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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *RUELLIA TUBEROSA* STEM BARK EXTRACTS FOR ANTIDEPRESSANT ACTIVITY

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ABSTRACT

The present study reports physicochemical characterization, antidepressant activity of extracts from *Ruellia tuberosa* Stem Bark collected from local region of Nanded, Maharashtra, India. Different physical parameters like ash values, extractive value, Loss on drying, solubility etc were evaluated for powdered drug. The extracts were obtained from Soxhlet method by using water and methanol as solvents for extraction and subjected for preliminary physicochemical evaluation and antioxidant studies. Total phenolic and flavonoids content were also analyzed. The presence of primary and secondary metabolites such as carbohydrate, proteins, alkaloids, phenolic compounds, saponins was confirmed through preliminary phyto-chemical analysis. DPPH free radical scavenging assays showed strong antioxidant activities with increase in concentration of Ethyl acetate and methanol leaf extracts. Maximum percentage inhibition i.e. 80.97% was shown by methanolic extract at concentration of 150 µg/ml and was compared with Ascorbic acid as reference standard. The *In-Vivo* antidepressant activity of *Ruellia tuberosa* Stem Bark was evaluated by Despair Swim Test model in rats using Imipramine as a standard. Both the extracts at 200mg/kg Concentration showed significant to highly significant number of entries & time spent in P zone (from $P < 0.05$ to $P < 0.001$). The result suggest that *Ruellia tuberosa* Stem Bark extracts possess antidepressant activity and this might be due to flavonids. Phenolic compound, steroid and proteins present in extract.

Keywords: *Ruellia Tuberosa Stem Bark,* Ethyl acetate and Methanolic extract, Phytochemical screening, Antioxidant effect, antidepressant activity.

I. INTRODUCTION:

Depressed mood is a feature of some psychiatric syndromes such as major depressive disorder, but it may also be a normal reaction to life events such as bereavement, a symptom of some bodily ailments or a side effect of some drugs and medical treatments. Depression is more common in women than men. The report on Global Burden of Disease estimates the point prevalence of unipolar depressive episodes to be 1.9% for men and 3.2% for women and the one-year prevalence has been estimated to be 5.8% for men and 9.5% for women. In view of the morbidity, depression as a disorder has always been a focus of attention of researchers in India.

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).The life time risk of depression varies from 5% to 12% in men and 10% to 25% in women.

Depression is a state of low mood and aversion to activity that can affect a person's thoughts, behavior, feelings and sense of well-being. People with depressed mood can feel sad, anxious, empty, hopeless, helpless, worthless, guilty, irritable, ashamed or restless. They may lose interest in activities that were once pleasurable and experience overeating, loss of appetite, have problems concentrating, remembering details or making decisions and may contemplate, attempt or commit suicide. Along this insomnia, excessive sleeping, fatigue, aches, pains, digestive problems or reduced energy may also be present.

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According to World Health Organization, depression affects about121 million people worldwide and it is among the leading cause of disability. Suicide is one of the most common outcomes of depression to reduce the impact of depression; there is an urge to provide a cost effective treatment to the public. With the increased incidence of depression recently, natural herbs that have antidepressant effect have again more attention as alternative treatment for depression. Indians are among the world's most depressed..The life time risk of depression varies from 5% to 12% in men and 10% to 25% in women. It has been estimated that 15% of patients with severe depressive episodes commit suicide. An accurate diagnosis followed by effective treatment can improve this outcome. However, the adverse effects and cost, limits their uses. Hence, there is justifiable need to search for therapeutic agents relatively potent, safe, low price, easily available and natural in origin [1].

In the present study we have selected a plant namely *Ruellia tuberosa* belongs to family *Acanthaceae* and commonly known as cracker plant. It is a small plant with thick fusiform tuberous roots and striking funnel-shaped violet-colored flowers. Its fruit is a 2 cm (0.8 in) long sessile capsule containing about 20 seeds. Some of the names of the plant such as popping pod, duppy gun and cracker plant come from the fact that children like to play with the dry pods that pop when rubbed with spit or water. *Ruellia tuberosa* may be found in moist and shady environments. It grows, however, preferably in grasslands and road sides often as a weed in cultivated fields [2].

This plant has antinociceptive and anti-inflammatory properties. In folk medicine and Ayurvedic medicine it has been used as a diuretic, anti-diabetic, antipyretic, analgesic, antihypertensive, gastroprotective and to treat gonorrhea. It is also used as a natural dye for textiles. Some butterfly species, like the Lemon Pansy and the Mangrove Buckeye, feed on the leaves of Ruellia tuberose. phytochemicals are present in parts of *Ruellia tuberosa* are .Various Sterols –β-sitosterol,β-sitosteroglucoside , stigmasterol, Campesterol Triterpines-Lupeol,Betulin,21-Methyldammer triol. Coumarins- Indole, carboxyaldehyde .Flavonoids-Cirsimaritin, Cirsimarin,Cirsiliol 4′-glucoside, Sorbifolin,Pedalitin,Luteolin 7-O-glucoside, Apigenin 7-O-glucoside,Apigenin 7-O-glucuronide,Apigenin 7-O-rutinoside [3].

