

Antidepressant Activities of the Methanol Extract, Petroleum Ether and Ethyl Acetate Fractions of *Morus mesozygia* Stem Bark

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Received November 24th, 2012; revised December 30th, 2012; accepted January 13th, 2013

ABSTRACT

Morus mesozygia Stapf (Moraceae) is the only *Morus* species indigenous to Tropical Africa. It is believed in traditional medicine that the plant exhibits several medicinal properties. The antidepressant-like activities of the crude methanol extract, petroleum ether and ethyl acetate fractions were investigated using the Forced Swimming Test (FST) and Tail Suspension Test (TST) models. The petroleum ether fraction at a dose of 140 mg/kg was the most effective in both the FST and TST when compared to the activity of the reference drug Imipramine (250 mg/kg) by reducing the immobility duration of the animals by 66.8% and 49.4% respectively.

Keywords: *Morus mesozygia*; Antidepressant Activity; Forced Swimming Test; Tail Suspension Test

1. Introduction

Morus mesozygia Stapf (Moraceae) is commonly referred to as the Black mulberry, [1] Ewe aye (Yoruba, South-west Nigeria) [2] and it is the only species of the genus *Morus* native to Tropical Africa while *Morus alba* and *Morus nigra* were introduced into the region for their edible fruits and as food for silkworm [1]. *M. mesozygia* is a large tree of about 40 m high and characterised by white latex, ridges at the base and branchlets, whitish or glabrous hair.

Morus mesozygia is useful in construction, furniture, dug-out canoes and walking sticks. The wood is also useful as fuelwood and for charcoal making. In traditional folklore medicine, *M. mesozygia* is used in the treatment of arthritis, debility, stomach trouble, rheumatism, malnutrition, venereal disease and as a pain killer [1]. Previous studies reported the isolation of moracin R, S, T, U from the methanol extract of the stem of *M. mesozygia* and their antioxidant activities [3]. In addition, 3beta-acetoxyurs-12-en-11-one, moracin Q, moracin T, artocarpesin, cycloartocarpesin were isolated from the stem-bark of *Morus mesozygia* while moracin KM, LM, and SC were isolated from the twigs [4].

The aim of this study is to investigate the antidepressant-like effects of the methanol extract, petroleum ether and ethyl acetate fractions of the stem bark of *Morus*

mesozygia.

2. Materials and Methods

2.1. Plant Collection and Authentication

Fresh stembark samples of *Morus mesozygia* were collected in the month of August 2011 at Olokemeji in Ekiti State, Nigeria and authenticated at the Forestry Research Institute of Nigeria Ibadan (FRIN) by Mr Kola Adebajo.

2.2. Drying and Extraction

The stem bark was air dried in the sun and milled into a fine powder. The powdered sample was extracted by cold extraction method using 80% methanol. The extract was concentrated under reduced pressure to give 112.6 g yield, the dried methanol extract was reconstituted in water and successively partitioned with petroleum ether and ethyl acetate to yield the pet ether and ethyl acetate fractions.

2.3. Animals

White albino rats of both sexes weighing between 98 and 180 g were obtained from the Department of Veterinary Physiology and Pharmacology, University of Ibadan, Nigeria. The animals were maintained at 25°C, 12-hour light and dark cycle. The animals had access to food and water. The experiment was conducted in accordance with

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the International regulations for handling and use of laboratory animals.

2.4. Phytochemical Screening

Phytochemical screening was carried out using standard procedures [5,6].

2.5. Antidepressant Study

The Forced Swimming Test (FST) and Tail Suspension Test (TST) were the two animal models adopted in this study. These methods are known to reflect a state of despair or lowered mood in humans [7].

1) Forced Swimming Test (FST)

The Forced Swimming Test adopted from the method of Porsolt was used in this study [8,9]. Five animals (n = 5) were used per group.

Group A: Rats received 1 ml of distilled water each.

Group B: Rats received 25 mg/kg of Imipramine.

Group C: Rats received 140 mg/kg of Ethyl acetate fraction.

Group D: Rats received 140 mg/kg of Petroleum ether fraction.

Group E: Rats received 140 mg/kg of methanolic crude extract.

Thirty minutes after administering extract, fractions, reference drug and control, individual rats were forced to

swim in an open cylindrical container (Diameter 20 cm, height 30 cm) containing 20 cm of water at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The total duration of immobility was recorded in a six-minute period. The rats were judged to be immobile when ceased struggling and remained floating motionless in the water, making only those movements necessary to keep their heads above water. A decrease in the duration of immobility was indicative of an antidepressant-like effect [8].

