EVALUATION OF PHYTOCONSTITUENTS, ANTIOXIDANT AND ACUTE ORAL TOXICITY OF WHOLE PLANTS OF *D. ANNULATUM* & *S. BENGALENSE* AND FLOWER OF *R. TUBEROSE*

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ABSTRACT

From the ancient times plants have provided huge amount of important ingredients in most of the traditional medicinal systems. The present study was based on the evaluation of phytoconstituents, antioxidant and acute oral toxicity profiles of the methanolic extracts of three indigenous Indian medicinal plants, *e.g., R. tuberosa, D. annulatum,* and *S. bengalense.* The whole plants of *Dichanthium annulatum*, *Saccharum bengalense* and *Ruellia tuberosa* were collected from Hooghly, West Bengal, India. Central National Herbarium, Botanical Survey of India, Ministry of Environment and Forest, Govt. of India has identified specimens of whole plants of *D. annulatum* (Ref No. CNH/50/2014/Tech.II/103) and R. tuberosa (Ref No. CNH/2017/Tech. II/19). Soxhlet apparatus was used for the extraction process. Qualitative phytochemical analyses of *D. annulatum*, *S. bengalense* and *R. tuberosa* methanolic extracts were performed according to the methods described previously. Total phenolic, total flavonoid content, free radical scavenging and acute oral toxicity studies were performed. In results, phenolics, flavonoids and alkaloids were present as major groups of important secondary metabolites and carbohydrates as important biomolecules in the methanolic extracts of *Dichanthium annulatum* Whole Plant (DAW), *Saccharum bengalense* Whole Plant (SBW), and *Ruellia Tuberose* Flowers (RTF). No death of the animals was observed within this dose regimen up to 72 hours of routine observation for all the three extracts, *e.g.,* DAW, SBW or RTF. Therefore, $1/10^{th}$ to$1/20^{th}$ of these doses, *i.e.,* 200 to 100 mg/kg for DAW, 400 to 200 mg/kg for SBW and 100 to 50 mg/kg for RTF were considered safe. It concluded that all these three plants *Dichanthium Annulatum* Whole Plant (DAW), *Saccharum Bengalense* Whole Plant (SBW), and *Ruellia Tuberose* Flowers (RTF) are rich source of flavonoids, phenolic contents etc. and found safe in acute oral toxicity profile.

Keywords: Acute oral toxicity, antioxidant, phytoconstituents, total phenolic content, total flavonoid content and mice.

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INTRODUCTION

From the ancient times plants have provided huge amount of important ingredients in most of the traditional medicinal systems [1]. Almost every plant contains huge variety of chemical compounds like ascorbic acid, carotenoids, flavonoids and phenolics that exhibit antioxidant and radical scavenging effects [2]. Activation of a number of different cellular or molecular

components of the immune system is associated with generation of ROS and RNS and therefore generation of local as well as systemic oxidative stress [3].

Ruellia tuberosa

The plant *Ruellia tuberosa* belongs to the Acanthaceae family and was introduced as a decoration to an Indian garden. It is a native of Central America [4]. The West Indies, Central America, Guyana, and Peru all use it medicinally [5]. Due to its explosive activity, the plant earned the English nickname Cracker plant.

It has been employed in traditional medicine as a diuretic, antidiabetic, antipyretic, analgesic, and anti-hypertensive, as well as a quencher of thirst and an antidote [6,7].

Common names: Snapdragon root, Duppy gun, Bluebell, Daniel's great gun, large bell-flower, Minnie root, popping pod & sheep potato.

Taxonomy [8]

Kingdom	- Planata
Division	- Magnoliophyta
Class	- Magnoliopsida
Order	- Scrophulariales
Family	- Acanthaceae
Genus	- Ruellia
Species	- *tuberosa*

Saccharum benghalense

Saccharum benghalense is synonym for *Tripidium bengalense* that is also called as munj grass that grows in desert regions and along river banks. The grass is tall, with smooth, greenish brown panicles. The grass is overgrown and can reach heights of up to 7 feet. The stem is used to make moorhas and chiks [9]. It is native to Myanmar, Bangladesh, Nepal, Afghanistan, Pakistan, northern India, and Iran [10].