The main aim of this project based on Phytochemical investigation and Pharmacological Evaluation of Ruellia tuborosa *stem bark extracts for 'Antidepressant Activity'.*

II. MATERIAL AND METHODS:

1. Collection, identification and authentication of plant material

Fresh leaves were collected in the month of October from local region of Nanded district and the plant was authenticated by Dr.S. S. Bodke, Associate Professer & Head of Department of Botany & Horticulture, Yeshwant Mahavidyalaya, Nanded. A voucher specimen of plant was preserved in the herbarium (NPC/M. Pharm/herbarium/2019-20/H-4) for further reference. Collection, authentication, identification, processing and storage have been done according to standard procedure for the plant material.

2. Processing of crude drug:

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.

3. Preparation of Extracts:[4].

Three extracts of Ruellia tuborosa *stem bark* powder were prepared

1. Pet ether
2. Ethyl acetate extract
3. Methanol extract

The extract obtained and the dried mass was weighed and recorded. The percentage of yield was calculated.

$$\text{(\%) yield} = \frac{\text{Wt. of extract}}{\text{Wt. of powdered drug}} \times 100$$

A. Preparation of Ethyl acetate extract

Ethyl acetate extract of powdered leaves was prepared in Soxhlet extractor according to the standard method till colorless solution was observed in siphon tube. 300 gm of the powdered and 1000 ml Ethyl acetate was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container till use. Percentage yield of extract was calculated.

B. Preparation of Methanol extract

Methanolic extract of powdered leaf was prepared in Soxhlet extractor according to the standard method till colorless solution was observed in siphon tube. 150gm. of the powdered and1000 ml Methanol was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container till use. Percentage yield of extract was calculated.

III. PHYTOCHEMICAL EVALUATION:

A. CHEMICAL TEST [4].

1. Detection of alkaloids:

Extracts were dissolved individually in dilute HCl and filtered.

Dragendorff's test:

To 2-3 ml Filtrate, add few drops of Dragendorff's reagent. Orange brown Ppt. formed indicates the presence of alkaloids.

Hager's test:

To 2-3 ml Filtrate Hager's reagent Formation of yellow precipitate indicates the presence of alkaloids

Tannic acid test:

Test solution treated with tannic acid solution gives buff colored precipitate the presence of alkaloids.

2. Detection of proteins & amino acid:

Million's test:

Mix 3 ml test solution with 5 ml Million's reagent. White precipitate warm precipitate turns brick red precipitate dissolves giving red colored solution indicates the presence of protein.

Ninhydrin test:

To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

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Biuret test:
To 3 ml test solution adds 4% NaOH and few drops of 1% Copper sulphate solution. Violet color appears.

3. **Detection of carbohydrates:**
Extracts were dissolved individually in 5 ml of distilled water and filtered. The filtrates were used to test for the presence of carbohydrates
Molish's test:
Filtrates were treated with 2 drops of alcoholic α - naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.
Barfoed's test:
Mix equal volume of Barfoed's reagents and test solution. Heat for 1-2 min in boiling water bath and cool Red precipitate is observed
Benedict's test:
Filtrates treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
Fehling's test:
Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A &B solutions. Formation of red precipitate indicates the presence of reducing sugars.
4. **Detections of glycosides:**
Extracts were hydrolysed with dil.HCl, and then subjected to test for glycosides.
Modified Borntrager's Test:
Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink color in the ammonical layer indicates the presence of anthranol glycosides.
5. **Detection of tannins:**
To 2-3 ml of aqueous or alcoholic extract, add few drops.
5% Ferric **chloride test:** deep blue – black color
Lade acetate **sol. Test:** White precipitate
6. **Detection of Flavonoids:**
Lead acetate test:
Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.
Shinoda test:
To dry powder or extract add 5 ml 95% ethanol few drops conc. HCL and .0.5 gm. magnesium turnings. Orange, pink, red to purple color appears. Add t-butyl alcohol before adding the acid to avoid accidents from a Violent reaction and magnesium, only flavones give a deep red to magenta color while flavones and flavones give weak pink to magnetic color is observed.
7. **Detection of phytosterols:**
Salkowski's test:
Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.
8. **Detection of Saponin:**
Foam test:
Shake the drug extract or dry powder vigorously with water. Persistent foam observed indicates the presence of saponin.

IV. DEVELOPMENT OF TLC FINGERPRINT [4].

1. **Introduction:**
Thin layer chromatography is a method of analysis in which the stationary phase, a finely divided solid, is spread as a thin layer on a rigid supporting plate and the mobile phase, a liquid, is allowed to migrate across the surface of the plate by capillary action by gravity or pressure. TLC separation takes place in the open layer with each component having the same total migration time but different migration distance. Numerous fixed adsorbents have been used, including Silica gel, Cellulose, Polyamide, Alumina, Ion exchange and chemically bonded silica gel. Mobile phase consists of a single solvent or a mixture of solvents.
The stationary phase of the TLC is prepared using various techniques such as pouring, dipping and spraying. The prepared plates are allowed for setting (air-drying). This is done to avoid cracks on the surface of adsorbent. After setting the plates are activated by keeping in an oven at 100 to 120^{0}C for one hour. Activation of TLC plates is nothing but removing water/moisture and other substances from the surface of any absorbent, by heating at temperature around 110^{0}C so that adsorbent activity is retained. TLC studies were carried out using various extracts to confirm the presence of different phytoconstituents in the extract.
Analysis
In TLC qualitative analysis of the unknown compound is done by comparing the R_f values. As solutes never travel the full length of the stationary phase in TLC all the R_f value depends on the amount of the stationary phase, the humidity, layer thickness, solvent quality, saturation of chamber, development distance, temperature, amount of substance added, and the presence of impurities.

