WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 12, 641-650. Research Article ISSN 2277– 7105

COMPARATIVE EVALUATION OF *Tabernaemontana divaricata* LEAVES EXTRACTS FOR ANTIDEPRESSANT ACTIVITY

[1]Priyanka P. Tadkase, [1] Prof. Dr. Nitin B. Ghiware, [2]Haidarali Shaikh, [1]Vidhya V. Mali, [1]Ashwini V. Gowande

[1]Department of Pharmacology, Nanded Pharmacy College, Nanded.

[2]Centre for Research in Pharmaceutical Sciences, Nanded Pharmacy College, Nanded.

Article Received on
22 April 2018,
Revised on 13 May 2018,
Accepted on 03 June 2018
DOI: 10.20959/wjpr201812-12592

***Corresponding Author**
Prof. Dr. Nitin B Ghiware
Head, Dept of Pharmacology,
Nanded Pharmacy College,
Nanded, Maharashtra. Pin-431605.

ABSTRACT

Depression is considered as an affective disorder characterized primarily by change of mood. The study is aimed to comparative evaluation of *Tabernaemontana divaricata* leaves ethyl acetate (TDEA) and methanol (TDME) extracts for antidepressant activity by using wistar rats. The plant *Tabernaemontana divaricata* belonging to family Apocynaceae is used to treat various diseases like diarrhea, athralgia, asthma, epilepsy, aphrodisiac, leprosy, paralysis, piles etc. It is also traditionally claimed as antihypertensive, anthelmintic, hair growth promoter, tonic to the brain, liver, spleen etc. In the present study Despair swim test is used as model to induce depression in wistar rats. The TDEA at doses (100 mg/kg, 200 mg/kg) and TDME at dose (100 mg/kg, 200 mg/kg) show significant decrease in immobility time in sec on day 15 as compared to day 1 and 8. Methanol extract (200 mg/kg) on day 15 shows equivalent activity compared to standard Imipramine (25 mg/kg). The present study reveals that *Tabernaemontana divaricata* leaves have potential to reduce depression and it could be readily available source of treatment at low cost.

KEYWORDS: *Tabernaemontana divaricata, Ethyl acetate extract, Methanol extract, Antidepressant activity.*

INTRODUCTION

Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration.[1] It is associated with significant socioeconomic problems, morbidity and

mortality.[2] Depending on the nature and severity of the symptoms, depression is classified into mild, moderate and severe depression.[3] Depression can be treated by antidepressants and Talk therapy called psychotherapy other treatments for depression are Electroconvulsive therapy (ECT), Transcranial magnetic stimulation (TMS) and Light therapy may relieve depression symptoms in the winter time.[1]

According to World Health Organization, depression affects about 121 million people worldwide and it is among the leading cause of disability. Suicide is one of the most common outcomes of depression. According to WHO sponsored study, while around 9% of people in India had an extended period of depression in their lifetime, nearly 36% suffered from Major Depressive Disorder (MDD).[4] The life time risk of depression varies from 5% to 12% in men and 10% to 25% in women. An accurate diagnosis followed by effective treatment can improve this outcome. The adverse effects and cost of synthetic drug, limits their uses. Hence, there is justifiable need to search for therapeutic agents' relatively potent, safe, low price, easily available and natural in origin.[5]

