

# Antidepressant Activity of Methanolic Extract of *Amaranthus Spinosa*

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## ABSTRACT

**Introduction:** Depressive disorder is a prevalent psychiatric disorder, which affects 21% of the world population. The presently using drugs can impose a variety of side-effects including cardiac toxicity, hypopnesia, sexual dysfunction, body weight gain, and sleep disorder. During the last decade, there is a growing interest in the therapeutic effects of natural products on mental disorders. *Amaranthus spinosa* was investigated for antidepressant activity.

**Methods:** Antidepressant activity of methanolic extract of *Amaranthus spinosa* (MEAS) was investigated by using Forced swimming test (FST) and Tail suspension test (TST) models. Escitalopram and Imipramine were used as reference standards.

**Results:** It has been observed from our study that both the MEAS at higher concentration showed significant ( $p < 0.01$ ) reduction in immobility in tail suspension and forced swim model of depression comparable to Escitalopram and Imipramine.

**Discussion:** However further study is needed to understand mechanism of action and to identify active component responsible for antidepressant like activity.

## 1. Introduction

**A**maranthus spinosa Linn., (Amaranthaceae), juice was used by tribal of Kerala, India to prevent swelling around stomach while the leaves are boiled without salt and consumed for 2-3 days to cure jaundice (Hema et al., 2006). In Indian traditional system of medicine (Ayurveda) the plant is used as antipyretic, laxative, diuretic, digestive, antidiabetic, anti-snake venom, antileprotic, blood diseases, bronchitis, piles and anti-gonorrhoeal (Kirtikar and Basu 1987). The Chinese use *A. spinosa* as traditional medicine to treat diabetes and seeds used as poultice for broken bones. Some tribes in India apply *A. spinosa* to induce abortion (Grubben and Denton 2004).

*A. spinosa* is also used as reported to possess anti-inflammatory (Olumayokun et al., 2004), antimalarial (Hilou et al., 2006), Antiandrogenic (Murgan et al., 1993b), immunomodulatory (Tatiya et al., 2007), anti-diabetic, anti-hyperlipidemic, spermatogenic activities of stem (Sangameswaran and Jayakar, 2008) and effect on hematology (Olufemi et al., 2003) and biochemical changes in Epididymis (Murgan et al., 1993a). The betalains in stem bark of *A. spinosa* were identified as amaranthin, isoamaranthine, hydroxycinnamates, rutin, quercetin and kaempferol glycosides (Hilou et al., 2006; Ibewuiké et al., 1997; Rastogi and Mehrotra, 1999; Stintzing et al., 2004; Ashok Kumar et al., 2008). It also contains amaranthoside, a lignan glycoside, amaricin, a coumaroyl adenosine along with stigmaterol glycoside, betaine such as glycinebetaine and trigonelline (Blunden

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et al., 1999; Azhar-Ul Haq et al., 2006). Betalains are well known for their antioxidant, anticancer, antiviral and antiparasitosis properties (Kapadia et al., 1995; Kapadia et al., 1996; Patkai et al., 1997).

According to the World Health Organization report, mood disorders are the second leading cause worldwide of disability adjusted life years and the leading cause of years lived with disability in all ages. Each drug used to treat this disorder has a success rate of about 60%. In addition, most therapies require several weeks of treatment before improvement of signs and symptoms is observed and there are numerous side effects caused by antidepressants (Wong and Licinio, 2001). Thus, the high prevalence of depression and the fact that a significant proportion of individuals do not respond well to any currently marketed antidepressants or treatments support the need for new therapeutics to treat depression. Numerous antidepressant compounds are now available, presumably acting via different mechanisms including serotonergic, noradrenergic and/or dopaminergic systems (Elhwuegi, 2004). Medical plant therapies may be effective alternatives in the treatment of depression, and has progressed significantly in the past decade (Zhang, 2004).