$$R_f = \frac{\text{Distance from origin to the point of maximum intensity}}{\text{Distance from origin to the solvent front}}$$

R_f = Retention factor

2. **Total Phenolic Content [5, 6].**
Total Phenolic Content was determined by using the **Folin-Ciocalteu assay**. An aliquot (1m) of extract or standard solution of Gallic acid [2, 4, 6, 8, 10µg/ml] was added to 10 ml of volumetric flask, containing 9ml of distilled water. A blank reagent using distilled water was prepared. 0.5 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 2 ml of 2% $NaHCo_3$ solution was added to the mixture. The volume was then made up to the mark. After incubation for 120 minutes at room temperature, the absorbance against the reagent blank was determined at 746 nm with an UV-Visible spectrophotometer.
3. **Total Flavonoids Content [5, 6].**
Total Flavonoid Content was measured by the alluminium trichloride colorimetric assay. An aliquot (1ml) of extracts or standard solutions of Rutin (50, 100, 150, 200 and 250µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.3 ml 5% $NaNO_2$, after five minutes 0.3 ml 10 % $AlCl_3$ was added. After five minutes, 2 ml 1M NaOH was added and the volume was made up to 10 ml with

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distilled water. The solution was mixed and absorbance was measured against the blank at 258 nm.

4. ***In vitro* anti-oxidant activity [7, 8].**

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions.

2,2 Diphenyl- 1 picryl-hydrazylradical scavenging (DPPH) Activity:

Principle:

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H. This transformation results in a color change from purple to yellow, which is measured spectrophotometrically. The disappearance of the purple color is monitored at 517 nm. The free radical scavenging activity can be measured by using 1, 1-diphenyl-2-picryl-hydrazyl.

Reagents Required:

1) DPPH
2) Pure Methanol

Preparation of samples and standard solutions:

Accurately weighed 10 mg of Acetone and Methanolic extracts and the standard ascorbic acid and dissolved separately in 10 ml of phosphate buffered saline. These solutions were serially diluted with methanol to obtain the lower dilutions.

Procedure:

The reaction mixture (3.0 ml) consists of 1 ml of 0.1mM DPPH solution in methanol was mixed with 1 ml of drug solution and 1.0 ml of methanol. The reaction mixture was vortexes and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. A reaction mixture without test sample was served as control.

The percentage of inhibition can be calculated using the formula:

$$\%\ \text{inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,

$A_{control}$: Absorbance of control.

A_{test}: Absorbance of test.

V. ANIMAL USED:

For the study *Wistar rats* of either sex, of weight 150-200gm were selected.

Test group:

For the study seven groups of animals were made. **(1613/PO/Re/S/12/CPCSEA).**

Route of administration: Oral route and ip. Route of administration.

Housing Condition:

Animals were housed seven groups in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (golden feed, New Delhi) and water regularly. Animals were divided randomly into six treatment groups; each group consisting of six rats

VI. DESPAIR SWIM TEST MODEL

Purpose & rationale:

Behavioral despair was proposed as a model to test for antidepressant activity by Porsolt et al. (1977, 1978). It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility

Animal Grouping and drug administration:

Group I : Control normal saline solution (0.9% NaCl) 1 ml/kg orally
Group II : Standard (Imipramine 10 mg/kg) orally
Group III : *Ruellia tuberosa* stem barkEthyl acetate extract (RTEA) 100 mg/kg orally
Group IV : *Ruellia tuberosa* stem barkEthyl acetate extract (RTEA) 200 mg/kg orally.
Group V : *Ruellia tuberosa* stem bark Methanol extract (RTME) 100 mg/kg orally
Group VI : *Ruellia tuberosa* stem bark Methanol extract (RTME) 200 m

Procedure [9]:

Wistar rats of both sexes weighing 150–250g are used. They are brought to the laboratory at least one day before the experiment and are housed separately in Makrolon cages with free access to food and water. Naive rats are individually forced to swim inside a vertical Plexiglas cylinder (height: 40 cm; diameter: 18 cm, containing fresh water to a height of 15 cm and maintained at 25 °C). Rats placed in the cylinders for the first time are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2–3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. The total duration of immobility was recorded in next 4 minutes of total 6 min test. After 6 min in the water the rats are removed and dried with cloth before being returned to their home cages. 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. An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. Plant extracts or standard drug Imipramine (25 mg/kg) is administered one hour prior to testing. The similar procedure was conducted on 1^{st}, 8^{th} and 15^{th} days of experiment.

Evaluation

Evaluation was done on the basis of duration of immobility time in sec in total 6 min test on respective days of experiment i.e. on day 1^{st}, day 8^{th} and day 15^{th}.

VII. STATISTICAL ANALYSIS

The data were expressed as mean + standard of mean (SEM).Statistical analysis were performed by one way analysis of variance (ANOVA).

