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STANDARDIZATION AND SOLVENT COMPARATIVE STUDY OF *RUELLIA TUBEROSA L*

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ABSTRACT

Background: Medicinal plants research shows the potential to serve as a niche for novel active ingredients, and plants in particular are regarded as highly valuable in ethno medicine and is sources for the screening of bioactive compounds against various pathological conditions. The study on medicinal plants started with extraction procedure that plays an important critical role to the extraction outcomes. *Ruellia tuberosa L*. (Family: Acanthaceae), known as cracker plant, is traditionally used as diuretic, anti-hypertensive, emetic in gall bladder disease, kidney disorder. **Aim and Objective**: Solvents are important for extracting potential compounds from natural sources, the frequency of solvent used for extraction is portrayed and the results are discussed in this article. This research was designed with the aim of describing and comparing the best solvent used for extraction of *Ruellia tuberosa L*. and standardize and evaluate *Ruellia tuberosa L*. both qualitatively and quantitatively on the basis of organoleptic characters, physical characters and phytochemical screening. **Methods:** *Ruellia tuberosa L*. plant leaves, flowers, roots, seed are collected from erode and it's surrounding area (Tamilnadu). The plant parts were washed, air dried and coarsely powdered. The *Ruellia tuberosa L*. extract was prepared by using maceration and followed by Soxlet extraction process. Different types of solvents are used for extraction. Identified which solvent is best for extraction of *Ruellia tuberosa L*. and organoleptic

characters, physiochemical properties, phytochemical screening was done. **Result:** We plan to determine qualitative and quantitative evaluation of *Ruellia tuberosa L.* using a wide variety of solvents to extract and also plan to find out which solvent is best suited for this extraction process. **Conclusion:** At the end of this research we will find out the organoleptical properties, physiochemical properties and phytochemical constituents of *Ruellia tuberosa L.* This research may be helpful in isolation of specific phytoconstituents and its related medicinal activity studies.

Future studies

1. Isolation of specific constituents
2. In vitro or invivo study

KEYWORDS

Extraction, Maceration, *Ruellia tuberosa L.*, solvents, phytoconstituents.

1. INRODUCTION

Phytochemical screening helps not only to reveal the constituents of the plant extract but also helpful in searching for bioactive agents those can be used in the synthesis of useful drugs. *Ruellia tuberosa L.*, (Acanthaceae) also known as minnieroot, is a short-lived perennial plant with funnel-shaped striking violet bracteate flowers on dichotomous cymes. Fruit is subcylindrical puberulent capsule having more or less 20 seeds per locule, thick fusiform tuberous roots in cluster. In traditional medicine, it has been used as anti-diabetic, anti-inflammatory, antinociceptive, antipyretic, analgesic, antihypertensive, antioxidant, insecticidal, anticancer, and antidotal toxic agents. The plant is reported to contain phytochemicals such as Coumarin, phenolic compounds, Oleic acid, methyl esters, steroids, terpenoids, long-chain aliphatic compounds, and flavonoid etc.[1,2,3]

The leaves extract of *Ruellia tuberosa L* has anti-diuretics and anti-oxidant activity.[4]

Root extract from *Ruellia* has anti-diuretic activity. These include reduce blood glucose levels, reduce malondialdehyde (MDA levels) and repair on the kidney histopathologic profile.[5]

Phytochemical investigation of study revealed that root extracted with n-hexane contained triterpenoid compounds.[6]

Preliminary phytochemical screening of ethyl acetate extract of *Ruellia tuberosa L* reveals presence of saponin, tannins, and flavonoids.[7]

Subramanian and Nair reported that *Ruellia tuberosa L* leaves have only traces of apigenin and luteolin but its flowers contain malvidin-3, 5-diglucoside in appreciable amount.[8]

Also the flower buds have the maximum proportion of flavonoids yielding 3% of agigenin 7-O-glucuronide. Other flavones identified were apigenin-7-O-glucoside, apigenin-7-O-rutinoside and luteolin 7-O-glucoside.[8]

Aerial parts of *Ruellia tuberosa L* yielded 21-methyldammar-22-en-3β,18,27-triol atriterpenoid.[9]