Taxonomy

Kingdom	- Planata
Class	- Liliopsida
Order	- Poales
Family	- Poaceae
Genus	- Saccharum (Tripidium)
Species	- *benghalense*

Dichanthium annulatum

It belongs to Poaceae (Graminae) family. It belongs to the Poales order, is perennial, and is also referred to as marvel grass. In India, these species appear promising for grazing or hay-making following reseeding into damaged grassland. In addition, it was used to uneven lawns. A high-quality fodder that can withstand brief flooding or drought is *Dichanthium annulatum* [11]. The discovery of many phenolic substances like Lignans, flavonoids, and flavolignan has recently sparked an increased interest in the phytochemistry of the family Poaceae (Graminaea) [12]. Poaceae plants have been utilised in folk medicine for a variety of ailments, including

hypertension, diabetes, inflammation, anthelmintic, astringent, ulcerative, diuretic, and antioxidant effects.

Taxonomy [13]

Kingdom	- Planatae
Division	- Tracheophyta
Class	- Magnoliopsida
Order	- Poales
Family	- Poaceae
Genus	- Dichanthium
Species	- *annulatum*

Phenolic compounds identified and isolated from *D. Annulatum* fruit has been suggested as potential herbal candidate for amelioration of acute and chronic inflammation previously in many studies due to their modulatory action on free radical generation and are therefore correlated to its anti-arthritic and anti-osteoporotic activities [14]. A recent study, reported that besides potent immunosuppressive activities, the polyphenols and flavonoids from *S. bengalense* extracts, namely quercetins and its derivatives have been significantly correlated to its anti-arthritic activity [15][16].

The present study was based on the evaluation of phytoconstituents, antioxidant and acute oral toxicity profiles of the methanolic extracts of three indigenous Indian medicinal plants, *e.g., R. tuberosa, D. Annulatum,* and *S. bengalense.*

MATERIALS AND METHODS

Plant collection and identification

The whole plants of Dichanthium Annulatum, Saccharum bengalense and Ruellia tuberosa were collected from Hooghly, West Bengal; India. Central National Herbarium (CNH), Botanical Survey of India, Ministry of Environment and Forest, Govt. of India has identified specimens of whole plants of D. Annulatum (Ref No. CNH/50/2014/Tech.II/103) and R. tuberosa (Ref No. CNH/2017/Tech. II/19).

Preparation of plant extracts

The Whole Plant of *Dichanthium Annulatum*, Whole Plant of *Saccharum bengalense* and flower petals of *Ruellia tuberosa* were isolated and were dried under shade. Ten grams of finely ground plant materials (Whole Plant of *D. Annulatum,* Whole Plant of *S. bengalense* and flower petals of *R. tuberosa*) were soaked into 30 ml methanol (70%) at 30ºC for 12 hr with shaking. The methanol was then allowed to evaporate completely under sterile conditions; this extraction protocol of the original materials was repeated three times. Each final residue was then dissolved into 10 ml methanol (70%) and filtered through Whatman‟s No.1 filter paper. Each filtrate was then centrifuged at 2000 rpm for 10 min. Supernatant was collected and air-dried to completeness under sterile conditions. The final yields were 7.1% for *D. Annulatum*, 12.15% for *S. bengalense* and 6.5% for *R. tuberosa* respectively

Preliminary identification of phytochemical constituents presents in extracts

Crude plant extracts were dissolved into suitable solvents and used for qualitative analysis of key phytochemical constituents such as alkaloids, flavonoids, phenolics, saponins, steroids, tannins, proteins, glycosides, and carbohydrates etc. Qualitative phytochemical analyses of *D. Annulatum*, *S. bengalense* and *R. tuberosa* methanolic extracts were performed according to

the methods described previously [17].

Test for carbohydrates- A volume 3 ml of the sample solution was taken into a test tube and 3 drops of Molisch's reagent were added carefully. About 3 ml of concentrated H_2SO_4 was added by the side of the test tube. Appearance of purple ring at the junction of the two liquid layers indicated the presence of carbohydrates.

Test for tannins- A volume of 5 ml of 45% ethanol was added to 2 g of the sample and boiled for 5 minutes. It was cooled and filtered. 3 drops of Pb-acetate were added to 1 ml of the filtrate. Precipitation of gelatinous material confirmed the presence of tannins.

Test for saponins- An amount of 0.5 g of sample was added to 5ml of distilled water and the solution was shaken vigorously for frothing. To the froth 3 drops of olive oil was added. Formation of emulsion concluded the presence of saponins.