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VIII. RESULTS:

1. Phytochemical tests of *Ruellia tuborosa Linn* stem bark extracts

Table 1: Observations for Phytochemical qualitative analysis

Sr. no	Test for	Pet ether	Ethyl acetate	Methanol
1	**Alkaloids**			
	Wagner test	-	-	+
	Hager's test	+	+	+
2	Proteins			
	Millon's test	+	+	+
	Xanthoprotein test	+	+	+
3	Carbohydrate			
	Molish's test	+	+	+
	Barfoed's test	+	+	+
	Benedicts test	+	+	+
4	Glycosides			
	Borntrager's test	-	+	+
	Keller killani test	+	+	+
5	Tannins and Phenolic comp.			
	Ferric chloride test	-	+	+
	Lead acetate sol^{n}test:	+	+	+
	Dil.Nitric acid test	+	-	+
6	Flavonoids			
	Alkaline test	+	-	+
	Shinoda test	+	+	+
	Steroids			
8	Salwoski test	-	-	+
	Libberman test	-	-	+
9	Amino acid			
	Ninhydrin test	+	+	+
	Tyrosin test	+	-	-
	Tryptophan test	-	+	+

Present +; Absent -; above observation table shows the presence of phytoconstituents in the extracts. It reveals all three (i.e. petroleum ether, methanolic and ethyl acetate) extracts contains carbohydrate, proteins, alkaloids, flavonoids, tannins, steroids and amino acid. Methanolic extract shows the more number of phytoconstituents.

2. TLC Fingerprinting:

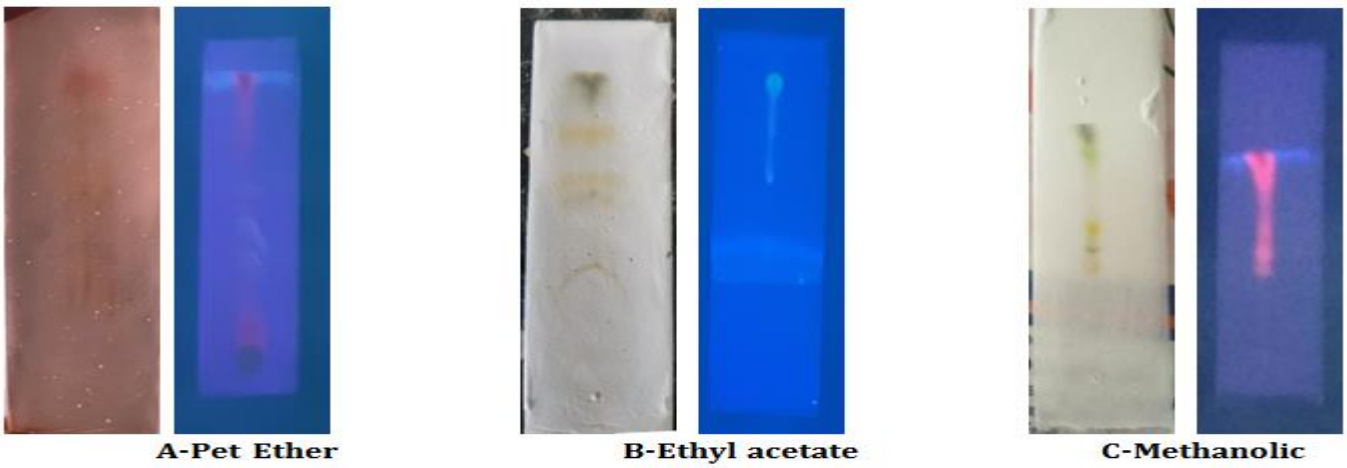

Fig. 2: TLC plates of A: Pet Ether Extract, B: Ethyl Acetate Extract, C: Mehanolic Extract

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Table 2: TLC R_f values of *Ruellia tuberosa* Linn stem bark extracts

Sr. No.	Extracts	Solvent system	Proportions	Spraying reagent	R_f Value
1	RT- PE	Benzene:Chloroform:Ethyl acetate	(5:3:2)	Sulphuric acid	0.48
2	RT- PE	Benzene:Chloroform:Ethyl acetate	(5:3:2)	Sulphuric acid	0.53
3	RT- PE	Benzene:Chloroform:Ethyl acetate	(5:3:2)	Sulphuric acid	0.72
4	RT- PE	Benzene:Chloroform:Ethyl acetate	(5:3:2)	Sulphuric acid	0.85
5	RT- PE	Benzene:Chloroform:Ethyl acetate	(5:3:2)	Sulphuric acid	0.95
6	RT-EA	Benzene:Methanol:Ethyl acetate	(8.5:0.5:1)	Sulphuric acid	0.5
7	RT-EA	Benzene:Methanol:Ethyl acetate	(8.5:0.5:1)	Sulphuric acid	0.63
8	RT-EA	Benzene:Methanol:Ethyl acetate	(8.5:0.5:1)	Sulphuric acid	0.66
9	RT-EA	Benzene:Methanol:Ethyl acetate	(8.5:0.5:1)	Sulphuric acid	0.85
10	RT-EA	Benzene:Methanol:Ethyl acetate	(8.5:0.5:1)	Sulphuric acid	0.95
11	RT-ME	Benzene: Chloroform: Ethanol	(8:1:1)	Sulphuric acid	0.63
12	RT-ME	Benzene: Chloroform: Ethanol	(8:1:1)	Sulphuric acid	0.65
13	RT-ME	Benzene: Chloroform: Ethanol	(8:1:1)	Sulphuric acid	0.67
14	RT-ME	Benzene: Chloroform: Ethanol	(8:1:1)	Sulphuric acid	0.84
15	RT-ME	Benzene: Chloroform: Ethanol	(8:1:1)	Sulphuric acid	0.87

