44]. For instance, it was reported that piperidine alkaloid, pipeline isolated from the fruits of Piper longum exhibited antidepressant-like effects in animal model of depression [45]. Similarly, it was demonstrated that mitragynine, an indole alkaloid isolated from Mitragyna speciosa possessed antidepressant effects in mice [44]. A wide range of phenolic compounds interact directly with neurotransmitter systems [46] and may exert antidepressant effects possibly through monoamine oxidase inhibition resulting in increase in the levels of 5-HT, DA, and noradrenaline in the brain [47].

One of the important parameters for quality control of herbal drugs is the test for contamination of heavy metals (HM) due to environmental pollution [48]. Heavy metals constitute dangerous health hazard if ingested [48], directly impairing mental and neurological functions in humans [49]. For instance, lead exposure is known to disrupt catecholaminergic systems [50] and depression and anxiety disorders are strongly associated with disturbances in these systems [51]. Many important medicinal plants such as St. John’s Wort, Hypericum perforatum L. used for centuries, as anti-depressant agent [52] has now been demonstrated to contain high contents of Cd [53]. Thus, it becomes imperative to determine the level of toxic metals in medicinal plant materials. In the samples of AFVU, Ni, Cu and Cr were not detected while Pb (0.00 ± 0.00 mg/100g) and Cd (0.03 ± 0.006 mg/100g) were below the recommended limit of 10 mg/kg and 0.3 mg/kg respectively [49]. Thus the extract might be considered safe from Ni, Cu, Cr, Pb and Cd toxicities even if ingested over a long period of time as antidepressant drugs are normally used. Several studies have implicated deficiency of trace elements such as zinc [54] and magnesium [55] in pathophysiology and therapy of depression. Also, according to recent studies, trace elements exert their antidepressant effects through the neurotransmitter pathway; for example, the contribution of serotonergic system to the antidepressant effect of zinc [56], the monoaminergic and nitrergic systems are also involved in the antidepressant effects of magnesium [57-58]. Therefore, the presence of Zn and Mg may at least in part be responsible for the antidepressant effect of AFVU.

Considering the substantial antidepressant-like effect of this aqueous fraction of this plant in the two models reported here, it is imperative to carry out further studies to isolate pure compound(s) that can be tested further as a practical approach to discovering novel antidepressant agent. For the first time, the current effort provides new data on the phytochemical constituents of this plant in addition to providing scientific basis for its ethnomedicinal use.
5. Conclusions

This study confirm the antidepressant activity of aqueous fraction of *Vigna Unguiculata* in mice and its mechanism of its actions is suggested to be mediated through noradrenergic, serotonergic, dopaminergic and nitergic pathways.

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