Test for flavonoids- An amount of 0.5 g of sample was added to 10 ml of ethyl acetate and was heated on boiling water bath for about a minute. The mixture was filtered and then shaken with 1% NH_4Cl. Appearance of yellow color on addition of dilute ammonia indicated the presence of flavonoids.

Test for alkaloids- An amount of 5 g of the sample was dissolved in a mixture of 10ml ammoniacal chloroform and 5ml chloroform and was filtered. The filtrate thus obtained was shaken with 10 drops of 0.5 M H_2SO_4. Appearance of creamish white precipitate confirmed presence of alkaloids.

Test for steroids- A volume of 2 ml of acetic anhydride was added to 0.5 g of the sample and 2 ml of 0.5 M H_2SO_4 was added by the side of the test tube. Change in color indicated presence of steroids.

Test for phenolics- An amount of 0.5 g of the sample was added to 2 ml of distilled water and was warmed, to which 2 ml of $FeCl_3$ was added. Appearance of green or blue color indicated the presence of phenolics.

Test for glycosides- An amount of 0.5 g of the sample was added to 1 ml of glacial acetic acid containing traces of $FeCl_3$ and 1 ml of concentrated H_2SO_4 was added by the side of the test tube and observed for the formation of reddish-brown color at the junction of the two layers. In presence of glycosides the upper layer turned bluish green.

Test for protein- A volume of 3 ml of sample solution was taken into a test tube. One ml of concentrated HNO_3 was added, it was heated and cooled under tap water and made alkaline with NaOH for appearance of yellow color which indicated presenceof proteins.

Estimation of total phenolic content in extract

Total phenolic content in the three different extracts was estimated by Folin-Ciocalteu procedure (Singleton and Rossi, 1965) using gallic acid (purchased from Sigma, MO) as standard to constitute the standard curve. Gallic acid was dissolved into ethanol and then diluted to 25, 50, 100, 150 and 200 µg/ml concentrations and on the other hand 10 mg of each extract were accurately weighed and transferred into a 10 ml volumetric flask and the volume was made upto the mark with methanol. The diluted standard solutions of gallic acid of different concentration and the extracts at a volume of 2 ml were separately mixed with 1 ml of Folin- Ciocalteu (purchased from Sigma, MO) and 0.8 ml of sodium carbonate (7.5 g % in water) and were incubated for 30 min at room temperature to complete the reaction. The absorbance of the reaction mixture was measured at 765 nm with dual beam UV-Vis spectrophotometer against blank [18].

Calculations of total phenolic content (TPC) from quercetin standard curve was done by using

the concentration values obtained for each extract after interpolating to the X-axis and then using the formula:

$$TPC = \frac{R \times DF \times V \times 100}{W}$$

Where, R = Result obtained from standard curve; DF= Dilution factor; V= Volume of stock solution; 100 – for 100 g dried sample; W= weight of the plant used in the experiment. The phenolic content in each extract were calculated as mean±SD (n=3) and expressed as mg quercetin equivalents (QE)/ g dry extract (w/

Estimation of total flavonoid content in extract

Total flavonoid content in the three different extracts was estimated by aluminium chloride colorimetric method according to the previously described protocols (Chang et al., 2002), using quercetin (purchased from Sigma, MO) as standard to constitute the calibration curve. Briefly readily purchased quercetin was dissolved into 80% ethanol and then diluted to 25,50, 100, 150 and 200 µg/ ml concentrations, on the other hand 10 mg of each extracts were accurately weighed and transferred into a 10 ml volumetric flask and the volume was made upto the mark with methanol. The diluted standard solutions of quercetin of different concentration and the extracts at a volume of 500 µl were separately mixed with 1.5 ml of 95% ethanol, 100µl of 10% aluminum chloride, 100 µl of 1 M potassium acetate and 2.8 ml of distilled water and were incubated for 30 min at room temperature to complete the reaction. The absorbance of the reaction mixture was measured at 415 nm (λ_{max} of quercetin) with dual beam UV-Vis spectrophotometer against blank (containing all the reagents excepting the sample aluminium chloride). The amount of flavonoid was calculated from linear regression equation obtained from the quercetin calibration curve [19]. The total flavonoid content in methanolic *A. vasica* Whole Plant extract (AVE), *S. bengalense* fruit extract (EOE) and *R. tuberosa* flower extract (CTE) were calculated using the following linear regression equation obtained from the standard plot of quercetin: $\boldsymbol{y=0.0067x+0.52,\ r^2=0.969}$, where *y* is absorbance and *x* is the amount of quercetin in µg. Calculations of total flavonoid content (TFC) from quercetin standard curve was done by using the concentration values obtained for each extracts after interpolating to the X-axis and then using the formula:

