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 nitric pathways. The EC assured its safety; while phenols and alkaloids were the most abundant phytocconstituents 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, Cameroon, 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 antinoceptive 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 fluorescein 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 phenol 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 flavonoids content was expressed as mg of AE/g of extract on a dry weight basis using the standard curve.

Determination of Total Phenolics Content

The total phenolics content of AFVU was estimated using Folin-Ciocalteu 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 Folinis-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 and 16 scans [17].
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 garlic acid equivalent/g AFVU by reference to standard curve (y = 0.0042x + 0.0221, R² = 0.9947), tannin (28.65 ± 5.09 mg garlic 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 garlic 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 |
|---------------------------------------------------------------|
|                          | 87.58 ± 1.02 mg of AE/g of extract |
| Total Alkaloid content | 86.32 ± 2.34 mg of GAE/g of extract |
| Total phenol            | 28.65 ± 5.09 mg of GAE/g of extract |
| Tannin content          | 18.56 ± 6.92 mg of QE/g extract    |

*Values are means of triplicate determination ± Standard deviation; where AE is atropine equivalent, GAE is garlic 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 2. Mineral composition analysis of AFVU with the AAS

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

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=O- 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 3. FTIR interpretation of compounds in AFVU

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

**Figure 1.** FT-IR Spectrum of AFVU
Antidepressant Activity and Mechanism of Aqueous Extract of *Vigna Unguiculata* ssp. *Dekindtiana* (L.) Walp Dried Aerial Part in Mice

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 \[p<0.05, \text{F}(4, 25) = 27.943, 24.073, 26.224, 37.143, 41.725 and 34.421\] 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 \[p<0.05, \text{F}(4, 25) = 58.844\] potentiated the antidepressant-like effect of AFVU in the FST.

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

| Group   | Dose (mg/kg) | Duration of Immobility (sec) | Duration of Immobility (sec) |
|---------|--------------|------------------------------|------------------------------|
|         |              | (Mean ± S.E.M)               |                              |
| Control | (10 ml/kg)   | 158.2 ± 7.0                  | 164.5 ± 13.4                 |
| AFVU    | 400          | 154.5 ± 6.1                  | 147.5 ± 4.4                  |
| AFVU    | 600          | 94.2 ± 7.6*                  | 105.5 ± 4.2*                 |
| AFVU    | 800          | 99.0 ± 4.9*                  | 128.0 ± 4.4*                 |
| Imipramine | 20          | 125.3 ± 2.9*                  | -                            |
| Fluoxetine | 20          | 125.5 ± 3.7*                  |                              |

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

Table 5. The effects of AFVU on locomotor and rearing behaviours in mice in the open field for 5 min

| Group       | Doses (mg/kg) | Number of squares crossed (Mean ± S.E.M.) | Number of rearing (Mean ± S.E.M.) | Total (Mean ± S.E.M.) |
|-------------|---------------|------------------------------------------|----------------------------------|---------------------|
| Control     | (10 ml/kg)    | 89.3 ± 7.8                               | 35.0 ± 2.9                       | 124.3 ± 9.9         |
| AFVU        | 400           | 88.5 ± 5.2                               | 32.2 ± 3.6                       | 120.7 ± 7.6         |
| AFVU        | 600           | 100.0 ± 8.4                              | 38.0 ± 2.8                       | 138.0 ± 11.0        |
| AFVU        | 800           | 90.0 ± 2.8                               | 37.3 ± 1.8                       | 127.3 ± 3.6         |
| Diazepam    | 1             | 94.0 ± 3.6                               | 18.0 ± 3.7*                      | 112.0 ± 4.4         |

Each value represents Mean ± SEM, n=6.
*p<0.05 compared to the vehicle [F(4, 25)=7.257, p = 0.05] (ANOVA, Dunnett’s)
Each column represents Mean ± SEM, n=6. AVFVU is aqueous fraction of *V. unguiculata* and VEH is normal saline.
*p<0.5 compared to vehicle, #p<0.05 compared to AFVU treated group (ANOVA, Student-Newman-Keuls post hoc test).

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 LD<sub>50</sub> 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], α<sub>1</sub>-adrenoceptors [35], α<sub>2</sub>-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 α<sub>1</sub>-, α<sub>2</sub>-adrenoceptors, 5-HT<sub>2</sub>, 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 nitric 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 nitricergic pathways.