Therefore, the present work aimed to evaluate firstly the antidepressant-like effect of the methanolic extract of *Amaranthus spinosus* in the models predictive of antidepressant action,

## 2. Methods

### 2.1. Collection of Plant Material and Extraction

The fresh plant of *A. spinosus* was collected from Chickballapur, and was authenticated by Prof. B.K. Venkatesh, Department of Botany, Government First grade College, Chickballapur, Karnataka. A voucher specimen (SKVCP 11) was deposited in college herbarium. The whole plant was shade dried and coarsely powdered. The coarse powder was subjected to extraction with methanol by soxhlet apparatus and extract was concentrated to dryness in vacuum.

### 2.2. Preliminary Phytochemical Screening

The methanol extract of *A. spinosus* was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, proteins and phenolic compounds (Kokate, 1986).

General procedure of qualitative chemical tests			
S.No.	Chemical tests	Observation	Inference
1.	<b>Test for Carbohydrates</b> <u>Molisch's test (General Test)</u>  To the 2 to 3 ml extract, few drops of $\alpha$ -Naphthol solution in alcohol was added followed by addition of conc.H <sub>2</sub> SO <sub>4</sub> from the sides of the test tube.	Violet ring is formed at the junction of two liquids	Presence of Carbohydrates
	<u>Fehling's Test</u>  1 ml. of Fehling's A and 1 ml Fehling's B was mixed in the test tube. Equal volume of extract was added and heated in the boiling water bath for 5-10 min.	First yellow, then brick red precipitate is Observed	Presence of reducing Sugars
	<u>Benedict's Test</u>  Equal volume of Benedict's reagent and extract in the test tube was added and heated in a boiling water bath.	Solution appears green, yellow or red	Presence of Reducing sugars
	<u>Barfoed's Test</u>  Equal volume of Barfoed's reagent and extract was mixed and heated for 1-2 min. in boiling water and cool.	Red precipitate is observed	Presence of Monosaccharides
2.	<b>Test for Proteins and aminoacids</b> <u>Biuret Test</u>  To 3 ml of extract 4% NaOH and few drops of 1% CuSO <sub>4</sub> Solution was added.	Violet or Pink colour develops	Presence of Proteins
	<u>Ninhydrin Test</u>  3 ml extract and 3 drops of Ninhydrin solution was heated in boiling water bath for 10 min.	Purple or bluish colour appears	Presence of Amino acids

3. a)	<b>Test for Fats and Fixed Oils</b> A small quantity of extract was pressed between filter papers.	Oil stains on the paper	Presence of fixed Oils
4. a)	<b>Test For Steroid</b> <u>Liebermann-Burchard reaction</u> 2 ml of the extract was mixed with chloroform and 1-2 ml acetic anhydride and 2 drops of Conc. H <sub>2</sub> SO <sub>4</sub> was added through the sides of the test tube.	Purple ring with acid solution turning green	Presence of Steroid
5. a)	<b>Test For Glycosides</b> <u>Cardiac Glycosides</u> <u>Keller Kiliani test</u> To 2 ml extract, glacial acetic acid, one drop of FeCl <sub>3</sub> and Conc. H <sub>2</sub> SO <sub>4</sub> was added.	Reddish brown layer appears at the junction of two liquids	Presence of Cardiac Glycosides
b)	<u>Legal's Test:</u> The extracts were treated with sodium nitroprusside in pyridine and methanolic alkali. The formation of	pink to red color	Presence of cardiac glycosides.
c)	<u>Anthraquinone glycosides Borntrager's test</u> To the extract dil. H <sub>2</sub> SO <sub>4</sub> was added. The mixture was boiled and then filtered. To the cold filtrate, equal volume of benzene or chloroform was added. It was then shaken well. The organic solvent was separated and ammonia was added.	Ammoniacal layer turns pink or red	Presence of Anthraquinone glycosides
d)	<u>Saponin glycoside Foam test</u> The drug powder or the extract was shaken vigorously with water.	Persistent foam is observed	Presence of Saponin glycosides
6.	<b>Test for Phenolic Compounds and tannins</b> To the extract FeCl <sub>3</sub> was added.	Deep blue-black colour formed	Presence of Phenolic Compounds
7. a)	<b>Test for Flavonoids</b> <u>Shinoda Test</u> To the extract, 0.5g of magnesium turnings and few drops of Conc.HCl were added from the sides of the test tube.	Pink colour observed	Presence of Flavonoids
8. a)	<b>Test for Alkaloids</b> <u>Dragendroff's test</u> To the filtrate few drops of dragendroff's reagent was added.	Orange brown ppt. is formed.	Presence of Alkaloids.