Table 3: Total phenolic content of *Ruellia tuberosa* Linn stem bark extracts

Sr. No.	Conc. µg/ml	Extracts	Phenolic content (mg GAE/g DW)	Flavonoid content (mg RE/g DW)
1	100	Petroleum ether	32.94	28.57
2	100	Ethyl acetate	58.82	55.23
3	100	Methanol	70.58	69.52

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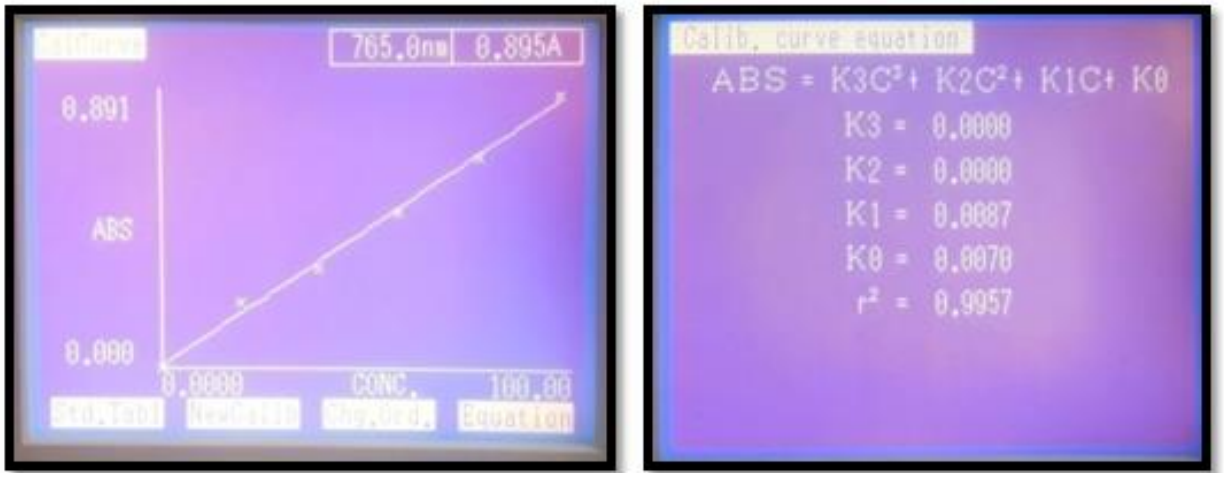
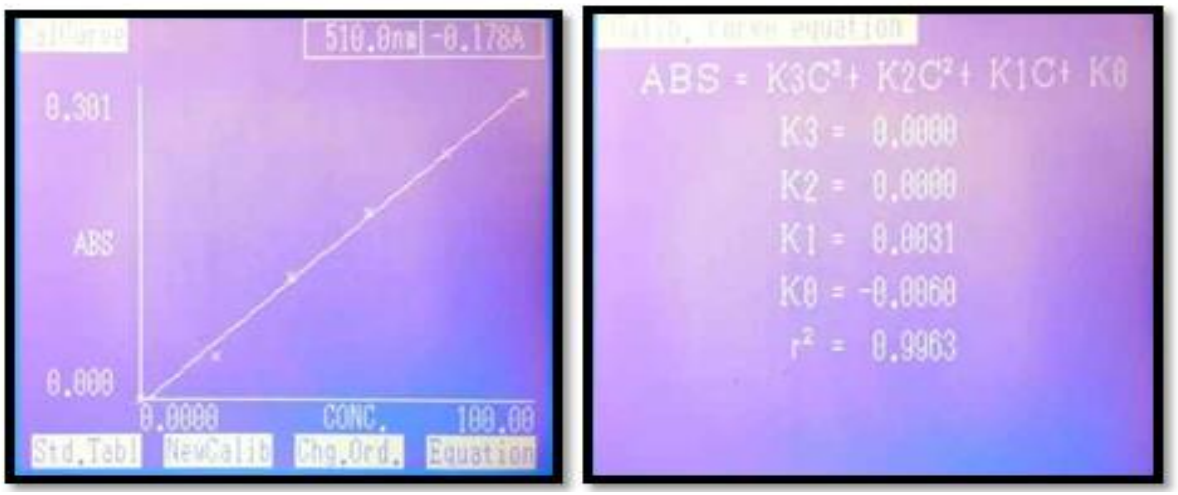


A B

Fig. 3: A-Calibration curve of Gallic acid; B- Equation of Gallic acid



A B

Fig. 4: Calibration curve of Rutin; B- Equation of Rutin

Pharmacological evaluation of *Ruellia tuberosa* Linn stem bark extracts

Anti-Oxidant Activity

The antioxidant activity of *Ruelia tubarosa* was determined by *in-vitro* methods such as, DPPH free radical scavenging assay method. The assays were carried out in triplicate and average value was considered. The results were compared with Ascorbic acid as a reference standard.

