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Experimental Evaluation of Antidepressant activity of Aqueous & Methanolic Flower Extracts of *Tridax procumbens Linn* in Mice

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Abstract

Objective: To investigate Antidepressant activity of aqueous and methanolic extract of *Triax procumbens* plant flowers in mice. **Methods:** The Antidepressant activity of aqueous and methanolic extract of *Triax procumbens* plant flowers were tested by Forced Swim Test (FST) and Tail Suspension Test (TST) in albino mice and the results were compared for the both extracts. Imipramine was used as the standard drug for comparison. **Results:** Phytochemical screening showed presence of carbohydrates, alkaloids, flavanoids, tannins, glycosides, phenols. AETP (Aqueous Extract of *Tridax procumbens*) & METP (Methanolic Extract of *Tridax procumbens*) did not produce any lethal effect even upto 2000mg/kg, p.o during Acute Oral Toxicity study. In FST (Forced swim test) and TST (Tail Suspension test), METP (Methanolic Extract of *Tridax procumbens*) showed diminution of duration of immobility time in 200mg/kg but not in 100mg/kg. **Conclusion:** From the above finding concluding that, shortening of immobility time in the FST and TST indicating, METP showed more antidepressant activity acting either by the enhancement of central 5-HT or catecholamine neurotransmission compared to AETP (Aqueous Extract of *Tridax procumbens*) in mice.

Keywords: *Tridax procumbens,* Aqueous Extract of *Tridax procumbens* , Methanolic Extract of *Tridax procumbens*, Forced swim test, Tail suspension test

Introduction

Depression is one of the major mental disorders characterized with symptoms such as regular negative moods, decreased physical activity, feelings of helplessness, sluggish thought and cognitive function [Galdino et al., 2009]. According to the World Health report, approximately 450 million people suffer from a mental or behavioral disorder. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020.(1)

Depression is caused by chemical imbalances in the brain which may be hereditary, stressful life changes, stroke, Parkinson's disease, or multiple sclerosis, stroke, social isolation, medical conditions such as hypothyroidism (underactive thyroid), medications (such as sedatives and high blood pressure medications), cancer, major illness, or prolonged pain and sleeping problems.(2)

Despite the development of new molecules for pharmacotherapy of depression, it is unfortunate that this disorder goes undiagnosed and untreated in many patients. Although the currently prescribed molecules provide some improvement in the clinical condition of patients, it is at a cost of having to bear the burden of their adverse effects.

Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders. On one hand these agents have less adverse effects, and on the other hand they have been shown to be comparable in efficacy to their synthetic counterparts.[3, 4]

Synthetic antidepressants are often associated with their anticipated side effects like dry mouth, inability in driving skills, constipation and sexual dysfunction and majority of patients are reluctant to take this treatment [Singh Rudra Pratap et al., 2012]. Nature plants, such as *Hypericum perforatum, Cissampelos sympodialis, Terminalia bellirica Roxb, Bacopa monniera, Ginkgo biloba, Pueraria lobata* may be an important source of new antidepressant drugs and the safety of nature plant extracts maybe better than that of synthetic antidepressants.[5,6]

Tridax procumbens commonly known as **coatbuttons** or **tridax daisy** is a species of flowering plant in the daisy family. The primary chemical constituents are carbohydrates, alkaloids, flavanoids, tannins, glycosides, phenols, they also contain iron, manganese and zinc [Danielle Ryan et al., 2007]. In traditional medicine *Tridax procumbens* used for wound healing, staunching bleeding and treatment of diarrhoea,backache and bronchial catarrh.

Essential oils extracted from T. procumbens are reported to have insecticidal activity against Musca domestica, Culex quinquefasciatus, Dysdercus similis and Supella spp. Aqueous extracts inhibit aflatoxin production by Aspergillus flavus and a petroleum ether extract from flowers protects cowpea seeds from damage by the bruchid Callosobruchus maculatus . T. procumbens is sometimes used as green feed for poultry in Nigeria.

The Anti depressant activity of *Tridax procumbens* is mentioned in Indian system of traditional medicine but there is no scientific evidence to prove its activity. Hence, the present study is designed to evaluate the antidepressant activity of *Tridax procumbens* using different animal models in mice.