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REFERENCES

[1] EL Cooper. CAM Bioprospecting: The 21st Century Pyramid. Evid Based Complement Altern Med. 2(2):125-127, 2005.

[2] F Firenzuoli, L Gori. Herbal medicine today: clinical and research issues. Evid Based Complement Altern Med. 4(1): 37–40, 2007.

[3] WHO. National policy on traditional medicine and regulation of herbal medicines: Report of a WHO global survey. World Health Organization, Geneva, Switzerland, 2005.

[4] B Beyene, H Deribe. Review on Application and Management of Medicinal Plants for the Livelihood of the Local Community. JRDM; 22: 33-39, 2010.

[5] DJ Newman, GM Cragg. "Natural products as sources of new drugs over the last 25 years". J Nat Prod. 70(3):461–477, 2007.

[6] LU Shariff. Modern herbal therapy for common ailments. Nature Pharmacy Series Spectrum Books Limited, Ibadan, Nigeria in Association with Safan Books (Export) Limited, United Kingdom; Vol. 1. 9–84, 2001.

[7] LA Ogunkami, OT Ogundipe, NQ Ng, CA Fakotokun. Genetic diversity in wild relatives of cowpea (*Vigna unguiculata*) as revealed by simple sequence repeats (SSR) markers. J Food Agric Environ. 6(3-4):263-268, 2008.

[8] M Rahmatullah, QT Tazin, JF Rumi, S Rahman, A Al-nahain, R Jahan. Oral glucose tolerance and antinociceptive activity evaluation of methanolic extract of *Vigna unguiculata ssp. unguiculata* beans. WJPSS. 3(8): 28-37, 2014

[9] LO Ojwang, L Yang, L Dykes, J Awika. Proanthocyanidin profile of cowpea (*Vigna unguiculata*) reveals catechin-O-galloside as the dominant compound. Food Chem. 139(1-4):35-43, 2013.

[10] RD Porstelt, A Bertin, M Jaffre. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther. 229:327–336, 1977.

[11] HEL Refaey, HS Amri. Effects of antidepressants on behavioral assessment in adolescent rats. Bahrain Med Bull. 33:1–12, 2014.

[12] National Institute of Health. Guide for the Care and Use of Laboratory Animals. National Research Council. National Academy Press, Washington, DC. 1985.

[13] F Shamsa, H Monsef, R Ghamooshi, M Verdi-an-rizi. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J Pharm Sci. 32:17-20, 2003.

[14] Z Jia, M Tang, J Wu. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64(4): 555–559, 1999.

[15] N Tamilselvi, P Krishnamoorthy, R Dhamotharan, P Arumugam, E Sagadevan. Analysis of total phenols, total tannins and screening of phyto components in *Indigofera aspalathoides* (Shivanan Vembu) Vahl EX DC. J Chem Pharm Res. 4(6):3259-3262, 2012.

[16] P Siddhuraju, K Becker. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J. Agric. Food Chem. 51(8):2144–2155, 2003.

[17] OA Fadare, OM Durosimi, R Fadare, OU Izvebkhai, IO Awoniyi, CA Obafemi. ATR-FTIR and HPLC Spectroscopic Studies and Evaluation of Mineral Content of *Carica papaya* Leaves and Flowers. J Phytotherapy. 1(1):1-7, 2015.

[18] D Lorke. A new approach to practical acute toxicity testing. Arch. Toxicol. 54(4): 275-287, 1983.

[19] G Wegener, V Volke, R Rosenberg. Endogeneous nitric oxide decreases hippocampal levels of the serotonin and dopamine in vivo. Br J Pharmacol. 130(3):575–80, 2000.

[20] IO Ishola, OE Agbaje, T Narendre, OO Adeyemi, R Shukla. Bioactivity guided isolation of analgesic and anti-inflammatory constituents of *Canisiss ferruginea* Vahl ex DC (Connaraceae) root. J. Ethnopharmacol. 142(2):383–9, 2012.

[21] A Dhir, SK Kulkarni. Possible involvement of nitric oxide (NO) signaling pathway in the antidepressant-like effects of MK 801 dizocilpine, a NMDA receptor antagonist in mouse forced swim test. Indian J Exp Biol. 46:164–170, 2008.