2) Tail Suspension Test

The tail suspension method used in this study was similar to those described by Steru *et al.*, 1985 [10]. The rats were suspended upside down on a metal rod at a height of 50 cm from the ground with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. The animals initially tried to escape by making vigorous movements but became immobile after a while. The animals were subjected to this kind of unavoidable and inescapable stress to reflect behavioural despair which in turn may reflect depressive disorders in humans. The total duration of immobility was rated within a 6 minutes period with each rat used only once.

2.6. Statistical Analysis

The results are expressed as mean \pm SEM and as a percentage (%) reduction in the immobility of the animals.

3. Results (Tables 1-2)

Table 1. Phytochemical screening of stembark of *Morus mesozygia*.

Anthraquinone	Flavonoids	Alkaloids	Tannins	Cardiac glycosides	Saponins	Cyanogenetic glycosides
+	+	+	+	+	+	-

+ = Present; - = Absent.

Table 2. Antidepressant-like effects of *Morus mesozygia* stem bark extract and fractions.

Extract/Dose (mg/kg)	FST	TST
Group A: Control Water only 1 ml	203.5 \pm 0.254	195 \pm 1.838
Group B Imipramine 25 mg/kg	133.5 \pm 3.139 34.4%**	183 \pm 0.678
Group C Ethyl acetate Fraction 140 mg/kg	96.0 \pm 2.319 28.1%* 52.8%**	134.5 \pm 2.913 26.5%* 31.0%**
Group D Petroleum ether 140 mg/kg	67.5 \pm 0.650 49.4%* 66.8%**	79.0 \pm 1.187 56.8%* 59.5%**
Group E Methanol 140 mg/kg	104.0 \pm 1.953 22.1%* 48.9%**	151.0 \pm 0.509 17.5%* 22.6%**

*Compared to reference drug Imipramine; **Compared to untreated control.

4. Discussion

Depression a chronic condition in clinical practice is associated with psychosocial and physical impairment [11]. This state of health has been attributed to deficiency in the functions of cerebral monoaminergic transmitters such as norepinephrine and/or dopamine located at synapses [12]. The World Health Organization has indicated that Depression will become the second condition leading to premature death or disability worldwide by 2020 [13].

In a previous study by Song *et al.*, 2009, the phytochemical profile of several species of several *Morus* species showed that members of the genus *Morus* are rich in alkaloids, flavonoids and polyphenols [14].

In this study, the phytochemical screening of the stem bark of *Morus mesozygia* showed the presence of saponin, cardiac glycosides, tannins, alkaloids, flavonoids and anthraquinone.

The Tail Suspension Test and Forced Swimming Test are considered as animal models for antidepressant drug screening [15,16]. These models mimic the unipolar type of depression in human which is characterized by depressive mood, difficulty in sleeping, fatigue, difficulty in making decisions and feeling worthless. These models are considered to be sensitive and relatively specific to all major classes of antidepressant drugs like tricyclics, selective serotonin reuptake inhibitors and monoamine oxidase inhibitors [8,17].

In this study, the effect of the ethyl acetate and petroleum ether fractions at a dose of 140 mg/ml were more effective than the reference drug Imipramine (25 mg/ml) when compared to the untreated control. The ethyl acetate fraction exhibited 28.1% and 26.5% reduction in immobility in the FST and TST when compared to Imipramine, 52.8% and 31.0% when compared to the untreated control. The petroleum ether fraction reduced immobility by 49.4% and 56.8% antidepressant effect when compared to Imipramine, 66.8% and 59.5% when compared to the untreated control in the FST and TST respectively.

In the FST and TST, the petroleum ether fraction was the most effective. The antidepressant-like effects exhibited by these fractions (petroleum ether and ethyl acetate) in the animal models used were not significantly different from each other. They however, exhibited a more pronounced effect than Imipramine (25 mg/kg) the reference drug.

Previous studies have reported the antidepressant-like effects of flavonoids such as quercetin glycosides [18-20].

The antidepressant-like effect exhibited by the stem bark of *Morus mesozygia* might be due to the presence of the alkaloids, saponins and flavonoids. Flavonoid glycosides have been reported to exhibit antidepressant-like

effects by being hydrolysed to their aglycones by mucosal and bacterial enzymes in the intestines which are then converted to conjugated metabolites during the absorption process [21,22] which then protects the brain function from central nervous system (CNS) disturbance [23].

Though the exact group of compound (s) responsible for the antidepressant-like effect is unknown, the pronounced antidepressant effect might be exerted by protecting brain function from CNS disturbance, [23] due to adaptogenic properties, by reducing NGF levels in the hippocampus which can contribute to the antidepressant-like effect on FST [24] or by exhibiting corticosteroid-like effects on the modulation of nerve transmission by altering the availability of neurotransmitters [25].

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