Twenty-five compounds were reported from GC-MS analysis of ethanol extract from tubers of *R.tuberosa*%: lupenol(68.14), stigmasterol(8.89), α-sitosterol(3.99), sucrose(2.24), 3α-bromo-cholest-5-ene(2.24),2-methyl-octadecane(2.10),2-methyl-nonadecane(1.93), hexacosane(1.43), heptacosane (1.29) as its prominent compounds.[10]

In the current work the *R.tuberosa* flower, leaves, roots and seed are extracted using maceration technique, followed by identification and characterization of the resulted extracts. Bioactive compounds such as terpenoids, flavonoids, saponins, tannins are secondary metabolite compounds that are usually dissolve in polar solvents thus in this study a comparative analysis is done to achieve maximum extraction of the plant.

Figure 01: Whole plant of *Ruellia tuberosa L* L.

2. LITERATURE REVIEW

2.1. Vernacularnames of *Ruellia tuberosa L.*[11]

- ✓ **Assamese**: Chatpati
- ✓ **Bangladesh**: potpoti
- ✓ **Marathi**: Ruwel
- ✓ **Sri Lankan**: Heenamukkara
- ✓ **Tamil**: Kiranthinayakam, pattaskai
- ✓ **Telugu**: Jurbulagadda

2.2. TaxonomicHierarchy[12]

- ✓ **Kingdom**: Plantae
- ✓ **Subkingdom**: Viridiplantae
- ✓ **Infrakingdom**: Streptophyta
- ✓ **Super division**: Embryophyta
- ✓ **Class**: Magnoliopsida
- ✓ **Superorder**: Asteranae
- ✓ **Order**: Lamiales
- ✓ **Family**: Acanthaceae
- ✓ **Genus**: *Ruellia L.*
- ✓ **Species**: *Ruellia tuberosa L.*

2.3. Classification and characteristics[12]

- ✓ **Plant division**– Angiosperms (flowering seed plants) (Dicotyledon)
- ✓ **Plant growth form**-Herbaceous plant
- ✓ **Lifespan**-Perennial
- ✓ **Mode of nutrition**-Autotrophic
- ✓ **Plant shape**-Shrubby
- ✓ **Preferred climate zone**-Tropical
- ✓ **Native Habitat**-Terrestrial

2.4. Description and ethnobotany

- ✓ **Growth form**-Perennial herb with erect stems up to 45cm tall
- ✓ **Roots**-The tuberous roots are thick and cylindrical finger –like

- ✓ **Foliage**-Leaves are oblong obovate and hairless on both sides (4-8cm long, 1.5-4.2cm wide). Leaf margin undulate to approximately entire (smooth edges). Leaves are arranged oppositely along the stem.
- ✓ **Stems**- Stems are 4 sided and hairy.
- ✓ **Flowers**-Pale blue to purple, trumpet shaped flowers are 2.2 to 5.5cm wide with 5 approximately round overlapping lobes (1.6cm long, 1.5cm wide)
- ✓ **Friuts**- Dry dehiscent fruits are known as capsules (1.8-2.5cm long, 0.3-0.4cm wide). Dark brown cylindrical pods are pointed at the tip and hairless except for glandular trichome at the tip. When a mature pod becomes wet, it opens with explosive dehiscence, catapulting the 7-8 disk shaped seeds into the air.
- ✓ **Habitat**-Found in disturbed sites, waste areas.

2.5. Ethnoclaim uses

1. Leaf

Leaf decoction is used in treatment of bronchitis (De Fillpps et al., 2004; Agnihotri et al., 2012; Samy et al., 2015).

Leaf extracts of *R. Tuberosa L* were reported to control lipid peroxide level and help strengthen antioxidant potential in diabetic rats (Manikandanu et al., 2010).

2. Root

Tuberous roots are ingredients in health tonic (De Fillpps et al., 2004; Samy et a., 2015) Root extract displayed anti-oxidant activity which were comparable with standards (chothani and Mishra, 2012).