$$TFC = \frac{R \times DF \times V \times 100}{W}$$

Where, R = Result obtained from standard curve; DF= Dilution factor; V= Volume of stock solution; 100 – for 100 g dried sample; W= weight of the plant used in the experiment. Theflavonoid content in each extract were calculated as mean±SD (n=3) and expressed as mg quercetin equivalents (QE)/ g dry extract (w/w).

Animal preparations

Male Swiss albino mice (20-22 g body weight, 3-4 weeks of age) were procured from for use in this study. All animals were housed in separate polystyrene cages in pathogen-free facilities maintained at 25 (±2) ºC, with 50-60% relative humidity, and 12 h light: dark cycle. All mice had *ad libitum* access to normal laboratory diet as recommended for mice by the National Center for Laboratory Animal Sciences, National Institute of Nutrition, India and filtered tap water. All experiments involving animals were conducted according to the protocols approved by Department of Animal Ethical Committee, under the supervision of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines (Registration No. IAEC/IV/Proposal/BB-2/2014, dated - August 26, 2014), Ministry of Environment and Forest, Government of India. In this study animals were utilized for

experimentation using three different models, *i.e.,* particulate antigen (*i.e.,* SRBC) challenged model, carrageenan-induced acute inflammation, and collagen-induced arthritis models.

DPPH free radical scavenging activity

The radical scavenging activity of each extract was determined in terms of percentage of DPPH scavenging, using ascorbic acid (purchased form MERCK) as a standard to calibratethe standard curve according to the methods by Iran shahi et al. (2009).The solution of DPPH was prepared by dissolving 4.3 mg of DPPH (1, 1-diphenyl –2- picrylhydrazyl) into 1 ml methanol (4.3 mg/ml); it was protected from light by covering the test tubes with aluminum foil. A volume of 150 µl DPPH solution was added to 3ml methanol and absorbance was taken immediately at 517 nm for control reading. A volume of 50 µl of standard ascorbic acid at different concentrations, *i.e.* 10, 25, 50, 75 and 100 µg /ml dissolved into water were mixed with 150 µl of DPPH, and the volume was made uniformly to 3 ml using methanol. On the other hand, 50µl of 10mg of each extract were made up to 3 ml Absorbance was taken after 15 min. at 517 nm using methanol asblank on UV-visible spectrometer Shimadzu, UV-1601, Japan. The IC_{50} values for each drug compounds as well as standard preparation were calculated [20]. The DPPH free radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = [\text{Control}_{Abs} - \text{Sample}_{Abs} / \text{ Control }_{Abs}] \text{ x } 100$$

The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations.

Acute oral toxicity study

The biosafety of the crude extracts used in this study were confirmed in an acute oral toxicity study. The extracts were suspended in sterile normal saline and stock solutions adjusted to ≈ 5-times the final dose that would deliver in a 200 µl gavage. 250, 500, 1000, 2000, 4000, or 8000 mg/kg body weight doses of each extract were administered in mice. According to previousmethod (Oliveira et al., 2008) *i.e.,* $1/10^{th}$ to $1/20^{th}$ of the dose below which no death or morbidity or toxic effects observed was selected as safe for use in the study [21].

RESULTS AND DISCUSSION

A. Qualitative Biochemical Analysis

Preliminary identification of major phytochemical constituents in methanolic extracts of *D.Annulatum* Whole Plant, *S. Bengalense* Whole Plant and *R. Tuberose* Flower

It was observed that phenolics, flavonoids and alkaloids were present in methanolic extracts of all the three plants. Methanolic extracts of *R. Tuberose* and *S. Bengalense* were rich in tannins as observed from the results; whilst saponins and glycosides were found in methanolic extracts of *S. Bengalense* and *R. Tuberose* respectively (**Table. 1**).