[22] Y Ergün, UGO Ergün, FO Orhan, E E Küçük. Co-administration of a nitric oxide synthase inhibitor and melatonin exerts an additive antidepressant-like effect in the mouse forced swim test. Med Sci Monit. 12(9):307-312, 2006.

[23] VH Potdar, SJ Kibile. Evaluation of Antidepressant-like Effect of Citrus Maxima Leaves in Animal Models of Depression. Iran J Basic Med Sci. 14(5):478–483, 2011.

[24] Z Rogoz, G Skuza, A Khodzinska. Anxiolytic like effects of the preferential dopamine D1 receptor agonists in an animal model. Pol J Pharmacol. 55(3): 449-454, 2003.

[25] M Mantovani, R Pertile, JB Calixto, AR Santos, AL Rodrigues. Melatonin exerts an antidepressant-like effect in the tail suspension test in mice: evidence for involvement of N-methyl-D-aspartate receptors and the L-arginine-nitric oxide pathway. Neurosci Lett. 343(1):1-4, 2003.

[26] TR Powell, C Fernandes, LC Schalkwyk. Depression-Related Behavioral Tests. Current Protocols in Mouse Biology. 2:119-127, 2016.

[27] HC Yan, X Cao, M Das, XH Zhu, TM Gao. Behavioral...
animal models of depression. Neurosci Bull. 26(4):327-37, 2010. doi: 10.1007/s12264-010-0323-7.

[28] PV Holmes. Rodent models of depression: Reexamining validity without anthropomorphic interference. Crit Rev Neurobiol. 15:143-174, 2003.

[29] JF Cryan, C Mombereau, A Vassout. The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. Neurosci Behav Rev. 29:571-625, 2005.

[30] V Tayal, BS Kalra, S Chawla. Evaluation of antidepressant activity of tramadol in mice. Indian J Pharmacol. 40(3):129–130, 2008.

[31] JV Esplugues. NO as a signaling molecule in the central nervous system. Br J Pharmacol. 135(5):1079–1095, 2002.

[32] P Santosh, R Venugop, AS Nilakash, S Kunjibihari, L Mangala. Antidepressant activity of methanolic extract of Passiflora foetida leaves in mice. Int J Pharm Pharm Sci. 3(1):112–115, 2011.

[33] MK Jayanti MK, C Prathima, JC Huralikuppi, RN Suresha, D Murali. Anti-depressant effects of Withania somnifera fat (Ashwagandha Ghrutha) extract in experimental mice. JIPBS. 3(1):33–42, 2012.

[34] F Ferrari, D Giuliani. Effects of (-) eticlopride and 7-OH-DPAT on the tail-suspension test in mice. J Psychopharmacol. 11(4): 339–44, 1997.

[35] EA Stone, D Quatermain. Alpha-1-noradrenergic neurotransmission, corticosterone, and behavioral depression. J Neurosci. 21(13):4875–82, 2001.

[36] NL Schramm, MP McDonald, LE Limbird. The alpha (2a)-adrenergic receptor plays a protective role in mouse behavioral models of depression and anxiety. J Neurosci. 2003.

[37] JP Redrobe, M Bourin. Clonidine potentiates the effects of 5-HT, 5-HT2A/2C and 5-HT1A/1B antagonists and 8-OH-DPAT in the mouse forced swimming test. Eur Neuropsychopharmacol. 8(3): 169–73, 1998.

[38] A Dhir A, SK Kulkarni. Involvement of nitric oxide (NO) signaling pathway in the antidepressant action of bupropion, a dopamine reuptake inhibitor, Eur J of Pharmacol. 568(1-3):177-85, 2007.

[39] AS Elhewegi. Central monoamines and their role in major depression. Prog Neuropsychopharmacol Biol Psychiatry. 28(3): 435–51, 2004.

[40] G Da Silva, A Matteussi, ARS Santos, JB Calixto, ALS Rodrigues. Evidence for dual effects of nitric oxide in the forced swimming test and in the tail suspension test in mice. Neuroreport. 11(7): 3699-702, 2000.

[41] R Vishnu, R Nisha, S Jamuna, S Paulsamy. Quantification of total phenolics and flavonoids and evaluation of in vitro antioxidant properties of methanolic leaf extract of Tarenna asiatica - an endemic medicinal plant species of Maruthamalai hills, Western Ghats, Tami Nadu. J Res Plant Science. 2(2): 196–204, 2013.

