EXPLORING THE PHARMACOGNOSTIC CHARACTERISTIC AND ANTIOXIDANT ACTIVITY OF PLANT RIVEA ORNATA Roxb.

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ABSTRACT

Although herbal drugs are known to elicit their response quite slowly but they are now overtaking allopathic medicine due to its high cost, drug resistance, side effect and development of tolerance. Here in this study some light on obscured herbal drug named *Rivea ornata* Roxb. For its pharmacognostical study and antioxidant activity.

KEYWORDS: *Rivea ornata* Roxb, pharmacognostical study, antioxidant, side effect, high cost.

INTRODUCTION

Medicinal plants can be cultivated within a home or community garden, and many grow wildly.[1,2] Some vegetables and fruits like berries, grapes, walnuts, olives, and foods like chocolate, wine, coffee and tea and popcorn, and some breakfast cereals contain large amounts of healthful antioxidant substances called polyphenols.

Hyper physiological burden of free radical causes imbalance in homeostatic phenomenon between oxidants and antioxidants in the body. The imbalance leads to oxidative stress that is being suggested as the root cause of aging and various human diseases like arteriosclerosis, stroke, diabetes, cancer and neurodegenerative diseases such as Alzheimer’s and Parkinsonism. Therefore research in recent past have accumulated enormous evidence advocating enrichment of body system with antioxidants to correct vitiated homeostasis and prevent onset as well as treat the disease caused due to free radical and related oxidative stress. Stress, smoking, drugs & diet generates excessive free radicals in human body.
Antioxidants have defined as the substance those in small quantities, able to prevent or greatly retard oxidation of easily oxidisable material such as fats. Antioxidants may exert their effect by different mechanisms such as suppressing the formation of active species by reducing hyperoxides (ROO) and H$_2$O$_2$ and also by sequestering metal ions scavenging active free radicals, repair and/or clearing damage.

MATERIAL AND METHOD

Authentication and Collection of plant
Dry plants of Rivea ornata were collected from the botanical garden of S.V.University, Tirupati in the month of May 2011 and its authentication was confirmed by Botanist, Dr. Madhava Chetty, S.V. University Tirupati. Herbarium of the plant has been deposited at Department of Pharmacognosy, B. Pharmacy College, Rampura, Kakanpura, Dist. Panchmahal, Gujarat, India for future reference.

Preparation of samples
Aerial parts of plant were used for Pharmacognostical studies. Aerial parts were collected, dried and powdered to 60# separately and stored in airtight containers and used for phytochemical and pharmacological studies.

Pharmacognostical studies

Macroscopical study$^{[3,4]}$
Macroscopic evaluation of the dry plant of Rivea ornata were studied and identified by comparing their morphological characters mentioned in the literature.

Microscopical study$^{[5,6]}$

a) Transverse sections of leaf and petiole of plant Rivea ornata
Microscopic evaluation of the leaf was carried out and the transverse section of the leaf and petiole were taken and various parts of it were observed under the microscope.

b) Surface preparation of leaf of Rivea ornata
Stomata, trichomes and epidermal cells are important identifying characteristics of leaf drug. In transverse sections their exact nature can’t be studied hence exposure of surface/epidermis becomes must for the detail microscopy studies. Type of stomata present, nature of epidermal cell wall, type of trichomes and their details can be studied only after exposing the epidermis.
This technique has significance in the determination of leaf constants, identification of crude drug and detection of adulterants.

c) Powder study of the herb *Rivea ornata*. Powder study of plant should be taken which is shown in fig 4.

**Quantitative microscopy of leaf**:\[7,8]\n
Quantitative microscopy of leaf of *Rivea ornata* was done and stomatal index, vein-islet number, vein termination number and palisade ratio were determined which are given in (table 1)

**a) Determination of stomatal number**

- Cleared the piece of the leaf (middle part) by boiling with chloral hydrate solution or alternatively with chlorinated soda. Peeled out upper and lower epidermis separately by means of forceps. Kept it on slide and mounted in glycerin water.
- Arranged a camera lucida and drawing board for making the drawings to scale and drew a square of 1 mm by means of stage micrometer.
- Put the slide with cleared leaf (epidermis) on the stage. Traced the epidermal cells and stomata.
- Counted the number of stomata present in the area of 1 sq. mm. Include the cell if at least half of its area lies within the square.
- Found out the result for each of the ten fields and calculated the average number of stomata per sq. mm.

Stomatal number is affected by various factors like age of the plant, size of the leaf, environmental conditions etc. Stomatal index is not much affected by these factors. It is relatively constant. Hence it is more significant in the evaluation of a leaf drug.

**b) Determination of stomatal index**

- Cleared the piece of the leaf (middle part) by boiling with chloral hydrate solution or alternatively with chlorinated soda.
- Peeled out upper and lower epidermis separately by means of forceps. Kept it on slide and mounted in glycerin water.
- Arranged a camera lucida and drawing board for making the drawings to scale and drew a square of 1 mm by means of stage micrometer.
- Put the slide with cleared leaf (epidermis) on the stage. Traced the epidermal cells and stomata.
- Counted the number of stomata, also the number of epidermal cells in each field.
- Calculated the stomatal index using the above formula.
- Found out the values for upper and lower surface (epidermis) separately.