Table 1. Preliminary identification of major phytochemical constituents in methanolic extracts of *D.Annulatum* Whole Plant, *S. Bengalense* Whole Plant and *R. Tuberose* Flower

Sl. No.	Phytochemicals	DAW	SBW	RTF
1.	Carbohydrates	+	+	+
2.	Tannins	_	+	+
3.	Saponins	_	+	_

4.	Flavonoids	+	+	+
5.	Alkaloids	+	+	+
6.	Steroids	_	_	_
7.	Phenolics	+	+	+
8.	Glycosides	_	_	+
9.	Proteins	_	_	_

B. Quantitative biochemical analysis

Estimation of total phenolic content (TPC) in methanolic extracts of *D. Annulatum* Whole Plant, *S. Bengalense* Whole Plant and *R. Tuberose* Flower

The TPC were also found in various concentrations in methanolic extracts of *D. Annulatum* Whole Plant, *S. Bengalense* Whole Plant and *R. Tuberose* Flower. Phenolic content was found to be highest in methanolic extract of *S. Bengalense* Whole Plants (90.5±1.5 mg GAE/g dry wt.) in comparison to methanolic extract *D. Annulatum* Whole Plant (76.23±1.23 mg GAE/g dry wt.) and *R. Tuberose*

Flowers (61.4±2.07 mg GAE/g dry wt.) which was significant at $p<0.05$ (**Figure 1**).

Assessment of total

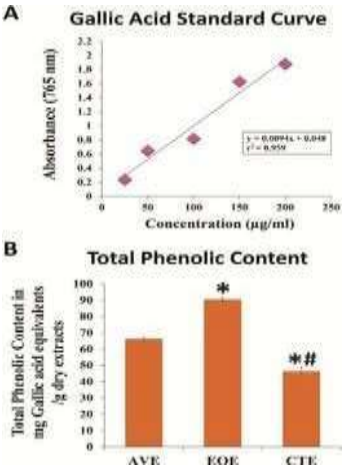

Figure 1. Total phenolic contents (TPC) (1B) in mg gallic acid equivalents (GAE)/g dry weight in different extracts were determined from gallic acid standard curve (1A). The data were shown as mean±SD; (n=3). DAW- methanolic extract of *D. Annulatum* leaf; SBW- methanolic extract of *S. Bengalense* Whole Plant pulps; and RTF- methanolic extract of *R. Tuberose* flower petal. *indicates significantly ($p<0.05$) different compared to DAW; #indicates significantly ($p<0.05$)

Estimation of total flavonoid content (TFC) in methanolic extracts of *D. Annulatum* Whole Plant, *S. Bengalense* Whole Plant and *R. Tuberose* Flower

The TFC in different extracts were found in present in various concentrations. Flavonoid content was found maximum in methanolic extract of *R. Tuberose* Flowers (67.2±0.72 mg

QE/g dry wt.) in comparison to *D. Annulatum* Whole Plant and *S. Bengalense* Whole Plants (significant at $p<0.05$), followed by total flavonoid content in methanolic extract *S. Bengalense* Whole Plants (62±0.83 mg QE/g dry wt.), and then methanolic extract of *D. Annulatum* Whole Plant (29.76±0.54 mg QE/g wt.) dry (**Figure 2**).