3. AIM AND OBJECTIVE

3.1. Aim

The aim of the present research is to study the pharmacognostical and phytochemical evaluation of *Ruellia tuberose Linn* (Acanthaceae).

3.2. Objective

The present work has been planned to carry out

PART-A

PHARMACOGNOSTICAL STUDIES
1. Pharmacognostical studies-implicates 1. Authentication and collection of plants 2. Macroscopy of the leaf 3. Behavioural characters with different reagents.

PHYSIO-CHEMICAL PARAMETERS
2. Physio-chemical parameters 1. Foreign matter 2. Loss on drying 3. Ash value

PART-B

PHYTOCHEMICAL STUDIES
1.Preparation extract ✓ Ethanolic extract ✓ Methanolic extract ✓ Acetone extract ✓ Chloroform extract
2.Quantitative analysis-preliminary phytochemical screening

4. MATERIALS AND METHODS

4.1. Chemicals used

- Ethanol
- Acetone
- Petroleum ether
- Methanol
- Chloroform
- Distilled water

4.2. Part- A

4.2.1. Plant collection and authentication

Fresh leaves, flower, root and seeds of *Ruellia tuberosa L* were collected from Erode district, Tamilnadu, during the month of march and was authenticated by Dr. M.U. Sharief, scientist 'E' and Head of Office, Botanical survey of India, Southern region, T.N.A.U Campus, Coimbatore-641 003, Tamilnadu. Authentication: BSI/SRC/5/23/2022/Tech.

4.2.2. Plant sample processing

1. Preparation of leaf powder

The leaves were collected and shade dried at ambient temperature for 5-6 days. It was powdered in a mixer. The powder was sieved in sieve number 60 and kept in a well closed container in a dry place as per standard guidelines.

2. Preparation of root powder

The roots were collected and shade dried at ambient temperature for about 2 weeks . It was powdered in a mixer. The powder was sieved in sieve number 60 and kept in a well closed container in a dry place as per standard guidelines.

3. Preparation of flower powder

The flowers were collected and shade dried at ambient temperature for 5 days. It was powdered in a mixer. The powder was sieved in sieve number 60 and kept in a well closed container as per standard guidelines.

4. Preparation of seed powder

The seeds were exposed from the seed coat and collected for shade drying. It was powdered in a mixer. The powder was sieved in sieve number 60 and kept in a well closed container as per standard guidelines.

4.2.3. Pharmacognostical studies

Morphological and micro morphological examination and characterization of medicinal plants have always been accorded due credentials in the pharmacognostical studies. Botanical identity of the plants is an essential prerequisite for undertaking the analysis of medicinal properties of any plant. A researcher may succeed in getting a new compound or may find many useful pharmacologically active properties in a plant. If the botanical identity of the plant happens to be erratic, the entire work on the plant becomes invalid. Thus it is needless to stress the botanical identity of the crude drug is the threshold in the processes of pharmacological investigations. The researcher should be equipped with all the possible diagnostic parameters of the plant on which the researcher plan to work.

4.2.4. Morphological studies of *Ruellia tuberosa L*

Leaf, flower, root and seed were studied individually for its morphological characters by organoleptic test.

4.2.5. Behavioural characters of the *Ruellia tuberosa L* (Leaf- crude powder)[13]

Dried powders of leaves, flowers seeds and roots were separately treated with different reagents reveals the presence of chromophoric compounds in them.

Procedure	Reagents used
The powder is treated with	Acetone
	Iodine solution
	Ammonia
	Ethanol
	Ferric chloride
	Acetic acid
	Chloroform
	Distilled water

4.2.6. Physiochemical parameters[13]

The powder was subjected to physiochemical parameters such as foreign organic matter, loss on drying, ash values and extractive values with different solvents in increasing order of polarity. The procedure was adapted as per WHO guidelines 1996, 1998, 2001 and James 1995.

1. Determination of foreign organic matter

Procedure

An accurately weighed 100g of air dried coarse drug was spread out in a thin layer. The sample drug was inspected with the unaided eye or with the use of 6x lens and the foreign organic matter was separated manually as completely as possible and weighed. The percentage of foreign organic matter was calculated with reference to the weight of the drug taken.