Tabernaemontana divaricata (Family: Apocynaceae) is referred as Crape Jasmine, evergreen small tree grown in tropical countries. In India, it is mainly cultivated as an ornamental plant.[6] The leaves are large, shiny and deep green in colour and the size is about 6- inches in length and 2-inches in width.[7] It is commonly called as Chandni in Hindi, Nandivriksha in Sanskrit, Togor in Bengali.[8,9] Alkaloids, flavonoids & terpenoids are the main secondary metabolites that exhibit many physiological and pharmacological properties on living cells.[10] In traditional medicine *Tabernaemontana divaricata* (L) R.Br. is used to treat various diseases like diarrhea, abdominal tumours, arthralgia, asthma, epilepsy, eye infections, fever, fractures, headache, inflammation, leprosy, mania, oedema, paralysis, piles. It is also used as anthelmintic, antihypertensive, aphrodisiac, diuretic, emmenagogue, hair growth promoter, purgative, remedy against poisons and tonic to the brain, liver and spleen.[11] To the best of our knowledge, no scientific data regarding the antidepressant effect of *T. divaricata* leaves. Thus the present study was undertaken for comparative evaluation of *Tabernaemontana divaricata* leaves petroleum ether, ethyl acetate, methanolic extracts for antidepressant activity on wistar rats.

MATERIALS AND METHODS

Plant material and extraction

Fresh leaves were collected from local region of Nanded district, Maharashtra, India. Plant was authenticated by Dr. S. S. Bodke, Associate Professer & Head Department of Botany & Horticulture, Yeshwant Mahavidyalaya, Nanded. A voucher specimen of plant was preserved in the herbarium (NPC/M.Pharm/herbarium/2017-18/H-4) for further reference.

Preparation of extract

The collected leaves were dried under shade, segregated and further crushed to coarse powder by mechanical grinder and the powder was passed through No. 14 sieve. The dried powdered leaves of TD (300g) were first defatted with Petroleum Ether (60-80°C) and later extracted with ethyl acetate & methanol by continuous hot extraction method in soxhlet apparatus The extracts obtained were subjected to standardization and then utilized for evaluating *in-vitro* anti-oxidant and *in-vivo* antidepressant activity.

PHYTOCHEMICAL EVALUATION

Total Phenolic assay[12,13]

The total phenolic content of *Tabernaemontana divaricata* leaves extracts was determined by using Folin- Ciocalteu method. A gallic acid calibration curve was designed by preparing the aliquot of (5, 10, 15, 20, and 25µg/ml) in distilled water from standard 1 ml solution of gallic acid was added to 10 ml volumetric flask in methanol i.e.100 µg/ml. Reagent blank using distilled water was prepared. 0.5 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 5 ml of 7% Na_2CO_3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 765 nm with an UV-Visible spectrophotometer. Total phenolics content was expressed as mg Gallic acid Equivalents (mgGAE/g). All the experiments were performed in triplicate.

Flavonoid Assay[12,13]

Total flavonoid content of *Tabernaemontana divaricata* leaves extracts was measured by the aluminium chloride colorimetric assay. A standard quercetin calibration curve was framed by preparing aliquot of (5, 10, 15, 20 and 25 µg/ml) from standard 1 ml solution of quercetin was added to the 10 ml volumetric flask. To the flask 0.3 ml 5% NaNO2, after five minutes 0.3 ml 10 % AlCl3 was added. After five minutes, 2 ml 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was

measured against the blank at 330 nm. Appearance of pink colour showed the presence of flavonoids content.[14] Total flavonoid content was expressed as mg quercetin equivalents (QE). All the experiments were performed in triplicate.

***In-vitro* Antioxidant activity DPPH free radical-scavenging assay[15-17]**

The *Tabernaemontana divaricata* leaves extracts capability to scavenge DPPH radicals was measured according to the method as previously described with some modifications. Antioxidants react with 1, 1- diphenyl-2-picryl-hydrazyl (DPPH) radical and convert it to 1, 1-diphenyl -2-picryl hydrazine. The degree of change in colour from purple to yellow can be used as a measure of the scavenging potential of antioxidant extracts.[18] Initially, absorbance of DPPH solution (0.1 mM in methanol) was measured at wavelength 514 nm. Various concentrations of extracts (50µg/ml & 100µg/ml) were mixed with 1 ml of 0.1 mM DPPH radical solution in methanol and made up final volume of 10 ml with methanol. A plane sample was considered as a control. The mixture was shaken vigorously and incubated in the dark for 90 min at room temperature. For all the experiment each concentration of assay was run in triplicate. Ellagic acid, gallic acid, ascorbic acid and butylated hydroxytoluene (BHT) was used as a standard. The absorbance was measured at wavelength 514 nm by using uv-visible spectrophotometer. The percent radical scavenging activity of tested samples was expressed by using following formula.