Materials and Methods

Plant material collection and authentication

The flowers of *Tridax procumbens* were collected from the premises of Bhaskara Institute of Pharmacy komatipalli, Bobbili ,Vizianagaram dist, Andhra Pradesh in December 2015.It was authenticated by **Dr.M.Vasu Babu**, Sr.Lecturer in Botany,Govt Degree college Vizianagaram.The botanical nomenclature of the plants was duly identified by using standard floras and also cross-checked with Herbarium records. The Plant material was shade dried for 10 days and pulverized.

Preparation of extract

The collected flowers of *Tridax procumbens* were shade dried at room temperature and grinded coarsely. The flowers were extracted by percolator using water as solvent & by Soxhelet apparatus using methanol. The resulting extract was concentrated in vacuum under reduced pressure and dried in desiccators. Thus, the prepared extract was used for further pharmacological evaluation.[8,9]

Materials

Imipramine was procured from Sigma life sciences, Bangalore.

Preliminary Phytochemical analysis

The both aqueous and methanolic extracts of flowers of *Tridax procumbens* was subjected for phytochemical screening and found that carbohydrates, alkaloids, flavanoids, tannins, glycosides, phenols were present

Animals

Albino mice of either sex weighing between 25-35gm were used in this study.All the animals were acclimatized in the quarantine room a NIN Animal house (National Institute of Nutrition ,Hubsiguda,Hyderabad),for 7 days and housed in groups of 5 under standard husbandry conditions like room temperature 23±2°C,relative humidity 30-70% and light/ dark cycle of 12 hours.

All the animals were fed with synthetic standard diet (National Institute of Nutrition, Hubsiguda, and Hyderabad) and water under still be supplied ad libitum under strict hygienic conditions. All the

experimental protocols were approved by Institutional Animal Ethical Committee (IAEC) of Andhra University. All the animals' studies were performed as per the rules and regulations in accordance to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals)

All animals were fasted 3h prior to oral administration of vehicle/standard/test compounds during the experiment were carried out during the light period (9:00 to 17:30h) to avoid circadian rhythm.

Fig 1: Albino Mice

Acute Oral Toxicity study

OECD (Organization for Economic Cooperation and Development) guidelines (425) state that, before establishing pharmacological activity of the New Chemical Entity is mandatory to establish maximum tolerated dose in mice [OECD 2001]. The purpose of the sighting study is to allow selection of appropriate starting dose for the main study. The starting dose for a sighting study was selected from the fixed dose levels of 5, 50, 300, 2000mg/kg as a dose expected to produce evident toxicity. [10]

In vivo Models for Antidepressant activity

Forced Swim Test

Animals were divided into 4 groups of 5 animals in each, weighing between 25-35 gms

The extracts of both AETP (Aqueous Extract of *Tridax procumbens*) & METP (Methanolic Extract of *Tridax procumbens*)

Group I- Control (Distilled water 10ml/kg,p.o)
Group II - Standard (Imipramine 10mg/kg,p.o)
Group III- Low dose (ETP (Extract of *Tridax procumbens*) 100mg/kg,p.o)
Group IV- High dose (ETP (Extract of *Tridax procumbens*) 200mg/kg,p.o)

For the forced swim test (FST), mice of the either sex were individually forced to swim in an open cylindrical container (diameter 10cm, height 25cm) containing 19cm of water at 25±1°C. Treatment was given 60min prior to study as described by study design all animals were forced to swim for 6min and the duration of immobility was observed and measured during the final 4min interval of the test. Each mice was judged to the 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.[(11)]

Figure 2: Representation of mice in Forced Swim Test

Tail suspension test

Animals were divided into 4groups of 5 animals in each, weighing between 25-35gms

The extracts of both AETP and METP

Group I- Control (Distilled water 10ml/kg,p.o)
Group II - Standard (Imipramine 10mg/kg,p.o)
Group III- Low dose (ETP (Extract of *Tridax procumbens*) 100mg/kg,p.o)
Group IV- High dose (ETP (Extract of *Tridax procumbens*) 200mg/kg,p.o)

Figure 3: Representation of mice in TST

The tail suspension method used in this study was similar to those described by steru et al., (1985). Treatment was given 6min prior to study as described by study design. Mice were suspended on the edge of the table, 50cm above the floor, with the help of adhesive tape placed approximately 1cm from the tip of tail. The total duration of immobility induced by tail suspension was recorded during 6min of the 10min period. Animal was considered to be immobile when it did not show any movement of the body, hanged passively and completely motionless.[12]

Statistical Analysis

Results will be presented as mean ± SEM (Standard Error Mean. The data will be subjected for statistical analysis by One way analysis of variance (ANOVA) followed by Dunnet's t test and P<0.05*, 0.01** and 0.001*** were considered as significant.