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### 2.3. Animals

Male Swiss Wistar rats weighing 150-250 gm were acclimatized to the experimental room at temperature  $23 \pm 2$  °C, controlled humidity conditions (50-55%) and 12 h light and 12 h dark cycle. They were caged with a maximum of two animals in polypropylene cage and were fed with standard food pellets (Kamadenu Enterprises, Bangalore) and water ad libitum. All the studies conducted were approved by the institutional animal ethical committee of Sri K.V.College of Pharmacy, Chickballapur, Karnataka, according to prescribed guidelines of

CPCSEA, Government of India (Reg. No. 117/1998/CPCSEA).

### 2.4. Acute Toxicity Studies

Methanol extract of *A. spinosus* (was studied for acute oral toxicity as per revised OECD (2002) guidelines No. 423. Animals were observed for four hours hourly for behavior changes and daily for fourteen days. The extract was devoid of any toxicity in rats when given in dose up to 2000 mg/kg by oral route. Hence, for further studies 200-400 mg/kg doses of extract were used.

## 2.5. Antidepressant Activity

### Experimental Design for anti-depressant activity:

The rats were divided five groups (n=6). Drugs/ vehicle were administered to the animals 60min prior to study.

**Group I:** Negative control, administer saline 2 ml/kg orally.

**Group II:** Positive control and receive standard drug Escitalopram (10 mg/kg orally).

**Group III:** Receive standard drug Imipramine (10 mg/kg orally)

**Group IV:** Receive MEAS 100 mg/kg orally

**Group V:** Receive MEAS 200 mg/kg orally

#### 2.5.1. Forced Swim Test

For the forced swim test (FST), Rats of either sex were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm) containing 19 cm of water at  $25\pm 1^\circ\text{C}$ . Treatment was given 60min prior to study as described by study design. All animals were forced to swim for 6 min and the duration of immobility was observed and measured during the final 4 min interval of the test. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect (Porsolt et al., 1977)

#### 2.5.2. Tail Suspension Test

The tail suspension method used in this study was similar to those described by Steru et al., (1985). Treatment was given 60 min prior to study as described by study design. Mice were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a 6 min of the 10 min period. Animal was considered to be immobile when it did not show any movement of the body, hanged passively and completely motionless.

## 2.6. Statistical Analysis

All the values were expressed as Mean  $\pm$  S.E.M. the results were analyzed statistically by one-way ANOVA followed by Dunett Multiple comparison test,  $P < 0.05$  was considered significant.

## 3. Results

### 3.1. Preliminary Phytochemical Screening

On preliminary phytochemical analysis of MEAS showed the presence of flavonoids, saponins, glycosides, terpenoids amino acids, alkaloids, carbohydrates, phenolic compounds and proteins.

### 3.2. Acute Toxicity Studies

Methanolic extract of *Amaranthus spinosus* showed no behavioural changes nor mortality at dose 2000 mg/kg.

### 3.3. Antidepressant Activity

The antidepressant effects of methanolic extract of *Amaranthus spinosus* (100 and 200 mg/kg) and Escitalopram and imipramine were studied by observing the changes in the duration of immobility in the two models: Forced swim test (FST) and Tail suspension test (TST). In both TST and FST, MEAS 100 and 200 mg/kg, p.o. produced significant reduction ( $p < 0.01$ ) in the immobility period when compared with that of control group animals that received only the vehicle. The results are tabulated in Table 1.

## 4. Discussion

Depression is an important psychiatric disorder that affects individuals' quality of life and social relations directly. Depression is characterized by emotional symptoms such as hopelessness, apathy, loss of self-confidence, sense of guilt, indecisiveness, and amotivation, as well as biological symptoms like psychomotor retardation, loss of libido, sleep disturbances, and loss of appetite. When the symptoms are very severe, major depression is considered.