$$\% \text{ scavenging activity} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

Antidepressant activity

Animals

Albino Wistar rats of either sex weighing 160 to 180 g for *in vivo* antidepressant activity was brought from Wockhardt, Aurangabad after the IAEC approval. The animals were maintained at (22 ± 2°C) & 42% humidity under standard laboratory conditions in an animal house (Registration No 1613/PO/Re/S/12/CPCSEA) with 12h light/dark with ad libitum food & water. All animals were brought to the laboratory environment for at least one week before the experiment.

Drug treatment

The animals were divided into six treatment groups (n = 6). Rat in group I, which served as control received vehicle (1 ml/kg), group 2 received imipramine (25 mg/kg), which referred

as the standard drug while groups 3 & 4 were given TDEA (100 and 200 mg/kg) and groups 5 & 6 were given TDME (100 and 200 mg/kg) respectively, 30 min before DST carried out.

Despair Swim Test[19]

Procedure: Wistar rats of either sex weighing 160–180 g are used for behavioral despair model as per Porsolt et.al (1977, 1978). Rats are individually forced to swim in cylinder (height: 40 cm; diameter: 18 cm, containing fresh water to a height of 15 cm). First time they are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. The total duration of immobility was recorded in next 4 minutes of total 6 min test. The water is changed after each test because urine and the other chemicals released by the first rat will affect the swimming pattern of the next rat.[2] An animal is considered immobile when it float passively and its nose just above the surface. TDEA and TDME extracts (100 mg/kg, 200 mg/kg) and Imipramine (25 mg/kg) are administered one hour prior to testing respectively. The similar procedure was conducted on 1st, 8th and 15th days of experiment.

STATISTICAL ANALYSIS

All the data represent as mean ± S.E.M. values. The data were analyzed by student t-test and one way analysis of variance (ANOVA). Whenever ANOVA was significant, further multiple comparisons were made using Tukey's test as the post hoc test. Statistical analysis was performed using the GraphPad InStat software version 5. The levels of statistical significance ranged from $p<0.05$ to $p<0.001$. $p>0.05$ was considered as non-significant (ns) compared to Control group.

RESULTS

Estimation of phenols, flavonoids and antioxidant activity

Methanolic extract contained more phenolic (48.80 mg GAE/g DW) and flavonoids (18.46 mg QE/g DW) as compared with ethyl acetate (43.60 mg GAE/g DW, 15.67 mg QE/g DW) and petroleum ether (39.46 mg GAE/g DW, 14.44 mg QE/g DW) respectively as depicted in Table 3.

Antioxidant activity shows methanolic extract inhibit more oxidative radicals at 50 mg/kg and 100 mg/kg (75.4%, 81.25%) than ethyl acetate (59.33%, 62.31%) and petroleum ether extracts (54.44%, 74.52%) respectively, when compared with ascorbic acid are shown in Table 4,5.

Estimation of *Tabernaemontana divaricata* on immobility time in DST

The effects of oral administration of *Tabernaemontana divaricata* on immobility time in sec in the Wistar rats DST are shown in Table 6. TDEA and TDME at doses (100 mg/kg and 200 mg/kg) and Imipramine (25 mg/kg) all significantly reduced immobility time compared with Control (Normal saline solution 0.9% NACL 1mg/ml) wistar rats $p< 0.05$ to $p<0.001$ and $p>0.05$ was considered non-significant (ns).