Results

Preliminary Phytochemical screening

The extract of flowers of *Tridax procumbens* was subjected for phytochemical screening and found that carbohydrates, alkaloids, flavanoids, tannins, glycosides, phenols were present. The results were shown below in table:1

S.No	Phytochemical constituents	Inference	
		AETP	METP
1	**Test for carbohydrates**		
	Molisch's test	+	-
	Fehling's test	+	-
	Barfoed's test	+	-
	Benedict's test	+	-
2	**Test for Alkaloids**		
	Dragendorff's test	-	+
	Wagner's test	-	+
	Mayer's test	-	+
	Hager's test	-	+
3	**Test for Tannins**	+	+
4	**Test for Flavonoids**		
	Schinoda test	-	+
5	**Test for Terpenoid**	-	-
6	**Test for Protiens**		
	Biuret test	-	-
7	**Test for phenols**	+	+
8	**Test for saponins**	-	-
9	**Test for Glycosides**	+	+

+ indicates presence; - indicates absence

Acute Oral Toxicity study

The aqueous and methanolic flowers extract of *Tridax procumbens* was found to be safe up to the dose level of 2000mg/kg, po, and did not produce any toxic symptoms. The survived animals were sacrificed and complete absorption of drug through GIT (Gastro Intestinal Tract) was observed. Hence 1/20th and 1/10th of Maximum Therapeutic Dose (2000mg/kg) were selected for the pharmacological models.

Forced Swim Test

The result of the effect of aqueous and methanolic flowers extracts of *Tridax procumbens* on the duration and % inhibition of immobility is shown in Table 2,3. The animals were treated with distilled water 10ml/kg p.o as control,100mg/kg, p.o of AETP and METP and 200mg/kg, p.o of AETP and METP, Imipramine 10mg/kg, p.o.as standard.

Table 2: Percentage inhibition of immobility time in Forced swim test - Aqueous extract

S.No	Treatment	% of immobility			
		30min	60min	120min	240min
1.	Control (Distilled water10ml/kg)	50.5	52.4	51.3	50.2
2.	Standard(Imipramine 10mg/kg)	25.6	28.5	26.3	27.5
3.	Low dose (100mg/kg)	13.5	14.8	16.3	17.2
4.	High dose (200mg/kg)	9.3	8.2	7.6	5.9

n=5 in each group. Significance at p <0.005*, p<0.001** and ns-not significant Vs control group