[42] N Sreevidya, S Mehrotra. Spectrophotometric method for estimation of alkaloids precipitable with Dragendorf’s reagent in plant materials. J AOAC Int. 86(6): 1124–1127, 2003.

[43] M Johnson, N Janakiriama, SS Sathish. Phytochemical Analysis of Vitex altissima L. using UV-VIS, FTIR and GC-MS. International Journal of Pharmaceutical Sciences and Drug Research, 4(1): 56-62, 2012

[44] NF Idaru, MT Hidayat, MAM Moklas, F Sharida, AN Raudzah, AR Sharmima, E Apyani. Antidepressant-like effect of mitragynine isolated from Mitragyna speciosa Korth in mice model of depression. Phytomedicine. 18(5): 402–407, 2011.

[45] SA Lee, SS Hong, XH Han, JS Hwang, GJ Oh, KS Lee, MK Lee MK, BY Hwang, JS Ro. Piperine from the fruits of Piper longum with inhibitory effect on monoamine oxidase and antidepressant-like activity. Chem Pharm Bull. 53:832–35, 2005.

[46] DO Kennedy, EL Wightman. Herbal Extracts and Phytochemicals: Plant Secondary Metabolites and the Enhancement of Human Brain Function. Adv Nutr. 2(1):32–50, 2011.

[47] Y Xu, Z Wang, W You, X Zhang, S Li, PA Barish, MM Vernon, X Du , G Li, J Pan, WO Ogle. Antidepressant-like effect of trans-resveratrol: Involvement of serotonin and noradrenaline system. Eur Neuropsychopharmacol. 20(6): 405–13, 2010

[48] D Jебasingh, DD Jackson, S Venkataraman, BS Emerald. Physiochemical and toxicological studies of the medicinal plant Cyperus rotundus L (Cyperacea). Int J Appl Res Nat Prod. 5(4): 1-8, 2013.

[49] S Tong, YE Schirming, T Prapamontol. Environmental Lead Exposure: a Public Problem of Global Dimension. Bull World Health Organ. 78(9):1068-1077, 2000.

[50] SV Kala, AL Jadhav. Region-specific alterations in dopamine and serotonin metabolism in brains of rats exposed to low levels of lead. Neurotoxicology. 16(2): 297–308, 1995.

[51] BW Dunlop, CB Nemeroff. The role of dopamine in the pathophysiology of depression. Arch Gen Psychiatry. 64(3): 327–337, 2007.

[52] L Verotta. ‘Hypericum perforatum, a Source of Neuroactive Lead Structures’. Curr Top Med Chem. 3(2): 187-201, 2003.

[53] M Schneider, R Marquard. ‘Investigation on the Uptake of Cadmium in Hypericum perforatum L. (St. John’s wort)’. Acta Hortic. 426: 435-442, 1996.

[54] B Szewczyk, E Poleszak, P Wla, A Wrbel, E Blicharska, A Cichy A, M Dybala, A Święek, L Pomienny-Chamiolo, A Pietrowska, P Bratiski, A Pilec, G Nowak. The involvement of serotoninergic system in the antidepressant effect of zinc in the forced swim test. Prog Neuropsychopharmacol and Biol Psychiatry. 33(2):323–329, 2009.

[55] J Wójcik, D Dudek, M Schlegel-Zawadzka, M Grabska, S Marcinek, E Florek, W Piekoszewski, RJ Nowak, W Opoka, G Nowak. Antepartum/postpartum depressive symptoms and serum zinc and magnesium levels. Pharmarol Rep. 58(4):571–576, 2006.

[56] B Szewczyka, M Sowaa, A Czuprynb, JM Wieronska, P Brańska, K Sadliłk, W Opoka, W Piekoszewski, M Smialowska, J Skangiel-Kramsk, A Pilec A, G Nowak. Increase in synaptic hippocampal zinc concentration following chronic but not acute zinc treatment in rats. Brain Res. 1090: 69–75, 2006.

[57] GA Eby, KL Eby. Rapid recovery from major depression
using magnesium treatment. Med Hypothese. 67(2):362–370, 2006.

[58] CC Cardoso, KR Lobato, RW Binfaré, PK Ferreira, AO Rosa, AR Santos, AL Rodrigues. Evidence for the involvement of the monoaminergic system in the antidepressant-like effect of magnesium. Prog in Neuropsychopharmacol and Biol Psychiatry. 33(2): 235–242, 2009.