Post hoc analysis Tukey's multiple comparisons test found that TDME 200 mg/kg has significant difference when compared to Imipramine standard (25 mg/kg) but activity more than standard in DST. *Tabernaemontana divaricata* exhibited a slight but non significant dose-dependent decrease in immobility. The results indicated that TD showed significant antidepressant-like effects in the DST.

Table 1: Phytochemical evaluation of *Tabernaemontana divaricata* leaves extracts.

Chemical test	Petroleum ether	Ethyl acetate	Methanol
Alkaloids	+	+	+
Carbohydrates	+	+	-
Proteins	-	-	+
Amino acids	-	-	+
Saponins	-	+	+
Phenols	+	+	+
Glycosides	+	+	+
Steroids	+	-	+
Flavonoids	-	+	+

Table 2: TLC Analysis of *Tabernaemontana divaricata* leaves Extracts.

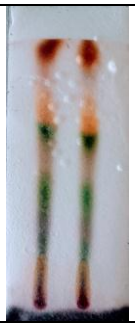
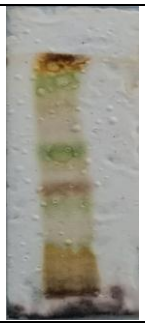
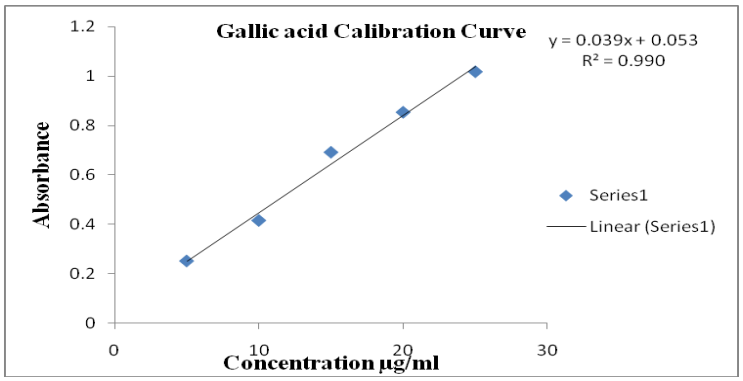
	Petroleum Ether	Ethyl Acetate	Methanol
Mobile Phase	**Benzene: Ethyl Acetate: Chloroform 4: 0.5 :0.5**	**Benzene: Chloroform 4.5: 0.5**	**Benzene: Ethyl Acetate: Chloroform 3.7: 0.5: 0.8**
			
Rf Value	0.07,0.10,0.24,0.32, 0.41,0.63,0.70,0.81, 0.90.	0.08,0.24,0.39,0.42, 0.60,0.85,0.96.	0.07,0.12,0.25,0.36, 0.42,0.46,0.65,0.69, 0.77,0.84,0.92.

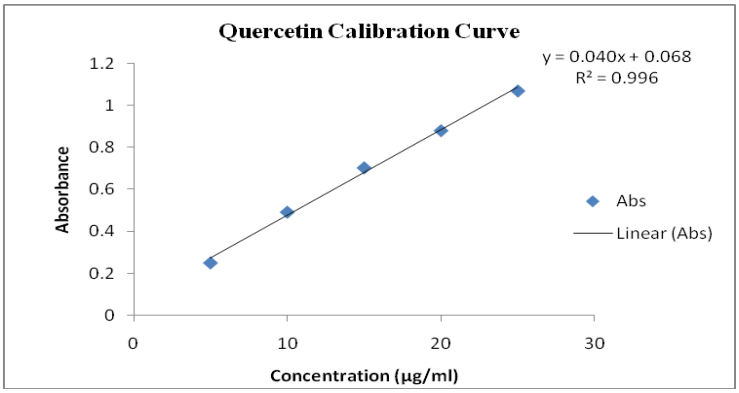
Table 3: Total phenolic and flavonoids content of *Tabernaemontana divaricata* leaves extracts.