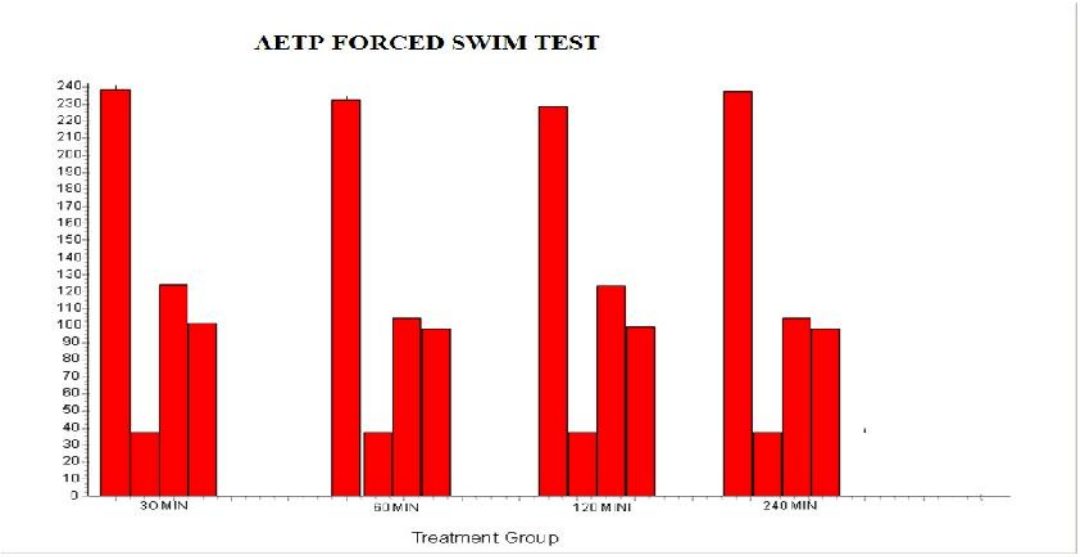

Figure 4: Effect of AETP on Immobility time in Forced swim test in mice

Table 3 Percentage inhibition of immobility time Forced swim test methanolic extract

S.No	Treatment	% of Immobility			
		30min	60min	120min	240min
1.	Control (Distilled water 10ml/kg)	50.5	51.4	52.3	50.1
2.	Standard(Imipramine 10mg/kg)	26.6	27.5	28.3	28.5
3.	Low dose (100mg/kg)	14.5	15.8	15.3	16.2
4.	High dose (200mg/kg)	8.3	8.2	6.6	5.9

n=5 in each group. Significance at p <0.005*, p<0.001** and ns-not significant Vs control group

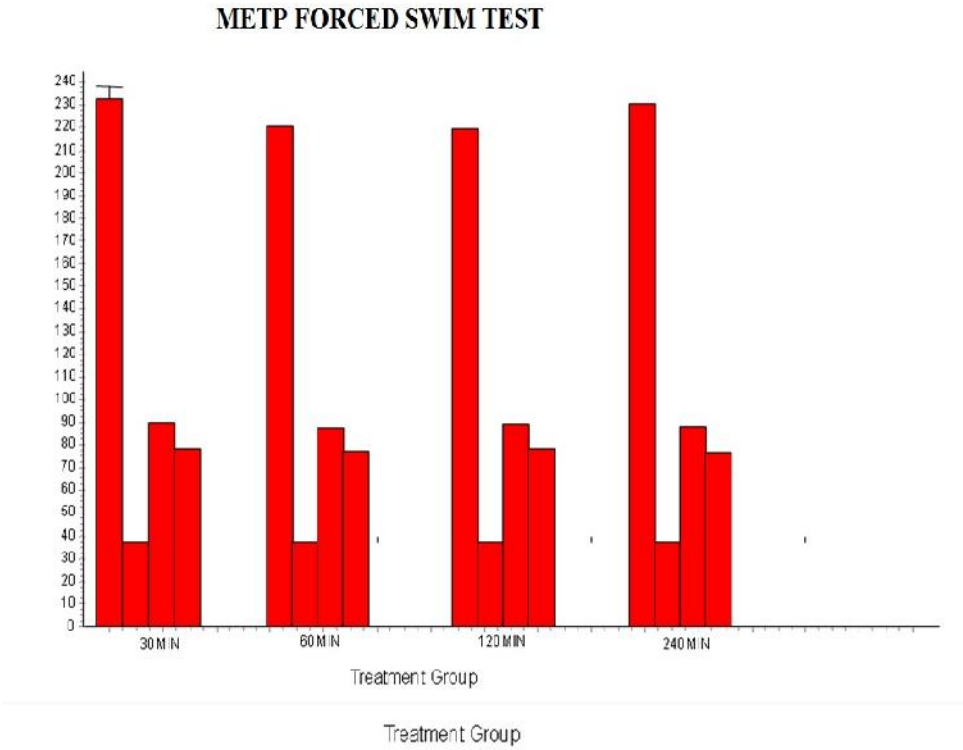

Figure 5 : Effect of METP on Immobility time in Forced swim test in mice

Tail suspension test

The result of the effect of aqueous and methanolic flowers extracts of *Tridax procumbens* on the duration and % inhibition of immobility is shown in Table 4,5.