Table 4: DPPH (2, 2-Dipheny1, 1-Picrylhydrazyl) radical scavenging activity

Conc. µg/ml	Gallic acid %inhibition	Ascorbic acid % inhibition	BHT % inhibition
25	93.07 ± 0.48	69.40 ± 0.67	88.46 ± 0.21
50	93.72 ± 0.12	71.76 ± 0.07	89.77 ± 0.28
75	94.50 ± 0.19	74.85 ± 0.73	93.90 ± 0.29
100	95.45 ± 0.15	81.57 ± 0.09	94.34 ± 0.14
125	95.34 ± 0.12	86.59 ± 0.22	94.48 ± 0.20

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Table 5: Comparative DPPH Scavenging assay method of *Ruelia tubarosa*

Sr. No.	Conc. (µg/ml)	Petroleum ether % Inhibition	Ethyl Acetate % Inhibition	Methanol % Inhibition	Ascorbic acid % Inhibition
1	25	42.32 ± 0.15	46.77 ± 0.15	56.87 ± 0.20	60.62 ± 0.26
2	50	54.44 ± 0.16	59.33 ± 0.26	75.4 ± 0.20	81.57 ± 0.09
3	75	62.58 ± 0.28	69.02 ± 0.16	72.68 ± 0.22	76.53 ± 0.19
4	100	74.52 ± 0.19	62.31 ± 0.22	81.25 ± 0.12	86.59 ± 0.22
5	125	79.15 ± 0.22	87.47 ± 0.87	92.23 ± 0.33	97.34 ± 0.28

DPPH (2, 2-dipheny 1, 1-picrylhydrazyl) radical scavenging activity Concentration Vs % inhibition

From the above table and graph it reveals that all among all 3 extracts of *Ruellia Tubrosa* Stem barks, Methanol extract have comparable percent DPPH scavenging activity (92.23 % inhibition) in comparison to standard ascorbic acid (97.34%). Methanolic extract shows better activity than the petroleum ether & Ethyl acetate extract.

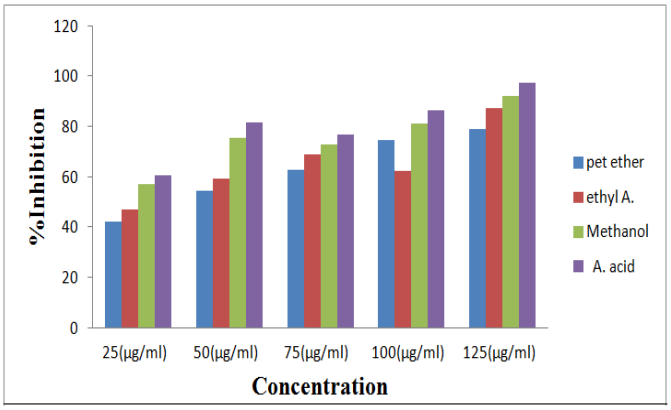

Chart 1: DPPH scavenging activity of *Ruellia Tubrosa* extracts

***In- Vivo* Antidepressant activity**

Table 6: Effect of Day 1 RTEA and RTME extracts on Immobility time in Despair Swim test model on wistar rats

Treatment Group	Despair swim test (Immobility time in sec)
	Day 1
Control (DMSO 2%)	86.66 ± 1.14
Imipramine 15 mg/kg	44.83 ± 1.35 **
RTEA 100 mg/kg	62.5 ± 1.60 *
RTEA 200 mg/kg	57.33 ± 1.60 **
RTME 100 mg/kg	52.33 ± 1.60 **
RTME 200 mg/kg	45.16 ± 0.94 **

Table 7: Effect of Day 8 RTEA and RTME extracts on Immobility time in Despair Swim test model on wistar rats

Treatment Group	Despair swim test (Immobility time in sec)
	Day 8
Control (DMSO 2%)	83.83 ± 1.49
Imipramine 15 mg/kg	42.83 ± 1.53**
RTEA 100 mg/kg	60.83 ± 0.94*
RTEA 200 mg/kg	53.66 ± 1.85 **
RTME 100 mg/kg	48.5 ± 1.47 **
RTME 200 mg/kg	43.5 ± 1.60 **

Table 8: Effect of Day 15 RTEA and RTME extracts on Immobility time in Despair Swim test model on wistar rats

Treatment Group	Despair swim test (Immobility time in sec)
	Day 15
Control (DMSO 1%)	81.83 ± 1.55
Imipramine 15 mg/kg	41.5 ± 1.83 **
RTEA 100 mg/kg	57.16 ± 1.40 *
RTEA 200 mg/kg	47.16 ± 1.70 **
RTME 100 mg/kg	43.66 ± 1.80 **
RTME 200 mg/kg	42.33 ± 1.60 **

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Table 9: Comparative effect of RTEA and RTME 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 (DMSO 2%)	86.66 ± 1.14	83.83 ± 1.49	81.83 ± 1.55
Imipramine 15 mg/kg	44.83 ± 1.35 **	42.83 ± 1.53**	41.5 ± 1.83 **
RTEA 100 mg/kg	62.5 ± 1.60 **	60.83 ± 0.94**	57.16 ± 1.40 **
RTEA 200 mg/kg	57.33 ± 1.60 **	53.66 ± 1.85 **	47.16 ± 1.70 **#
RTME 100 mg/kg	52.33 ± 1.60 **	48.5 ± 1.47 **#	43.66 ± 1.80 **#
RTME 200 mg/kg	45.16 ± 0.94 **#	43.5 ± 1.60 **#	3 ± 1.60 **#

(N=6) *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 (NS), indicated as No significant difference when compared to standard .*

