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# *Xylopia aethiopica* fruit extract exhibits antidepressant-like effect via interaction with serotonergic neurotransmission in mice

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α-Methyl-*p*-tyrosine (PubChem CID: 81824)
*p*-Chlorophenylalanine (PubChem CID: 73610)
d-cycloserine (PubChem CID: 6234)
d-serine (PubChem CID: 71077)
Desipramine (PubChem CID: 2995): Fluoxetine (PubChem CID: 3386)
l-arginine (PubChem CID: 6322)
$N_\omega$-nitro- l-arginine methyl ester (PubChem CID: 135193)
Noradrenaline (PubChem CID: 439260)
Reserpine (PubChem CID: 5770)

ABSTRACT

*Ethnopharmacological relevance:* *Xylopia aethiopica* has been used traditionally to treat some central nervous system disorders including epilepsy.
*Aim of the study:* Despite the central analgesic and sedative effects, there is little evidence for its traditional use for CNS disorders. This study thus assessed the antidepressant potential of *Xylopia aethiopica* ethanolic fruit extract (XAE).
*Material and methods:* Antidepressant effect was assessed in the forced swim test (FST) and tail suspension test (TST) models in mice. The role of monoamines in the antidepressant effects of XAE was evaluated by selective depletion of serotonin and noradrenaline, whereas involvement of NMDA/nitric oxide was assessed with NMDA receptor co-modulators; D-serine and D-cycloserine and NOS inhibitor, L-NAME.
*Results:* *Xylopia aethiopica* (30, 100, 300 mg kg$^{-1}$) dose dependently reduced immobility in both FST and TST. The reduced immobility was reversed after 5-hydroxytryptamine (5-HT) depletion with tryptophan hydroxylase inhibitor—*p*-chlorophenylalanine (*p*CPA) and after monoamine depletion with vesicular monoamine transporter inhibitor—reserpine. The observed antidepressant effect was not affected by catecholamine depletion with the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine (AMPT). Similarly XAE did not potentiate the toxicity of a sub-lethal dose of noradrenaline. XAE had a synergistic effect with the glycine$_B$ receptor partial agonist, D-cycloserine and nitric oxide synthase inhibitor, L-NAME. However established antidepressant effects of XAE were abolished by NMDA and NOS activation with D-serine and L-arginine.
*Conclusion:* This study shows that *Xylopia aethiopica* has antidepressant potential largely due to effects on 5-HT neurotransmission with possible glutamatergic effect through the glycine$_B$ co-binding site and nitric oxide synthase inhibition.



## 1. Introduction

Depression is a chronic debilitating disease with wavering symptomatology that affects over 120 million people globally (Poleszak et al., 2011). The WHO estimates that depression would have the highest burden of disease by the year 2030 (Kirsch et al., 2008; Cohn et al., 2012). In the USA, direct and indirect cost due to depression run up an estimated 83 billion dollars a year and similar economic implications exists in other countries (Greenberg et al., 2003). In West Africa, Ghanaian university students have the highest prevalence of depression in the sub-region (Asante and Andoh-Arthur, 2015). Although strides have been made in the search for antidepressants in the last few decades, the search for newer antidepressant is still relevant due to sub-optimal efficacies with clinical effects of current therapies not differing much from placebos (Khan et al., 2000; Kirsch et al., 2002). Over 40% of patients are refractory to current treatment whereas most antidepressants have slower onset of action (Rosenzweig-Lipson et al., 2007; Kirsch et al., 2008). Thus the race for better antidepressants is still relevant.

*Xylopia aethiopica* (Annonaceae) is a common spice in West Africa. It has been used traditionally for several disorders including neurological diseases like epilepsy (Souza and Dossa, 1988), inflammatory disorders: bronchitis, haemorrhoids and rheumatism (Igwe et al., 2003) and painful conditions such as neuralgia and lumbago. It has been shown to have anti-inflammatory effects (Obiri and Osafo, 2013), central analgesic (Ameyaw et al., 2014, Woode et al., 2013), sedative (Biney et al., 2014) and anticonvulsant effects (Okoye et al., 2013). The antiproliferative effects against human cervical cancer cells has been also reported

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(Choumessi et al., 2012) in addition to protective effect on γ radiation-induced liver and kidney damage (Adaramoye et al., 2010) while Woode et al. (2011) have also evaluated its effect on reproductive functions.

It is recognized that certain drugs that have been successful in the management of one neurological disorder have also found translational use in the management of other CNS disorders (O'Connor and Dworkin, 2009; Whiteside et al., 2010). For example, tricyclic antidepressants (TCAs) and the serotonin and noradrenaline reuptake inhibitor duloxetine is used as a first-line drug in managing neuropathic disorders like diabetic neuropathy and other chronic painful disorders such as fibromyalgia (O'Connor and Dworkin, 2009) while the anti-epileptic lamotrigine have also been used to manage mood and affective disorders (Ettinger and Argoff, 2007). Thus, the central analgesic and anticonvulsant effects of *Xylopia aethiopica* could suggest possible effects in other neurologic disorders like depression.

Activation of immune cells in the brain by induced inflammatory cytokines disturbs neuroendocrine function, neurotransmitter metabolism and neural plasticity leading to development of depression (Anisman et al., 2008; Dantzer et al., 2008; Raison et al., 2010). Recognizing significant nexus between inflammation and neurological disorders, the reported anti-inflammatory effects of *Xylopia aethiopica* as well as its CNS effects, this study evaluates the antidepressant potential of *Xylopia aethiopica* fruit extract and possible mechanisms underlining this.