Sr. No.	Conc. µg/ml	Extracts	Phenolic content (mg GAE/g DW)	Flavonoid content (mg QE/g DW)
1	100	Petroleum ether	39.46 ± 0.33	14.44 ± 0.29
2	100	Ethyl acetate	43.60 ± 0.13	15.67 ± 0.03
3	100	Methanol	48.80 ± 0.40	18.46 ± 0.05

Note: GAE/g DW and QE/g DW denotes Gallic Acid Equivalent per gram dry weight, Quercetin Equivalent per gram dry weight respectively.



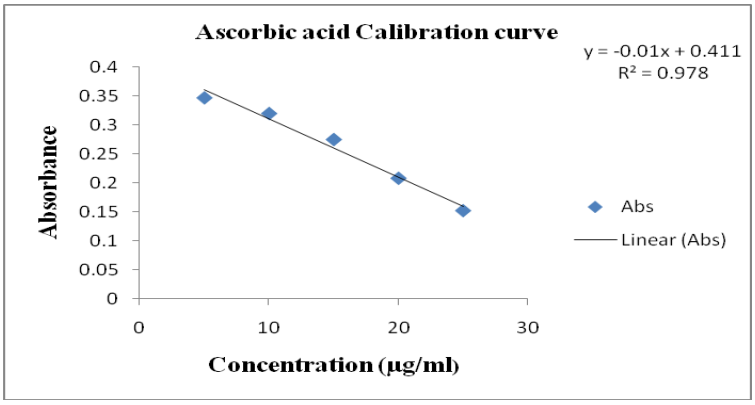
Graph No. 1: Calibration Curve of Gallic acid.



Graph No. 2: Calibration Curve of Quercetin.

Table 4: Total Antioxidant Content of Standard.

Sr. No.	Conc µg/ml	Ellagic acid % inhibition	Gallic acid % inhibition	Ascorbic acid % inhibition	BHT % inhibition
1	5	63.91 ± 0.03	93.07 ± 0.48	69.40 ± 0.67	88.46 ± 0.21
2	10	68.99 ± 0.50	93.72 ± 0.12	71.76 ± 0.07	89.77 ± 0.28
3	15	69.56 ± 0.20	94.50 ± 0.19	74.85 ± 0.73	93.90 ± 0.29
4	20	71.82 ± 0.09	95.45± 0.15	81.57 ± 0.09	94.34 ± 0.14
5	25	76.35 ± 0.50	95.34 ± 0.12	86.59 ± 0.22	94.48 ± 0.20



Graph No. 3: Calibration curve of Ascorbic acid.

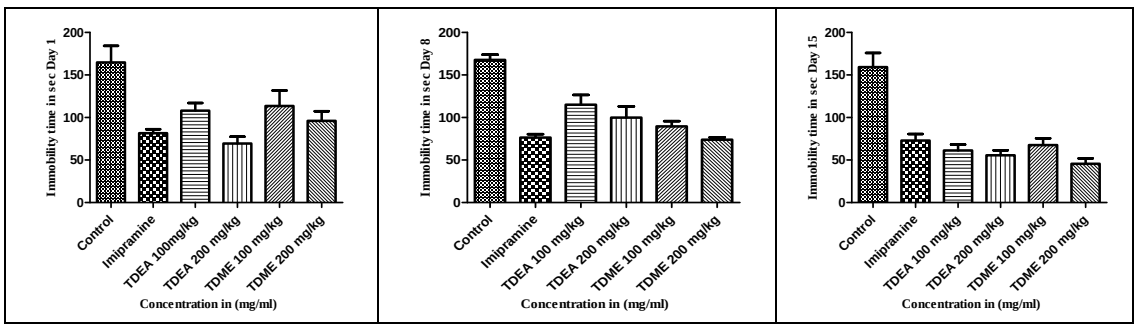
Table 5: Total Antioxidant Content of *Tabernaemontana divaricata* leaves Extracts.