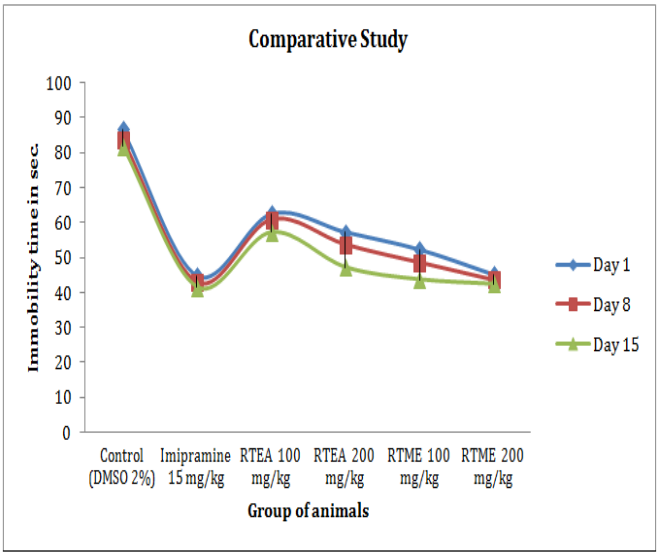

Chart 2: Comparison between all three (1st,8th,15th) days data time on Immobility in seconds

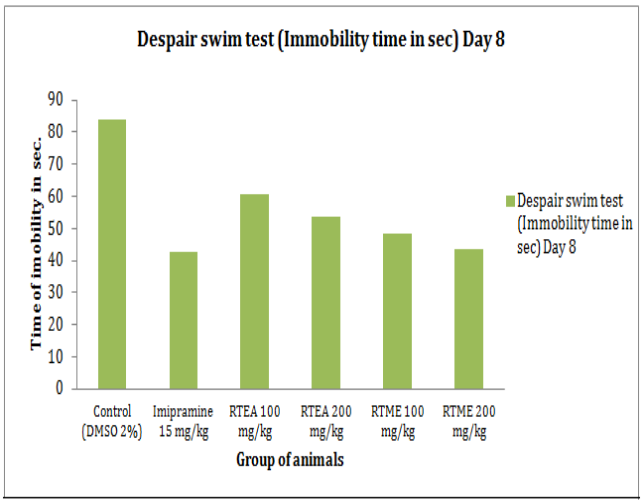

Chart 4: Effect of Ruellia tuberosa (RT) and Imipramine (15 mg/kg) on day 8 in DST. (The column represents mean duration of immobility recorded in a 6 min observation period).

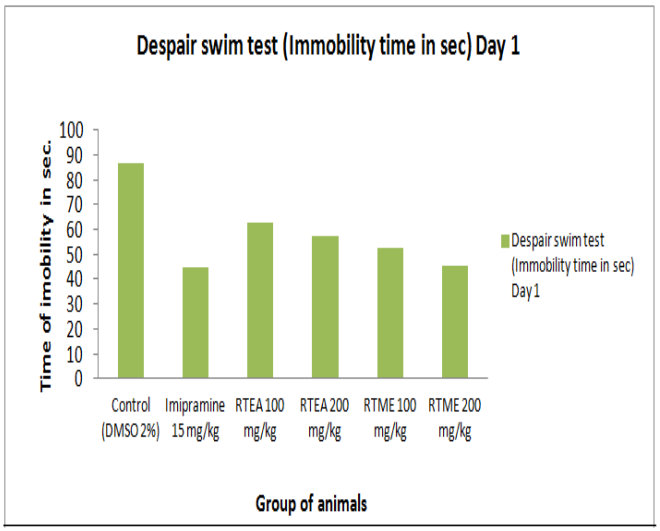

Chart 3: Effect of *Ruellia tuberosa* (RT) and Imipramine (15 mg/kg) on day 1 in DST. (The column represents mean duration of immobility recorded in a 6 min observation period).

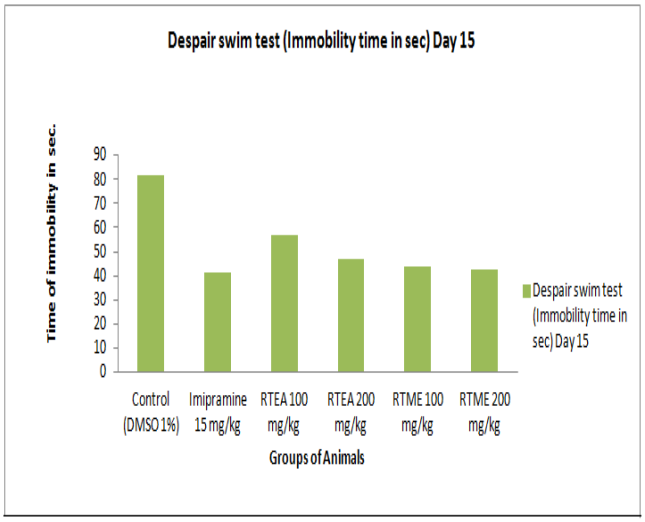

Chart 6: Effect of *Ruellia tuberosa* (RT) and Imipramine (15 mg/kg) on day 15 in DST (The column represents mean duration of immobility recorded in a 6 min observation period).