Medications such as tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), selective reversible inhibitors of monoamine oxidase A (RIMAs), and specific serotonin-noradrenaline reuptake inhibitors (SNRIs) are clinically employed for drug therapy (Fava, 2003). However, these drugs can impose a variety of side-effects including cardiac toxicity, hypopnea, sexual dysfunction, body weight gain, and sleep disorder (Antai-Otong, 2004; Baldwin et al., 2006; Khurana and Baudendistel, 2003; Park et al., 2005).

Escitalopram is classical selective serotonin reuptake inhibitors SSRIs, it is bound at the primary site of presynaptic serotonin transporter (SERT) with a very high

**Table 1.** Effect of *Amaranthus spinosus* on immobility time in Forced swim test and Tail Suspension test

Treatment	Dose (mg/Kg)	Forced Swim test Duration of Immobility (Sec)	Tail Suspension test Duration of Immobility (Sec)
Control		140.33±6.6	148.5±5.6
Escitalopram	4	60.75±1.38**	90.8±4.59**
Imipramine	4	63.5±1.5**	91±4.56**
MEAS	100	48±2.58**	106.4±3.2**
MEAS	200	59.6±1.310**	114.3±3.5**

Each value represents Mean ± S.E.M., n=6. \*\*p< 0.05 compared with control.

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affinity, and it has higher serotonergic activity than the classical SSRIs (Sanchez et al., 2004). Imipramine prevents reuptake of nor adrenaline and serotonin resulting in their increased availability in the synapse and therefore an increase in adrenergic and serotonergic neurotransmission (Tatsumi et al., 1997).

In this study, we used two animal models, FST and TST. Both the paradigms are widely accepted behavioral models for assessing pharmacological antidepressant activity (Bourin, 1990; Porsolt et al., 1977). Characteristic behavior scored in these tests is termed immobility, reflecting behavioral despair as seen in human depression (Steru et al., 1985; Willner, 1984). In addition, it is well known that many antidepressant drugs are able to reduce the immobility time in rodents (Porsolt et al., 1977). MEAS produced a marked reduction in immobility time at doses of 100 and 200 mg/kg in the rat FST and TST, with a profile comparable to that observed for the classical antidepressant drug ESC and imipramine. FST has not traditionally been viewed as a consistently sensitive model for detecting selective serotonin reuptake inhibitor activity, whereas these antidepressants are generally reported as active in the TST (Cryan et al., 2005). Moreover, TST is proposed to have a greater pharmacological sensitivity as compared with FST (Cryan et al., 2005; Thierry et al., 1986).

*A. spinosus*, contains amino acids namely, lysine, arginine, histidine, cystine, phenylalanine, leucine, isoleucine, valine, threonine, methionine, tyrosine and tryptophan (Anonymous, 1988). These amino acids contribute positively to the antioxidant activity (Hernandez-Ledesma et al., 2005; Chen et al., 1998; Saito et al., 2003; Chen et al., 1996; Cameron & Pauling 1979). *Amaranthus* also reported to contain beta-carotene, thiamine, riboflavin, niacin and ascorbic acid. Carotenoids serve as precursors of vitamin A, show antioxidant activity (De Pee et al., 1995).

Phytochemical analysis showed the presence of Flavonoids and phenolic compounds have been reported to have multiple biological effects such as Central nervous system disorders (Priyanka et al., 2012), antioxidant activity (Bors & Saran, 1987), analgesic (Mills & Bone, 2000), anti-inflammatory (Lilian Eugenia Peizer et al., 1998), inhibition of mast cell histamine release antiulcerogenic (Van Wauve & Goosens, 1989), cytotoxic, antihypertensive, hypolipidemic, antiplatelet and neurodegenerative diseases (Amresh et al., 2007). A study from Noldner and Schotz (2002) has indicated that rutin is essential for the antidepressant activity of *Hypericum perforatum* extract, a plant used in many countries for the treatment of mild to moderate forms of depression (Linde and Knüppel, 2005),

## Conclusion

The present study provides the first evidence indicating that methanolic extract of *Amaranthus spinosus* showed significant antidepressant activity in TST and FST models of depression. Further research is required to know the mechanism of its action.

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Table 1. Effect of *Amaranthus spinosus* on immobility time in Forced swim test and Tail Suspension test



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