% Foreign matter = Initial weight – Final Weight/Initial weight×100

2. Determination of moisture content (loss on drying)

Procedure

An accurately weighed 10 g of coarsely powdered drug was placed in a tarred evaporating dish. Then the dish was dried at 105°C for 5 h and weighed. The drying and weighing was continued at one hour intervals until the difference between the two successive weighing is not more than 0.25 %. The loss on drying was calculated with reference to the amount of powder taken.

Loss on Drying = weight of water in the sample/weight of wet sample×100

3. Determination of ash value

Ash value

The residue remaining after incineration is the ash content of the drug. The ash value is criteria to judge the identity or purity of the crude drug. A high ash value is indication of contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing.

- ✓ Physiological ash – It is derived from plant tissue itself.
- ✓ Non physiological ash – It is the residue of the adhering material to the plant. Eg. soil and sand.

Ash content

The residue remaining after incineration is the ash content of crude drug, which simply represents inorganic salts naturally occurring in the drug or adhering to it or deliberately added to it as a form of adulteration.

Determination of total ash

Procedure

An accurately weighed 3 g of air dried coarsely powdered drug was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450° C, until free from carbon then allowed to cool and weighed. The percentage of ash was calculated with reference to the air dried drug.

$$\% \text{ Total Ash} = \text{weight of ash/weight of sample} \times 100$$

Determination of acid insoluble ash

Procedure

The total ash obtained from the previous procedure was mixed with 25 mL of 2 M hydrochloric acid and boiled for 5 min in a water bath, and then the insoluble matter was collected in an ash less filter paper and washed with hot water, dried and ignited for 15 min at a temperature not exceeding 450° C, cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

$$\% \text{ of Acid insoluble ash} = \text{weight of acid insoluble ash/weight of sample} \times 100$$

Determination of water-soluble ash

Procedure

The total ash obtained from the previous procedure was mixed with 25 mL of water and boiled for 5 min in a water bath, and then the insoluble matter was collected in an ash less filter paper and washed with hot water, dried and ignited for 15 min at a temperature not exceeding 450° C, cooled in desiccators and weighed. The insoluble matter was subtracted from the weight of the total ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air dried drug.

5. Part - B

5.1. Maceration technique[13]

This is an extraction procedure in which coarsely powdered drug material, either leaves or stem bark or root bark is placed inside a container; the menstruum is poured on the top until completely covered the drug material. The container is then closed and kept for at least three days. The content is stirred periodically and if placed inside bottle it should be shaken time to time to ensure complete extraction. At the end of extraction, the micelle is separated from the marc by filtration or decantation. Subsequently the micelle is separated from the menstruum by evaporation in an oven or top of water bath. This method is convenient and very suitable for thermolabile plant material. The terms involved in maceration -marc, menstruum.

Marc- undissolved solid residue

Menstruum- solvent used for extraction

Types of menstruum- polar and non-polar

- ✓ **Polar**: solvents with dielectric constant above 15. Eg : water, methanol, acetone
- ✓ **Non polar**: solvents with dielectric constant less than 15 are generally non polar.

Eg: chloroform, hexane, benzene.

Types of maceration process

- ✓ **Simple maceration**: A process for tinctures made from organised drug Eg. Roots, stems, leaves etc
- ✓ **Maceration with adjustments**: A process for tinctures made from unorganised drugs such resins
- ✓ **Double maceration and triple maceration process**: For concentrated preparations.

5.2. Preparation of extracts of *Ruellia tuberosa L*,

Shade dried and powdered *Ruellia tuberosa L*

(Leaf, flower, root, seed)

Defatted using Petroleum ether (60-80^{o}C)

Residue is dried and extracted with
(Ethanol, methanol, Acetone, Chloroform)

By Maceration extraction Process

Filtered the macerated content

Extract was concentrated under reduced pressure to obtain a solid residue (dark green).