Assessment of total flavonoid contents

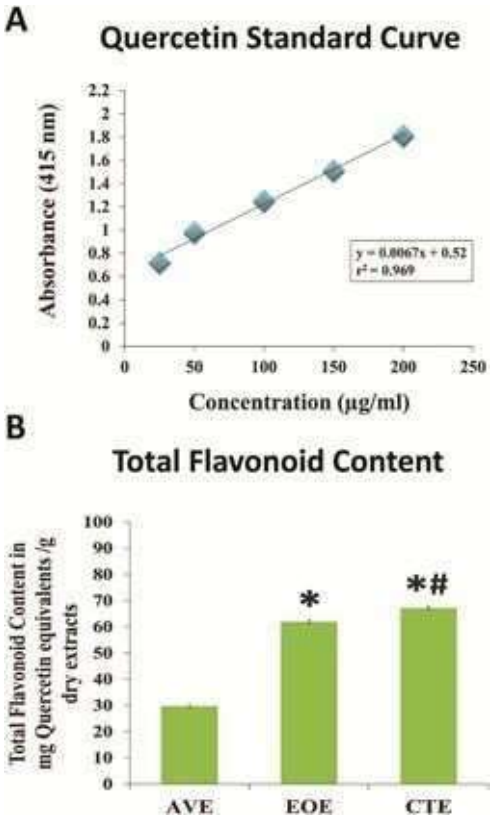

Figure 2. Total flavonoid contents (TFC) (**2B**) in mg quercetin equivalents (QE)/g dry weight indifferent extracts were determined from quercetin standard curve (**2A**). The data were shown as mean±SD; (n=3). DAW- methanolic extract of *D. Annulatum* leaf; SBW- methanolic extract of *S. Bengalense*, RTF- methanolic extract of *R.*
The data were shown as mean±SD; (n=3). DAW- methanolic extract of *D. Annulatum* leaf; SBW- methanolic extract of *S. Bengalense* Whole Plant pulps; and RTF- methanolic extract of *R. Tuberose* flower petal. *Significantly ($p<0.05$) different compared to DAW; #indicates significantly ($p<0.05$)

Estimation of DPPH scavenging activity of the methanolic extracts of *D. Annulatum* Whole Plant, *S. Bengalense* Whole Plant and *R. Tuberose* Flower

DPPH radical is a stable free radical (maximum absorption at 517 nm) which loses absorbance following acceptance of electron or free radical species, which results in a visually noticeable discoloration from purple to yellow. **Figure 3** shows that DPPH scavenging activities of the extracts were different. The methanolic extract of *R. Tuberose* Flowers (RTF) yielded highest

DPPH radical scavenging activity (86.24% inhibition) in comparison to methanolic

Assessment of DPPH scavenging

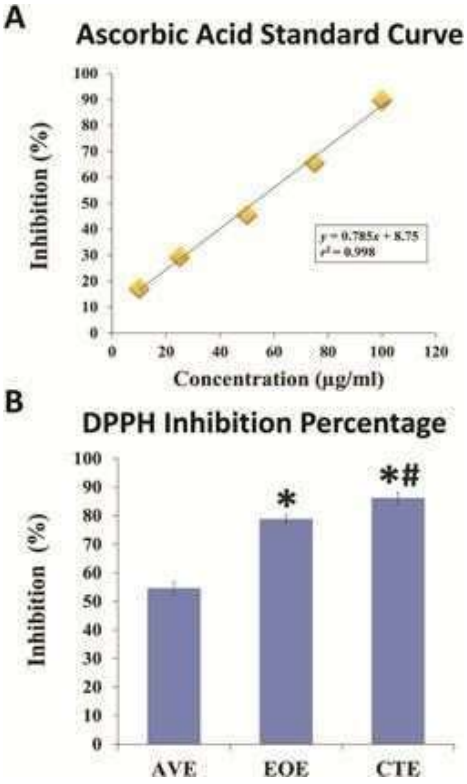

Figure 3. DPPH free radical scavenging activities (**3B**) in percentage inhibition in different extracts were determined from ascorbic acid standard curve (**3A**). The data were shown as mean±SD; (n=3). DAW- methanolic extract of *D. Annulatum* leaf; SBW- methanolic extract of *S. Bengalense* Whole Plant pulps; and RTF- methanolic extract of *R. Tuberose* flower petal. *indicates significantly ($p<0.05$) different compared to DAW; #indicates significantly ($p<0.05$) different extracts of *D. Annulatum* (54.67% inhibition) and *S. Bengalense* Whole Plants (78.87% inhibition).

Acute toxicity study with methanolic extracts of *D. Annulatum* Whole Plant, *S. Bengalense* Whole Plant and *R. Tuberose* Flower

In acute toxicity study with oral administration of graded doses (250, 500, 1000, 2000, 4000, or 8000 mg/kg body wt) of methanolic extracts of *D. Annulatum* Whole Plant, *S. Bengalense* Whole Plant and *R. Tuberose* Flower to mice showed no adverse effects in terms of observable changes in behavior, cutaneous effects, or gastrointestinal effects during the observation period at doses up to 2000 mg/kg for DAW, 4000 mg/kg for SBW and 1000 mg/kg for RTF upto 72 hr (**Table 2**).