## 2. Materials and methods

### 2.1. Animals

Swiss Webster mice (20–25 g, 8–10 weeks) were obtained from Noguchi Memorial Institute of Medical Research (NMIMR), University of Ghana, Accra and housed in the vivarium of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST), for acclimatization until they were used. Animals were housed 10 mice per cage with soft wood shavings as bedding in a 12/12 h day/night cycle. Food (normal mice *chow*: Agricare Ltd, Kumasi, Ghana) and water (normal tap water) was *ad libitum*. All experiments were carried out in accordance with NIH Guidelines for the Care and Use of Laboratory Animals with ethical approval from the Department of Pharmacology Animal Ethics Committee.

### 2.2. Extract preparation

Fresh unripe fruits of *Xylopia aethiopica* were harvested from KNUST Botanic Gardens (06° 41′6.38″ N; 01° 33′ 44.34″ W) in December 2013. Its authenticity was confirmed by comparison to voucher specimen (FP/09/77) at Department of Herbal Medicine, KNUST. It was shade-dried, milled and then 2 kg was cold macerated with 70% (v/v) ethanol for 72 h. The extract obtained was concentrated to a semisolid brownish mass (yield 32.5%). An HPLC fingerprint of the extract (Fig. 1) was obtained to characterize the extract as previously described by Adosraku and Kyekyeku (2011).

### 2.3. Forced swim test

The forced swim test was performed as outlined by Porsolt et al. (1977) and slightly modified. Animals (n=8) received either orally *Xylopia aethiopica* extract (XAE) 30, 100 or 300 mg kg$^{-1}$, fluoxetine (FLX) 3, 10 or 30 mg kg$^{-1}$, desipramine (DES) 3, 10 or 30 mg kg$^{-1}$ or distilled water 10 ml kg$^{-1}$. Based on preliminarily determined time of peak effect (TPE), behavioural experiments were conducted after 120 min for XAE and 60 min for DES, FLX and distilled water post drug treatment. Mice were gently placed in identical cylindrical plastic tanks (25 cm high, 10 cm internal diameter) containing water (23 ± 1 °C) 15 cm deep and allowed to swim for six minutes recording with a camera suspended 80 cm above the tanks. Duration of escape oriented behaviours (climbing and swimming) and immobility over the last four minutes of the test were quantified by an experienced observer blinded to all treatment groups using the public domain software JWatcher Version 1.0™ (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia. Available at ⟨http://www.jwatcher.ucla.edu/⟩).

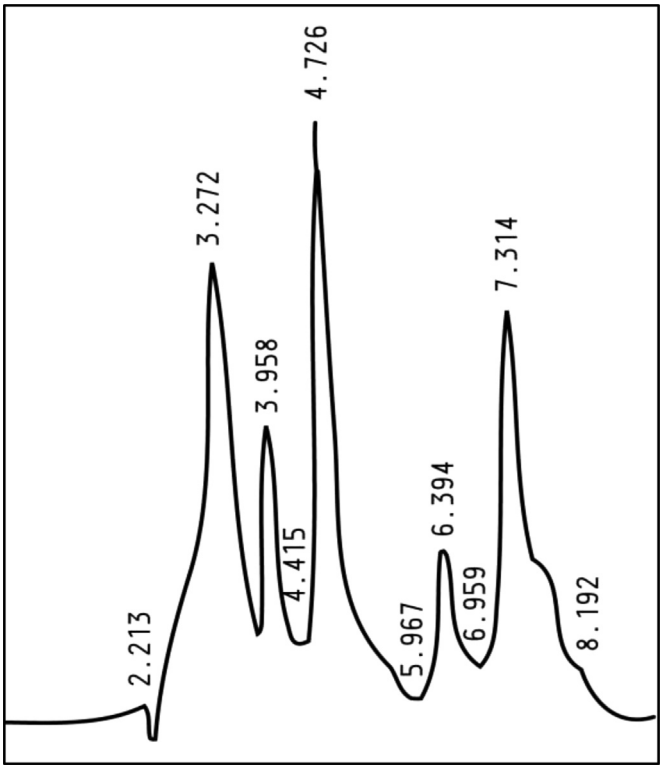


**Fig. 1.** HPLC fingerprint of ethanolic fruit extract of Xylopia aethiopica. Mobile phase: methanol and water (9:1) eluted isocratically at 0.5 ml min$^{-1}$ and absorbance of eluent was monitored at 206 nm.

### 2.4. Tail suspension test

Tail suspension test as earlier described was employed (Steru et al., 1985). Randomly-grouped Swiss mice (n=8) received XAE 30, 100 or 300 mg kg$^{-1}$, fluoxetine 3, 10 or 30 mg kg$^{-1}$, desipramine 3, 10 or 30 mg kg$^{-1}$ or vehicle 10 ml kg$^{-1}$. At time of peak effect, they were individually suspended at their tail (1 cm from the tip) with an adhesive tape on a horizontal bar raised 52 cm from a table top. Duration of escape-oriented behaviours (pedaling, curling and swinging) and immobility were recorded with a camera for 6 min and quantified with JWatcher™ by an experienced observer blinded to all treatment groups. Mice that climbed on their tail were gently pulled down and the test continued.

### 2.5. Effect of monoamines

The possible involvement of monoamines in the observed antidepressant-like effects of XAE was assessed by inhibition of storage or synthesis of monoamines based on previous work by O'Leary and colleagues (O'Leary et al., 2007). To deplete both cytoplasmic pools and vesicular stores of 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine (DA), mice were assigned