5.3. Leaf

1. Ethanolic extract

The shade dried and coarsely powdered leaf of*Ruellia tuberosa L* (Leaf) was defatted with petroleum ether (60-80^{o}C). The residue was dried and extracted with Ethanol (70%) by Maceration extraction until the complete extract of the material is obtained and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

2. Methanolic extract

The shade dried and coarsely powdered leaf of *Ruellia tuberosa L* (Leaf) was defatted with petroleum ether (60-80^{o}C). The residue was dried and extracted with Methanol by Maceration extraction until the complete extract of the material is obtained and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

3. Acetone extract

The shade dried and coarsely powdered leaf of *Ruellia tuberosa L*. (Leaf) was defatted with petroleum ether (60-80^{o}C). The residue was dried and extracted with Acetone by Maceration extraction until the complete extract of the material is obtained and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

4. Chloroform extract

The shade dried and coarsely powdered leaf of *Ruellia tuberosa L* (Leaf) was defatted with petroleum ether (60-80^{o}C). The residue was dried and extracted with Chloroform by Maceration extraction until the complete extract of the material is obtained and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

5.4. Root

1. Ethanolic extract

The shade dried and coarsely powdered Root of *Ruellia tuberosa L* (root) was defatted with petroleum ether (60-80^{o}C). The residue was dried for 2 weeks and extracted with Ethanol by Maceration followed by soxlet extraction until the complete extract of the material and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

2. Methanolic extract

The shade dried and coarsely powdered Root of *Ruellia tuberosa L* (root) was defatted with petroleum ether (60-80^{o}C). The residue was dried for 2 weeks and extracted with Methanol by Maceration extraction until the complete extract of the material is obtained and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

3. Acetone extract

The shade dried and coarsely powdered Root of *Ruellia tuberosa L*. (root) was defatted with petroleum ether (60-80^{o}C). The residue was dried for 2 weeks and extracted with Acetone by Maceration extraction until the complete extract of the material is obtained and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

4. Chloroform extract

The shade dried and coarsely powdered Root of *Ruellia tuberosa L*. (root) was defatted with petroleum ether (60-80^{o}C). The residue was dried for 2 weeks and extracted with Chloroform by Maceration extraction until the complete extract of the material is obtained and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

5.5. Seed

1. Ethanolic extract

The shade dried and coarsely powdered seed of *Ruellia tuberosa L.* (seed) was defatted with petroleum ether (60-80^{o}C). The residue was dried for 2 weeks and extracted with Ethanol by Maceration followed by soxhlet extraction until the complete extract of the material and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

2. Methanolic extract

The shade dried and coarsely powdered seed of *Ruellia tuberosa L.* (seed) was defatted with petroleum ether (60-80^{o}C). The residue was dried for 2 weeks and extracted with Methanol by Maceration extraction until the complete extract of the material is obtained and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

3. Acetone extract

The shade dried and coarsely powdered seed of *Ruellia tuberosa L.* (seed) was defatted with petroleum ether (60-80^{o}C). The residue was dried for 2 weeks and extracted with Acetone by Maceration extraction until the complete extract of the material is obtained and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

4. Chloroform extract

The shade dried and coarsely powdered seed of *Ruellia tuberosa L.* (seed) was defatted with petroleum ether (60-80^{o}C). The residue was dried for 2 weeks and extracted with Chloroform by Maceration extraction until the complete extract of the material is obtained and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

5.6. Flower

1. Ethanolic extract

The shade dried and coarsely powdered flower of *Ruellia tuberosa L* (flower) was defatted with petroleum ether (60-80^{o}C). The residue was dried for 2 weeks and extracted with Ethanol by Maceration followed by soxhlet extraction until the complete extract of the material and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

2. Methanolic extract

The shade dried and coarsely powdered flower of Ruellia tuberosa L. (flower) was defatted with petroleum ether (60-80^{o}C). The residue was dried for 2 weeks and extracted with Methanol by Maceration extraction until the complete extract of the material is obtained and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

3. Acetone extract

The shade dried and coarsely powdered flower of *Ruellia tuberosa L.* (flower) was defatted with petroleum ether (60-80^{o}C). The residue was dried for 2 weeks and extracted with Acetone by Maceration extraction until the complete extract of the material is obtained and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

4. Chloroform extract

The shade dried and coarsely powdered flower of *Ruellia tuberosa L* (flower) was defatted with petroleum ether (60-80^{o}C). The residue was dried for 2 weeks and extracted with Chloroform by Maceration extraction until the complete extract of the material is obtained and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

6. PHYTOCHEMICAL STUDIES[13]

The extract was subjected to qualitative analysis. Qualitative analysis includes phytochemical screening of secondary metabolites such as Alkaloids, Aminoacids, Flavonoids, steroids, Glycosides, Saponins, Fatty acids and extract were determined.