The animals were treated with distilled water 10ml/kg p.o as control,100mg/kg, p.o of AETP and METP and 200mg/kg, p.o of AETP and METP, Imipramine 10mg/kg, p.o as standard

Table 4 Percentage inhibition of immobility time in Tail suspension test of Aqueous extract

S.No	Treatment	% of inhibition
1.	Control (Distilled water 10ml/kg)	50.57
2.	Standard (Imipramine 10mg/kg)	35.65
3.	Low dose (100mg/kg)	28.32
4.	High dose (200mg/kg)	15.85

n=5 in each group. Significance at p <0.005*, p<0.001** and ns-not significant Vs control group

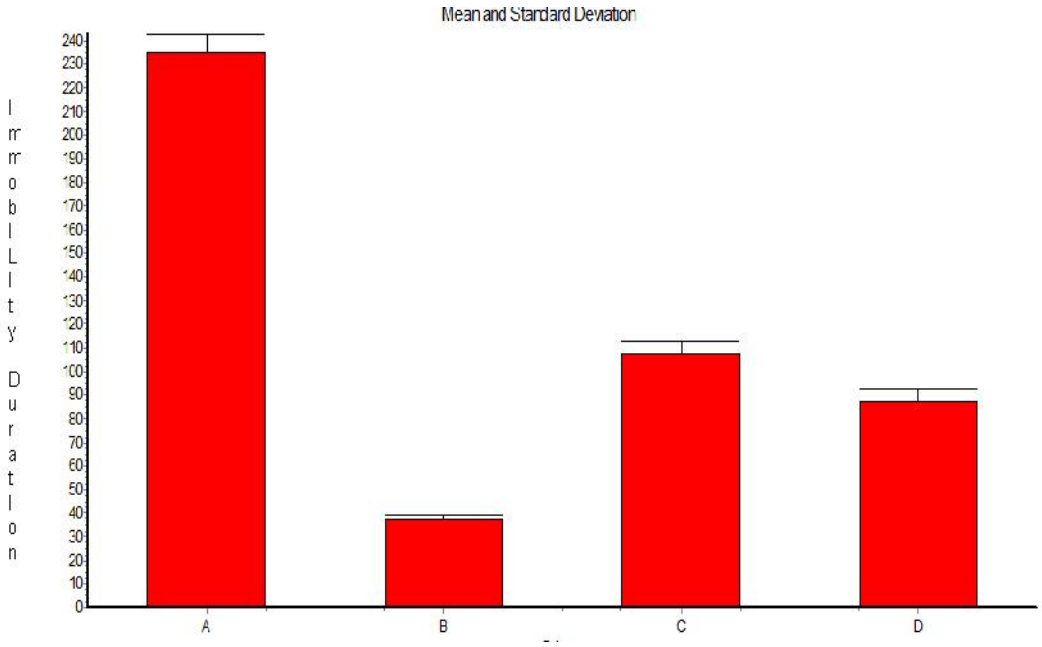

Figure 6: Effect of AETP on Immobility time in Tail Suspension test in mice A-control, B- Imipramine 10mg/kg , C- AETP 100 mg/kg, D- AETP 200 mg/kg

Table 5 Percentage inhibition of immobility time in Tail suspension test of methanolic extract

S.No	Treatment	% of inhibition
1.	Control (Distilled water 10ml/kg)	51.57
2.	Standard (Imipramine 10mg/kg)	33.65
3.	Low dose (100mg/kg)	27.32
4.	High dose (200mg/kg)	14.85

n=5 in each group.Significance at p <0.005*, p<0.001** and ns-not significant Vs control group