Sr. No.	Conc µg/ml	Petroleum ether % inhibition	Ethyl acetate % inhibition	Methanol % inhibition	Ascorbic acid % inhibition
1	50	54.44 ± 0.16	59.33 ± 0.26	75.4 ± 0.20	81.57 ± 0.09 (20 µg/ml)
2	100	74.52 ± 0.19	62.31 ± 0.22	81.25 ± 0.12	86.59 ± 0.22 (25 µg/ml)

Table 6: Effect of TDEA and TDME on Immobility time in Despair Swim test model on wistar rats.

Treatment Group	Despair swim test (Immobility time in sec)		
	Day 1	Day 8	Day 15
Control	164 ± 19.54	167 ± 6.31	159 ± 16.53
Standard	81 ± 4.59	76 ± 4.15	72 ± 7.74
TDEA 100 mg/kg	108 ± 8.84 *	115 ± 11.35 **	61 ± 7.08 **#
TDEA 200 mg/kg	69 ± 8.06 **#	99 ± 13.26 **#	55 ± 5.86 **#
TDME 100 mg/kg	113 ± 18.03 *#	89 ± 6.20 **#	67 ± 8.05 **#
TDME 200 mg/kg	96 ± 11.26 **#	73 ± 2.79 **#	45 ± 6.36 **$

*Values are expressed as Mean ± SEM. Significance when compared to control group indicated with symbol *P<0.05, **P<0.001. Compared to standard group indicated with symbol #P>0.05;$ indicates significant difference as compared to standard but activity is more than standard.*



Day 1	Day 8	Day 15

Fig. 1: Effect of *Tabernaemontana divaricata* (TD) extracts and Imipramine (25 mg/kg) on day 1, day 8 & day 15 in DST (The column represents mean duration of immobility recorded in a 6 min observation period).

DISCUSSION

In the present study, we investigated the antidepressant like effects of ethyl acetate and methanolic extract of *Tabernaemontana divaricata* leaves in wistar rats by using animal model Despair Swim test. The TDEA and TDME (100 mg/kg, 200 mg/kg) on day 15 shows more significant decrease in immobility time in sec compared to day 1 and day 8. On day 15, TDME (200 mg/kg) extract revealed equivalent activity as compared to standard imipramine (25 mg/kg). Phytochemical screening found that *Tabernaemontana divaricata* leaves constitute alkaloids, carbohydrates, proteins, amino acids, phenols, glycosides, steroids and flavonoids.

CONCLUSION

Different extracts of *Tabernaemontana divaricata* leaves shows significant antidepressant activity. The methanol extract shows more significant activity at respective doses compared to ethyl acetate extract. This is a baseline work; further investigation is needed at molecular level.

ACKNOWLEDGEMENT

Authors' gratefully thanks to Mr. S K Sarje, Department of Pharmacology, Nanded Pharmacy College, Nanded for his support to carry out successfully present project work.

REFERENCES

1. Srinivas GR, Swamy CD, Ananth VK, Sandeep DR, Bhargav RR. Antidepressant activity of *Piper nigrum* fruit extract and comparison with Imipramine mice models, J of Pharmacy Res, 2012; 5(7): 3910-3912.
2. Nagapati PB, Parashivamurthy BM, Evaluation of Antidepressant like Activity of Aqueous Extract of Fruits of *Emblica officinalis* (Amla) in Albino Mice. Int J of Recent Trends in Sci and Tech, 2013; 9(2): 290-294.
3. Tan PT, Halijah H, Antidepressant like Activity of *Banana Peel* Extract in Mice. American Med J., 2011; 2(2): 59-64.
4. Archit P, Rakhi T, Vinay GP, Dipesh S, Nirmala R. Antidepressant Activity of Simvastatin Behavioral Models of Depression in Rats. Int J of Res in Med Sci., 2015; 3(7): 1666-1671.
5. Sarvesh S, Anjula S, Hemant S, Pratap S, Dheeraj K, Amod KS et.al. An Experimental Study to Evaluate the Antidepressant Activity of *Mucuna pruriens* in Mice. World J of Phar and Pharm Sci., 2015; 4(5): 588-597.