6.1. Test for Alkaloids

EXPERIMENT	OBSERVATION	INFERENCE
✓ 2gm of the powdered material + 1gm of calcium hydroxide & 5ml water → made into smooth paste and set aside for 5 minutes. ✓ It was then evaporated to dryness in a porcelain dish on a water bath. ✓ To this 200ml of chloroform added and mixed well refluxed for half an hour on a water bath. ✓ Then it was filtered and chloroform was evaporated. ✓ To this 5ml of dilute hydrochloric acid added with following reagents.		
1.Mayer's reagent A small quantity of the extract + a few drops of Mayer's reagent	Cream colour precipitate	Presence of alkaloids

2.Dragendorff's test A small quantity of the extract + few drops of Dragendorff's reagent	Orange brown precipitate	Presence of alkaloids
3.Wagner's test A small quantity of extract + few drops of Wagner's reagent	Reddish brown precipitate	Presence of alkaloids
4.Hager's test A small quantity of extract + few drops of Hagner's reagent	Yellow precipitate	Presence of alkaloids

6.2. Test for Indole

EXPERIMENT	OBSERVATION	INFERENCE
Test solution + acetic acid and trace amount of anhydrous $FeCl_3$ under lay/ H_2SO_4	Intense blue at interface	Presence of indole

6.3. Test for Quinoline (Thalleioquin test)

EXPERIMENT	OBSERVATION	INFERENCE
✓ Extract + 1 drop of dilute sulphuric acid & 1ml of water. ✓ Add bromine water drop wise till the solution acquires permanent yellow colour + 1ml dilute ammonia, emerald green colour is produced. ✓ The powdered drug when heated with acetic acid in dry test tube, evolves red fumes, which condense in the top portion of the tube. ✓ The bark, when moistened with sulphuric acid and observed under ultraviolent light.	A blue fluorescence due to the methoxy group of quinine and quinidine	Presence of quinine and quinidine

6.4. Test for Sterols

EXPERIMENT	OBSERVATION	INFERENCE
1.Salkowski's test Few drops of concentrated sulphuric acid + extract of powdered leaf →shaken well and set aside.	Lower chloroform layer of the solution turned red in colour	Presence of sterols
2.Libbermann –Burchard's Chloroform solution + few drops of anhydrous acetic acid + 1ml of concentrated sulphuric acid added to the sides of the test tube and set aside for a while.	At the junction of the two layers a brown ring was formed. The upper layer turned green.	Presence of sterols

6.5. Test for Saponins

EXPERIMENT	OBSERVATION	INFERENCE
1.Froth test 0.1gm of powder + 5ml of distilled water →vigorously shaken in a test tube for 30 seconds & left undisturbed for 20 minutes.	Persistent forth.	Presence of saponins

6.7. Test for Acid

EXPERIMENT	OBSERVATION	INFERENCE
Small quantity of test solution + few drops of concentrated sulphuric acid.	Yellow orange.	Presence of flavonoids.