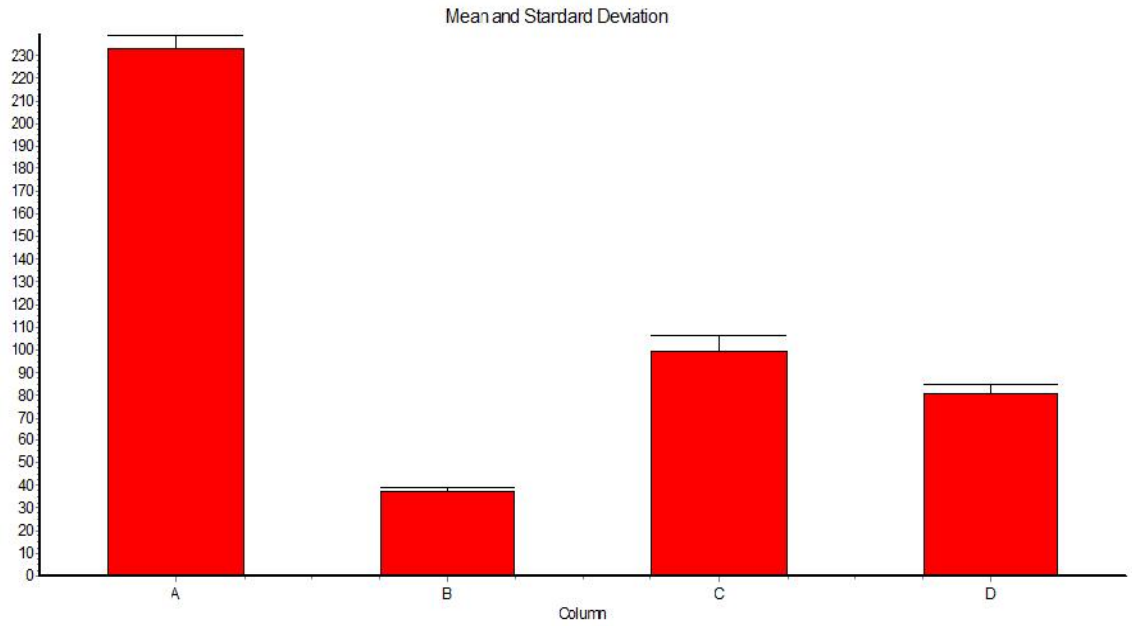

Figure 7 : Effect of METP on Immobility time in Tail Suspension test in mice
A-control B- Imipramine 10mg/kg C- METP 100 mg/kg D- METP 200 mg/kg

Discussion

Depression is a heterogenous mood disorder characterized with regular negative moods, decreased physical activity, feelings of helplessness and is caused by decreased brain levels of monoamines like noradranline, dopamine and serotonin. Therefore, drugs restoring the reduced levels of these monoamines in the brain either by inhibiting monoamine oxidase or by inhibiting reuptake of these neurotransmitters might be fruitful in the treatment of depression that has been classified and treated in a verity of ways. Although a number of synthetic drugs are being used as standard treatment for clinically depressed patients, they have adverse effects that can compromise the therapeutic treatment. Thus, it is worthwhile to look for antidepressants from plants with proven advantage and favourable benefits-to-risk ratio.

On the basis of the above information, both aqueous and non aqueous (methanolic) flowers extract of *Tridax procumbens* was selected for evaluating its antidepressant activity due to its traditional use in treatment of depression.

In Acute Oral Toxicity study, both AETP and METP did not show any lethal effect even up to the doses of 2000mg/kg, po and test doses of 100 & 200mg/kg, po were used for the Pharmacological activity.

On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviours. The two most widely used animal models for antidepressant screening are the forced swimming and tail suspension tests. These tests are quite sensitive and relatively specific to all major classes of antidepressants. In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Similarly in the FST, mice are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression. It has been seen that the TST is less stressful and has higher pharmacological sensitivity than FST [Santosh P et al., 2011].

Results showed that the administration of the METP produced a diminution of duration of immobility time of mice exposed to the both FST & TST than AETP. In the present study, the METP (200mg/kg, po) administered to mice produced significant antidepressant effect in both FST & TST than METP (100mg/kg,po) & AETP (both 100 & 200mg/kg,po) and their efficacies were found to be comparable to Imipramine (10mg/kg, po).

From all the above investigations, the Antidepressant activity of Methanolic flower extract of *Tridax procumbens* was found to be significant at 200mg/kg, po. The flavanoid and Alkaloid components of METP might be interacting with 5-HT in mediating the antidepressant effect of *Tridax procumbens.*

Conclusion

The AETP and METP contains carbohydrates,alkaloids, Tannins, flavanoids, glycosides, phenols. From the above findings, the Anti depressant activity of METP was significant at 200mg/kg , p.o in Forced swim test, Tail suspension test. Shortening of immobility time in the forced swimming and tail suspension tests indicating METP acting either by enhancement of central 5-HT and catecholamine neurotransmission. However, more extensive Pharmacological studies of this plant are required for complete understanding of the Anti depressant activity of Methanolic flower extract of *Tridax procumbens.*

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