6. Roja SD, Rama MV, Subhasini P. In-vitro Antimicrobial Activity of *Tabernaemontana divaricata* Flower. Int J of Pharm Res and Bio-Sci., 2015; 4(2): 48-53.
7. Kalaimagal C, Umamaheswari G. Bioactive Compounds from the Leaves of *Tabernaemontana divaricata* (L). Int J of Recent Sci Res., 2015; 6(4): 3520-3522.
8. Umarani V, Sudhakar M, Lakshmi BV. Antiulcer Activity of *Tabernaemontana divaricata* Linn In Rats. Int J of Pharm and Ind Res., 2012; 2(3): 235-241.
9. Nowshin NR, Mostafizur R, Khalequzzaman K. Antioxidant and Cytotoxic Potential of Methanol Extract of *Tabernaemontana divaricata* Leaves. Int Current Pharm J., 2012; 1(2): 27-31.
10. Qureshi Z, Masood M, Saxena R, Nagar H, Ahmed T, Manoj R. Evaluation of Wound Healing Activity of *Tabernaemontana divaricata* Leaves in Experimental Rats. Int J of Pharm and Therapeutics, 2016; 7(3): 111-120.
11. Chanchal NR, Balasubramaniam A, Sayyed N. Antidiarrheal Potential of *Tabernaemontana divaricata*. Phytopharmacology. Infosights Publishing, 2013; 4(1): 61-68.
12. Biju J, Sulajman CT, Satheesh G, Reddy VR. Total Phenolics and Flavonoids in Selected Medicinal Plants from Kerala. Int J of Phar and Pharm Sci., 2014; 6(1): 406-408.
13. Ovais US, Muhammad MA, Azwani MS, Nasyriq A. Determination of Total Phenolics, Flavonoids Content and Free Radical Scavenging Activities of Common Herbs and Spices. J of Pharmacognosy and Phytochemistry, 2014; 3(3): 104-108.
14. Lateef AK, Theophilus AD, Samuel SA, Julius KO, Idowu JO, Adesoji MA. Antidepressant Activity and Mechanism of Aqueous Extracts of *Vigna unguiculata* ssp. Dekindtiana (L.) Walp Dried Aerial Parts in Mice. Int J of Neurosci and Behavioral Sci., 2017; 5(1): 7-18.
15. Shaik GP, Shaik KS, Shaik YA, Imaduddin, Roshan S, Saffon AK. Evaluation of Antiepileptic and Antioxidant Activity of Methanolic Extract of *Terminalia Tomentosa* (Roxb) Wight and Arn in Rats. World J of Pharm Res., 2015; 4(2): 766-776.
16. Choudhary RK, Ajaya ES, Swarnka PL. Radical Scavenging Activity of Phenolics and Flavonoids in Some Medicinal Plants of India. J of Pharm Res., 2011; 4(3): 712-713.
17. Dildar A, Muhammad MK, Ramsha S. Comparative Analysis of Phenolics, Flavonoids, Antioxidant and Antibacterial Potential of Methanolic, Hexanic and Aqueous Extracts from *Adiantum Caudatum* Leaves. Antioxidants, 2015; 4: 394-409.
18. Saikia LR, Sristisri U. Antioxidant Activity, Phenol and Flavonoid Content of Some Less Known Medicinal Plants of Assam. Int J of Pharma and Bio Sci., 2011; 2(2): 383-388.
19. Gerhard HV, Wolfgang HV, Bernward AS, Jurgen S, Gunter M, Wolfgang FV. Drug Discovery and Evaluation. Pharmacological Assays. 2nd ed. Newyork: Springer Publications, 2002; 559-561.