6.8. Test for Tannins

EXPERIMENT	OBSERVATION	INFERENCE
✓ Powdered drug small quantity extracted with water. ✓ Aqueous extract + few drops of ferric chloride solution.	Bluish black colour	Presence of tannins

6.9. Test for Flavonoids

EXPERIMENT	OBSERVATION	INFERENCE
1.Shinoda's test ✓ Little of powdered drug+alcohol →heated and filtered. ✓ Test solution + magnesium turnings and few drops of concentrated hydrochloric acid. ✓ Boiled for five minutes.	Red colour	Presence of flavonoids
2.Alkali test Small quantity of test solution + 10% aqueous sodium hydrochloride	Yellow orange colour	Presence of flavonoids

6.10. Test for Terpenoids

EXPERIMENT	OBSERVATION	INFERENCE
✓ Little of powdered drug extracted with chloroform and filtered. ✓ Filtrate was warmed gently with tin and thionylchloride	Pink colour solution	Presence of terpenoids

6.11. Test for Glycosides

EXPERIMENT	OBSERVATION	INFERENCE
1.Liebermann's test 1ml of extract + 1ml of chloroform + 1ml acetic acid	Violet to blue colour and then to green colour	Presence of glycosides

6.12. Test for Anthraquinones

EXPERIMENT	OBSERVATION	INFERENCE
1.Borntrager's test ✓ Powdered drug boiled with sulphuric acid →filtered. ✓ Filtrate + benzene → shaken well. ✓ Organic layer separated → added with ammonia solution slowly.	Red colouration at the ammoniacal layer	Presence of anthraquinones
2.Modified Borntrager's test ✓ 0.1gm of powdered drug → boiled for 2 minutes with dilute HCl + few drops of $FeCl_3$ solution → filtered while hot and cooled. ✓ Filtrate extracted with benzene and benzene layer was separated and added with equal amount of NH_3.	No pink colour	Presence of anthraquinone glycosides

7. RESULT AND DISCUSSION

7.1. Macroscopical studies of *Ruellia tuberosa L*

The leaves and root, seed, flower of *Ruellia tuberosa L* was subjected to macroscopical studies and the results are presented in table 01 and 02,03,04.

Figure 02: Whole plant of *Ruellia tuberosa L* L.

Figure 03: Seed with leaf of *Ruellia tuberosa L* L.

Figure 04: Flower of *Ruellia tuberosa L.*

Figure 05: Root of *Ruellia tuberosa L* L.

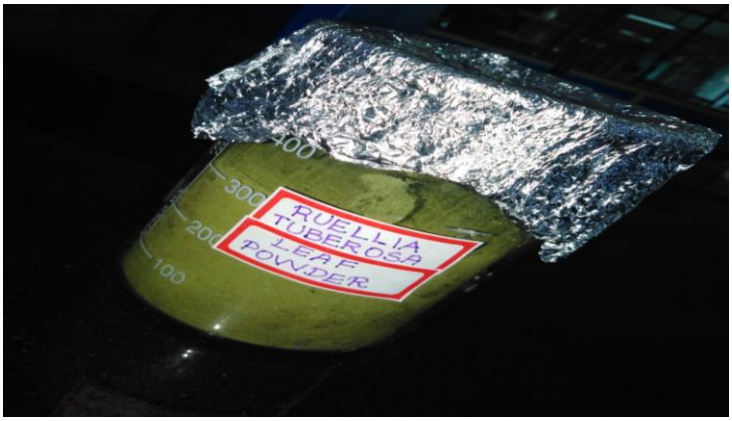

Figure 06: Leaf powder of *Ruellia tuberosa L.*

Figure 07: Extraction of *Ruellia tuberosa L.*

Table 01: Macroscopical studies of *Ruellia tuberosa L* (Leaf).

S.No	Parameters	Observation
1	Colour	Dark Green
2	Apex	Obtuse
3	Base	Attenuate
4	Arrangement	Opposite
5	Margin	Undulate
6	Shape	Elliptic
7	Length	12cm
8	Leaf blades length	4-6×1.5-2.5cm
9	Petiole	Ovate to oblong
10	Petiole length	1.5cm

Table 02: Macroscopical studies of *Ruellia tuberosa L* (Flower).

S.No	Parameters	Observation
1	Colour	Mauve to bluish purple
2	Lobes	Filiform
3	Margin	Undulate
4	Calyx	17-32mm long
5	Length	3-7mm long
6	Pedicels	10-20mm long

Table 03: Macroscopical studies of *Ruellia tuberosa L* (Seed).

S.No	Parameters	Observation
1	Colour	Brown
2	Capsules	20 seeds
3	Shape	Orbicular
4	Length	2.5-2mm

Table 04: Macroscopical studies of *Ruellia tuberosa L* (Root).