Table 2: Assessment of acute oral toxicity

S. N.	Drug treatment	Dose (mg/kg)	Toxicity	Mortality
1.	*Dichanthium Annulatum*	250	No adverse effects	0/6
	Whole Plant extract	500	No adverse effects	0/6
	(DAW)	1000	No adverse effects	0/6
		2000	No adverse effects	0/6
		4000	Increased water intake	0/6
		8000	Increased water intake	0/6
2.	*Saccharum bengalense*	250	No adverse effects	0/6
	Whole Plant extract	500	No adverse effects	0/6
	(SBW)	1000	No adverse effects	0/6
		2000	No adverse effects	0/6
		4000	No adverse effects	0/6
		8000	Increased water intake	0/6
3.	*Ruellia Tuberose*	250	No adverse effects	0/6
	Flower extract	500	No adverse effects	0/6
	(RTF)	1000	No adverse effects	0/6
		2000	Increased water intake	0/6
		4000	Increased water intake	0/6
		8000	Increased water intake	0/6

The results obtained in this study indicated that, phenolics, flavonoids and alkaloids were present as major groups of important secondary metabolites and carbohydrates as important biomolecules in the methanolic extracts of *Dichanthium Annulatum* Whole Plant (DAW), *Saccharum bengalense* Whole Plant (SBW), and *Ruellia Tuberose* Flowers (RTF). Presence of these biomolecules in methanolic, aqueous and other extracts from different parts of these plants were also confirmed previously by other authors after preliminary biochemical tests as was carried out in this study [22]. In the present study quantitative analysis of total contents of the phenolics confirmed that amongst DAW, SBW and RTF; total phenolic content was highest in SBW in comparison to DAW and RTF; which might be due to its citrus nature. Polyphenols or phenolics are biggest group of phytochemicals found in plants, and in many reports on rodents and other animal models' polyphenol rich diets has been suggested to have health related benefits. Polyphenols are plant derived secondary metabolites which functions to protect the host mainly from oxidative damages and are therefore considered as predominant contributors to the total antioxidant activities of Whole Plants, rather than ascorbic acid [23]. It was reported that high intake of Whole Plants and vegetables rich in polyphenols has been

linked to lowered risks of many chronic diseases including cancer, cardiovascular disease, chronic inflammation and many degenerative diseases related to oxidative stress from reactive oxygen and nitrogen species [24].

In the present study it was also observed that total flavonoid content was highest in the *R. Tuberose* Flowers in comparison to other two plant parts, *i.e.. S. Bengalense* Whole Plants and *D. Annulatum* Whole plant. Flavonoids are the largest and most diverse group of phytonutrients as considered to date. In case of all flavonoids the common structural pattern is a heterocyclic ring conjugated with a benzoic ring called the diphenyl propane, and difference in pattern of hydroxylation and glycosylations of the benzoic ring owes to diversification of the flavonoids into many major subclasses, such as flavones, flavonols, flavanones, flavanonols, isoflavones, flavonols or flavin 3-ols (catechins), anthocyanidins and chalcones. Three flavanol glycosides, kaempferol-3-β-glucoside, quecetin-3-β-glucoside, and myricetin-3-β- glucoside were isolated from the petals of *R. Tuberose*, amongst which particularly quercetin-3β- glucosides were identified by NMR studies, and LC/MS studies in highest quantities particularly in methanolic extracts of the *R. Tuberose* Flowers [25].

No death of the animals was observed within this dose regimen up to 72 hours of routine observation for all the three extracts, *e.g.,* DAW, SBW or RTF. Therefore, $1/10^{th}$ to$1/20^{th}$ of these doses, *i.e.,* 200 to 100 mg/kg for DAW, 400 to 200 mg/kg for SBW and 100 to 50 mg/kg for RTF were considered safe. The selected doses for DAW (200, 100 and 50 mg/kg); for SBW (400, 200 and 100 mg/kg); and for RTF (100, 50 and 25 mg/kg) in the present study therefore fall within the safe dose regimen. DPPH accepts electrons or hydrogen atoms from natural antioxidants upon its interaction and therefore this method determines the antiradical power of an antioxidant. Therefore, the highest DPPH scavenging activity of the methanolic extracts of *R. Tuberose* Flowers in comparison to *S. Bengalense* Whole Plants and *D. Annulatum* Whole plant can be positively correlated to its flavonoid contents, more precisely the quercetin glucoside contents.

It concluded that all these three plants *Dichanthium Annulatum* Whole Plant (DAW), *Saccharum Bengalense* Whole Plant (SBW), and *Ruellia Tuberose* Flowers (RTF) are rich source of flavonoids, phenolic contents etc. and found safe in acute oral toxicity profile.

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Nil.

CONFLICT OF INTEREST

None conflict of interest was given by the authors.

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