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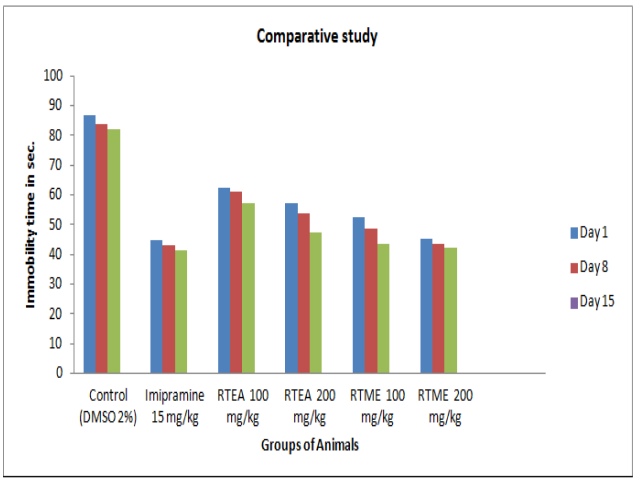

Chart 7: Comparison of effect of *Ruellia tuberosa* (RT) and Imipramine (15 mg/kg) on day 1, 8, 15 in DST (The column represents mean duration of immobility recorded in a 6 min observation period).

IX. DISCUSSION:

Stem bark of *Ruellia tuberosa* reported to have medicinal values including anticancer, antidiabetic, anti-inflammatory, antibacterial, antiulcer, hepatoprotective, antioxidant properties [10].

The phytoconstituents such as alkaloids, flavonoids, glycosides, saponins, tannins, quinines were reported as the basis of therapeutic properties. The other important constituents which include sitosterol, sitosteroglycoside quercetin, tercatain, tergallagin, terflavin, Apigenin [11-12].

To the best of our knowledge, no scientific data regarding the antidepressant effect of *Ruellia tuberosa* leaves thus the present study undertaken for comparative evaluation of *Ruellia tuberosa* Stem bark for Petroleum ether, ethyl acetate, Methanolic extract for antidepressant activity on Wistar rats.

Extracts shows presence of alkaloid, glycosides, tannin, carbohydrates, proteins, amino acids,flavonoids and steroids.

The total phenolic and flavonoid as compared with the ethyl acetate and petroleum ether extract.

The evaluation of antioxidant activity Ruellia Tuberosa Stem Bark extract was done by using DPPH radical scavenging assay method at concentration of 25,50,75,100,125µg/ml. The mehanolic extract showed maximum percentage inhibition at 5µg/ml as compared to ethyl acetate and petroleum ether. Gallic acid, ascorbic acid, and rutin were used as standard. The result indicates that the methanolic extract has more phytochemical constituent which is responsible for antioxidant activity.

Experimental Despair Swim Test model was used to test antidepressant activity of ethyl acetate and ethanolic extract of plant material. The RTEA and RTME at doses 100mg/kg nd 200mg/kg and Imipramine 15mg/kg all significantly reduced immobility time compared with control DMSO 1%. Wistar Rat $p<0.05$ to $p<0.001$ and $p>0.05$ was considered non-significant (ns).

Post hoc analysis Tukey's multiple comparisons test found that RTME 200mg/kg has significant difference when compared to Imipramine standard (15mg/kg) but activity more than standard in DST.Ruellia Tuberosa exhibited a slight but non-significant dose-dependent decrease in immobility. The result indicated that RT showed significant antidepressant-like effect in the DST.

On day 1 the oral administration of RTEA and RTME (100mg/kg, 200mg/kg) and Imipramine (15mg/kg) p.o. shows less significant decrease in duration of immobility compared to day 8.

On day 8 the oral administration of RTEE and RTME (100mg/kg, 200mg/kg) and Imipramine (15mg/kg) p.o. shows more significant decrease in duration of immobility compared to day 15.

On day 15 it shows more significant decrease in immobility time in sec compared to day 1 and day 8. On day 15 RTME (200mg/kg) showed significant difference when compared to Imipramine (15mg/Kg) but is same like standard.

X. SUMMARY AND CONCLUSION

It was planned to explore *Ruellia tuborosa* stem bark part for its antidepressant potential and accordingly three extract were prepared first deffacted with petroleum ether and further extracted with ethyl acetate and ethanol. These extracts were tested for its phytochemical estimation and antioxidant and antidepressant activity. Preliminary phytochemical evaluation of three extract was carried out for the determination of presence of phytoconstituents. It reveals that all three extract (i.e.Petroleum ether, ethyl acetate and ethanol) contain carbohydrate, glycosides, alkaloids, proteins, steroids, tannins, flavonoids. Presence of phytoconstituents was further confirmed by Thin Layer Chromatography.

The two extract were tested for *in vivo* antidepressant activity. The obtained results showed that all the extracts showed significant effect at respective doses of 100mg/kg and 200mg/kg according to body weight when compared with standard drug imipramine. The Methanolic extract at a dose of 200 mg/kg showed best result than other acetone extracts. It shows the antidepressant potential by Despair Swim Test (physical method) by immobility state produced by animals.

The animals are forced to swim in a restricted space from which they cannot escape are induced to a characteristics behavior of immobility. The mean immobility time for each group is observed on day 1, day 8, and day 15. From this study is concluded that the ethyl acetate and methanol Extracts of *Ruellia tuberosa* Stem Bark showed significant Antidepressant action when compared with control group and standard Imipramine treated groups.

The different extracts of *Ruellia tuberosa* stem bark showed 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 for determination of active constituents responsible for antidepressant activity.

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