Journal of Ethnopharmacology 207 (2017) 129–145

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Maerua angolensis stem bark extract reverses anxiety and related behaviours in zebrafish—Involvement of GABAergic and 5-HT systems

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ARTICLE INFO

Keywords:
5-HT
Maerua angolensis
Zebrafish
Anxiolytic
$GABA_A$ receptor

ABSTRACT

Ethnopharmacological relevance: *Maerua angolensis* DC (Capparaceae) has been employed in the management of several central nervous system (CNS) disorders including anxiety. This study evaluated the anxiolytic effects of the petroleum ether/ethyl acetate fraction stem bark extract and its possible mechanism(s) using zebrafish anxiety models.

Methods: Adult zebrafish, tested in the novel tank and light dark tests, have shown by previous authors to be sensitive to the anxiolytic effects of known anxiolytic drugs. Adult zebrafish were treated with *M. angolensis* extract, fluoxetine, desipramine, and diazepam followed by testing in the novel tank and light dark tests. We further assessed the effect of the extract on anxiety after inducing an anxiogenic phenotype using the ethanol-withdrawal and chronic unpredictable stress (CUS) tests. The anxiolytic effect was further investigated after pretreatment with flumazenil, granisetron, cyproheptadine, methysergide and pizotifen.

Results: *M. angolensis* extract, similar to fluoxetine and desipramine, demonstrated significant anxiolytic behaviour at doses that did not reduce locomotor activity significantly. Similar anxiolytic effects were recorded in the ethanol withdrawal-induced anxiety test. Furthermore, the anxiogenic effects induced by the CUS paradigm were significantly reversed by treatment *M. angolensis* extract and fluoxetine. The anxiolytic effects of *M. angolensis* extract were however reversed after pre-treatment with flumazenil, granisetron, cyproheptadine, methysergide and pizotifen.

Conclusions: Taken together, this suggests that the petroleum ether/ ethyl acetate fraction of *M. angolensis* possesses significant anxiolytic activity, which could partly be accounted for by an interaction with the serotoninergic system and the $GABA_A$ receptor.

1. Introduction

Anxiety is a normal adaptive mechanism employed by humans, smaller rodents as well as fish to cope with potential danger (Leonardo and Hen, 2008). However, in humans, this state of cognitive and behavioural preparedness can be maladaptive and impair the ability to respond optimally to the environment with associated negative economic and social implications (Leonardo and Hen, 2008).

Withdrawal-induced anxiety is a common problem with drug abuse both self-medicating and chronically treated patients with typical physiological signs such as increased anxiety, tremors, agitation, memory impairment and elevated cortisol levels (Holcombe et al., 2013; Saitz, 1998). The selective serotonin re-uptake inhibitors (SSRIs) and benzodiazepines have proven to be very effective in managing symptoms of increased anxiety states. However, adequate treatment of anxiety with current treatment has proved to be expensive and elusive

Abbreviations: 5-HT, 5- hydroxytryptamine; 5-HTT, 5-hydroxytryptamine transporter; AUC, area under curve; ANOVA, analysis of variance; CNS, central nervous system; CS, chasing stress; CUS, chronic unpredictable stress; Cypro, cyproheptadine; D_1, Dopamine receptor D_1 subtype; D_2, Dopamine receptor D subtype; DA, dopamine; DBE, dorsal body exposure; Dzp, diazepam; EtOH, Ethanol; Flz, fluoxetine-;; FMZ, flumazenil; FT-IR, Fourier transform infrared spectroscopy; GABA, gamma aminobutyric acid; $GABA_A$, GABA receptor subtype A; Gstn, granisetron; HS, heat stress; i.p., intraperitoneal; IR, Infrared spectroscopy; KNUST, Kwame Nkrumah University of Science and Technology; LD, light dark; MAE, *Maerua angolensis* extract; Met, methysergide; NE, norepinephrine; NT, novel tank;; Piz, pizotifen; RS, restrain stress; SEM, standard error of mean; SERT, serotonin transporter; SI, social isolation; SSRI, selective serotonin reuptake inhibitor; TC, tank change; Tx, treatment; W/, withdrawal

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http://dx.doi.org/10.1016/j.jep.2017.06.012
Received 15 November 2016; Received in revised form 19 May 2017; Accepted 8 June 2017
Available online 20 June 2017
0378-8741/

with a high incidence of relapse and ineffectiveness in co-morbid affective disorders like depression. This presents an opportunity to uncover novel sources of compounds that possess a better pharmacological profile with respect to efficacy and frequency of remission in clinically ill patients.

Several natural remedies possess diverse benefits in behavioural and mental disorders. Local remedies also serve as a cheap source of therapy and have been employed in the management of disorders such as anxiety, schizophrenia, and epilepsy. *Maerua angolensis* D.C (Capparaceae) is a local plant found in various parts of West and Central Africa with a myriad of uses for neurologic disorders (Iliya and Woode, 2014; Malami et al., 2014). The root and stem bark decoction is considered sedating and have been used in the management of pain, epilepsy and psychosis (Chhabra et al., 1989). Additionally, *Maerua angolensis* is in traditional medicine for ameliorating anxiety associated with other disease states such as schizophrenia.