S.No	Parameters	Observation
1	Colour	Off white to rusty brown
2	Shape	Cylindrical
3	Length	2.5cm to 8cm
4	Thickness	0.2 – 0.4cm
5	Root type	Elongated, slender, fusifor, tuberous.

7.2. Determination of physic chemical properties of *Ruellia tuberose L.*

Table 05: Physico-chemical evaluation of *Ruellia tuberose L.*

Physicochemical Parameters	Results in Percentage			
	Leaf extract	Flower extract	Root extract	Seed extract
Foreign matter	0.42%	0. 38%	0.65%	0.51%
Loss on drying	11.53%	11.54 %	11.22%	12.41%
Total ash	11.50%	11.60%	12.12%	11.54%
Water soluble ash	7.01%	6.69%	3.25%	3.30%
Acid insoluble ash	2.41%	2.01%	2.30%	2.12%

7.3. Determination of behavioural characteristics of *Ruellia tuberosa L* (leaf).

Table 06: Behavioural Characteristics of *Ruellia tuberosa L* (leaf).

S. No	Reagent used	Visible/ daylight	Short UV (254nm)	Long UV (366nm)
1	Acetone	Greyish	Bluish grey	Faint greyish
2	Iodine solution	Yellow	No colour	No colour
3	Ethanol	No colour	White	Creamy white
4	Distilled water	No colour	White	White

7.4. Preliminary phytochemical analysis of extracts of *Ruellia tuberosa L.*

Table 07: Preliminary phytochemical analysis of different extracts of *Ruellia tuberosa L.*

Constituents	Test	Parts	C_2H_5OH	CH_3OH	$CHCl_3$	C_3H_6O
Saponins	Froth test	Leaf	+	+	+	+
		Flower	+	+	+	+
		Root	+	+	+	+
		Seed	-	-	-	-
Alkaloids	Mayer's test	Leaf	+	+	+	+
		Flower	+	+	+	+
		Root	+	+	+	+
		Seed	+	+	+	+
	Wagner's test	Leaf	+	-	+	+
		Flower	+	+	+	+
		Root	+	+	-	+
		Seed	+	+	+	+
Quinoline	Thalleioquin Test	Leaf	+	+	+	+
		Flower	+	+	-	-

		Root	+	+	-	-
		Seed	-	+	-	-
Sterols	Salkowski's test	Leaf	+	+	+	+
		Flower	+	+	+	+
		Root	+	+	+	+
		Seed	-	+	+	+
Tannins	Ferric chloride	Leaf	+	+	+	+
		Flower	+	+	+	+
		Root	+	+	+	+
		Seed	+	-	-	-
Phenolic compounds		Leaf	+	-	+	+
		Flower	+	-	+	+
		Root	+	-	-	-
		Seed	+	+	+	-
Flavonoids	Shinoda's test	Leaf	+	+	+	-
		Flower	+	+	-	+
		Root	+	+	+	+
		Seed	+	-	+	-
Glycosides	Liebermann's test	Leaf	+	+	+	+
		Flower	+	+	+	+
		Root	+	+	+	+
		Seed	-	-	-	+

+ Phytoconstituent is present - Phytoconstituent is absent

8. CONCLUSION

The use of medicinal plants is a traditional practice in India thus, it is very important to evaluate the therapeutic use of plants through scientific methods and provide information about the species that could be used in the future for their useful medicinal properties.

The present study has showed that different solvent extracts of *Ruellia tuberosa L* leaves, flower, roots and seeds contained alkaloids, carbohydrates, flavonoids, glycosides , Phenol, Saponins, Steroids, Sterols. It also shows the organoleptic character, Physiochemical and physical properties of *Ruellia tuberosa L* leaf, flower, seed and roots.

We compared the solvent extracts of leaf, flower, root and seeds of *Ruellia tuberosa L*, in that ethanolic extract is better, because more amount of phytoconstituents are identified in ethanol extract.

Further studies are to be carried out with Isolation of specific constituents and invivo or invitro activity.

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