Zebrafish displays a high homology to humans with an intact and highly conserved neurotransmitter system (Panula et al., 2006) and exhibits similar neurobehavioural phenotypes that can easily be quantified (Bencan et al., 2009). It has hence become increasingly popular in evaluating anxiety phenotypes. Previous reports have shown that the adult zebrafish is sensitive to the effects of known anxiolytic agents in the light-dark and novel tank tests. The novel tank diving test, conceptually similar to the open field test in rodents, is the most extensively studied model of anxiety in the zebrafish. The novel tank test explores the fact that adult zebrafish spend about 50% of a 5 min session in the bottom of a novel tank (Maximino et al., 2012) an effect which is reduced by diazepam, buspirone, or acute ethanol treatment (Maximino et al., 2010b)

The light-dark test can be used to assess anxiety in zebrafish and is similar to the light dark test used to assess anxiety in rodents (Chakravarty et al., 2013). Behaviour in this task reflects a conflict between the preference of the animal for dark protected areas and an innate motivation to explore new environments. Spending longer periods in the dark chamber reflects an increased anxiety state. Additionally, an increased anxiety state is also indicated by a decrease in the latency to enter the dark chamber, similar to the light-dark box test for rodents (Maximino et al., 2010b).

Conventional in vivo anxiety models that employ rodents offer at best medium throughput assays and in addition employs relatively high amounts of compounds for testing. Screening carried out in zebrafish within the past decade have confirmed the ability of this model system to identify bioactive compounds in a target-independent manner, while using micro- to milligram quantities to establish pharmacological effect, thereby facilitating the discovery of potentially superior activity of novel compounds (Crawford et al., 2011).

In bioactivity testing, the zebrafish as a model organism have served as a screening platform for screening different neuroactive agents including crude plant preparation as seen in Torres-Hernandez *et al.*, 2016, where the authors demonstrated anti-seizure activity of crude root extracts of *Valeriana officinalis* (Torres-Hernández et al., 2016).

The current work assessed the anxiolytic potential of *Maerua angolensis* in chronic and acute models of anxiety in zebrafish. We also explored the involvement of the GABAergic and serotonergic systems in anxiolytic effects of *Maerua angolensis* extract.

2. Methods

2.1. Plant extraction and FT-IR analysis of crude extract

The stem bark of *Maerua angolensis* were collected from Kwahu Tafo (6.415360°N, 0.363160°W) in the Eastern region of Ghana and authenticated by Prof. Kofi Annan of the Department of Herbal medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology. A voucher specimen (KNUST/FP/12/051) has been kept at the herbarium of the Faculty.

Table 1
Schematic diagram representing experimental design of chronic unpredictable stress.

		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Week 1	Morning	TC	OC	RS	SI	DBE	RS	CS
	Evening	DBE	C	HS	CS	C	HS	O
Week 2	Morning	C	O	SI	HS	DBE	CS	HS
	Evening	TC	DBE	RS	TC	C	SI	OC
Week 3	Morning	Tx & testing	Tx	Tx & testing				

RS-Restrain stress, CS-Cold stress, HS-Heat stress, SI-Social isolation, OC-Overcrowding, DBE-Dorsal body exposure, TC-Tank change, C-Chasing stress Tx-Treatment.

The stem bark was chopped and room-dried for 14 days. The dried bark was then pulverized into a fine light-brown powder. About 1.8 kg of the powder was then extracted by cold percolation with petroleum ether and ethyl acetate mixture (50:50). The solvent mixture was drained 72 h later to obtain a dark green extract which was further concentrated in a rotary evaporator at 60 °C and under reduced pressure. The concentrate obtained was further dried in a hot air oven at 55 °C for 72 h to obtain a green semisolid mass (~8.5 g) which was then stored in a freezer at −40 °C until use.

To identify the possible functional groups that may be present in the sample, a triplicate Fourier transform infrared spectroscopy (FT-IR) spectra was generated using PerkinElmer® UATR Two and subsequently baseline corrected. The spectra between 400 and 1400 cm^{-1} is usually considered as the unique region for every compound/compound mixtures and hence can be used for identification and quality control.

2.2. Chemicals and drugs

Granisetron hydrochloride (Corepharma LLC, Middlesex, England); pizotifen maleate, methysergide (Novartis Pharmaceutical cooperation, Basel, Switzerland); fluoxetine (Eli Lilly and Co., Indianapolis, IN, USA); cyproheptadine (Actavis UK Ltd, Devon, UK); flumazenil (Roche Pharmaceutical Ltd, Garden City, UK.); desipramine, Tween 80, diazepam (Sigma-Aldrich Inc., St. Louis, MO, USA).

Maerua angolensis extract (MAE) and all compounds, except ethanol, were prepared by solubilizing the fine powder with Tween 80 *q.s.* Doses of the compounds used for this study were chosen based on pilot experiments and previous reports (Bencan et al., 2009; Maximino et al., 2013; Stewart et al., 2011).

2.2.1. Zebrafish

Adult zebrafish (3 months) of the wild type strain were obtained from a local aquarist (Aquarium Marshals Ltd, Accra). Fishes were acclimated in glass tanks (20 L) containing biologically filtered and dechlorinated water maintained at a 23–25 °C. Each tank received independent water in and out flow to minimize cross contamination. To mimic their natural habitat each housing tank was filled with gravels to a height of about 2 cm and a fresh water plant (*Cabomba aquatica*) was submerged in each tank. Fishes were maintained under a 14 h:10 h light dark cycle. Adult fishes were fed twice daily with commercial fish flakes alternated with high protein pellets.

Animals were kept and experimental procedures were performed in accordance to European Union recommended guidelines for experiments with zebrafish (EU Directive 2010-63-Experiments with zebrafish) and approved by the departmental research and ethics committee.

2.3. Sedative and locomotor activity

To assess the possible influence of the drug/extract on locomotor activity, the behaviour of adult zebrafish assessed after immersion in the respective solution of drug concentration. Briefly adult zebrafish (n