c) **Determination of palisade ratio**
- Cleared the piece of the leaf by boiling with chloral hydrate solution.
- Arranged the camera lucida and drawing board for making drawings.
- Using the 4 mm objective, traced off the outlines of four cells of the epidermis.
- Then, focused down to palisade layer and traced off sufficient cells to cover the tracings of the epidermal cells. Completed the outlines of those palisade cells, which were intersected by the epidermal walls.
- Counted the palisade cells under the four epidermal cells. (Included the palisade cell in the count when more than half was within the area of epidermal cells and excluded it when less than half was within the area of epidermal cells.)
- Calculated the average number of cells beneath a single epidermal cell, this figure was the “palisade ratio”.
- Repeated the determination for five groups of four epidermal cells from different parts of the leaf and found the average of the results for the five groups. This average was the “palisade ratio” of the leaf.

d) **Determination of vein-islet number**
- Cleared the piece of the leaf by boiling with chloral hydrate solution for about thirty minutes.
- Arranged the camera lucida and drawing board for making drawings to scale.
- Put stage micrometer on the microscope and using 16 mm objective, drew a line equivalent to 1 mm as seen through the microscope and constructed a square on this line.
- Moved the paper so that the square was seen in the eyepiece, in the centre of the field.
- Put the slide with the cleared leaf (epidermis on the stage)
- Traced off the veins, which were included within the square, completing the outlines of those islets, which overlapped two adjacent sides of the square.
- Counted the number of vein islets in the square millimeter. Where the islets were intersected by the sides of the square, included those on two adjacent sides and excluded those islets on the other sides. (To obtain a critical result for a leaf, 4 sq. mm. should be used, preferably in one large area of 4 sq. mm)
- Found the average number of vein islets from the four adjoining squares, to get the value for one sq. mm.

e) **Determination of vein-let termination number**
- Cleared the piece of the leaf by boiling with chloral hydrate solution for about thirty minutes.
- Arranged the camera lucida and drawing board for making drawings to scale.
- Put stage micrometer on the microscope and using 16 mm objective, drew a line equivalent to 1 mm as seen through the microscope.
- Constructed a square on this line.
- Moved the paper so that the square was seen in the eye piece, in the centre of the field.
- Put the slide with the cleared leaf (epidermis on the stage).
- Traced off the veins, which were included within the square, completing the outlines of those islets, which overlapped two adjacent sides of the square.
- Counted the number of vein-let terminations present within the square.
- Found the average number of vein-let termination number from the four adjoining squares to get the value for one sq. mm.

**Proximate analysis.**[9-14]
Proximate analysis aids to set up certain standard for dried crude drugs in order to avoid batch-to-batch variation and also to judge their quality. Their studies also give an idea regarding the nature of phytoconstituents present.(Table 2)

Proximate analysis of these crude drug powders was carried out using methods prescribed in the ayurvedic pharmacopoeia of India by subjecting them to various determinations like:

I. Total Ash
II. Acid insoluble ash
III. Water soluble ash
IV. Alcohol soluble extractive value
V. Water soluble extractive value
VI. Loss of moisture content

**Determination of ash value**

Ash values of powder of aerial part of *Rivea ornata* Roxb. were determined by the following method:

(a) **Determination of total ash**

2 gm of accurately weighed powder was incinerated in a crucible at a temperature 500-600°C in a muffle furnace till carbon free ash was obtained. It was then cooled, weighed and percentage of ash was calculated with reference to the air-dried drug.

(b) **Determination of acid insoluble ash:**

The total ash obtained above was boiled for 5 min with 25 ml of 2M hydrochloric acid and filtered using an ash less filter paper to collect insoluble matter. The ash obtained was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid-insoluble ash was calculated with reference to the air-dried powdered drug (60#).

(c) **Determination of water soluble ash:**

The total ash was boiled for 5 min with 25 ml of water and insoluble matter collected on an ash-less filter paper washed with hot water and ignited for 15 min at a temperature not exceeded 450 °C in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried powdered drug.

**Determination of extractive values:**

Extractive values of powder of aerial parts of *Rivea ornata* was determined by the following methods:

(a) **Determination of alcohol soluble extractive value**

4g of the air-dried powdered material were macerated with 100 ml of alcohol in a closed flask for 24 h, shaking frequently at an interval of 6 h. It was then allowed to stand for 18 h and filtered rapidly to prevent any loss during evaporation. 25 ml of the filtrate was evaporated to dryness in a porcelain dish and dried at 105°C to a constant weight. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.
(b) Determination of water soluble extractive value:
4g of the air-dried powdered material were soaked in 100ml of water in a closed flask for 1h with frequently shaking. It was then boiled gently for 1 h on water bath; cooled and weighed and readjusted the weight. 25 ml of the filtrate was evaporated to dryness in a porcelain dish and dried at 105˚C to a constant weight. The percentage of water-soluble extractive was calculated with reference to the air-dried powered drug (60#).

Determination of moisture content
Placed about 100gm aerial part of Rivea ornata Roxb. after accurately weighing in a tarred evaporating dish. After placing the above said amount of the drug in the tarred evaporating dish, dried at 105ºC for 5 hours, and weighed. Continued the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25%. Constant weight was reached when two consecutive weighing after drying for 50mins. and cooling for 30 minutes in a desiccator, showed not more than 0.01gm difference.

PHYTOCHEMICAL STUDIES
Preliminary Phytoprofiles.15,16
Successive solvent extraction: 10g of the air-dried powdered plant material was successively extracted with the following solvents of increasing polarity in a soxhlet apparatus.
  a. petroleum ether (60º - 80ºc)
  b. ethyl acetate
  c. chloroform
  d. methanol
  e. water

All the extracts were concentrated by distilling the solvents and the extracts were dried in an oven at 500C. Each time before extracting with the next solvent, the marc was dried in an air oven below at 500C. The marc was finally macerated with water for 24 hours to obtain the aqueous extract.

The completion of the extraction was confirmed by evaporating a few drops of extract from the thimble on watch glass to observe that no residue remained after evaporation of the solvent. The liquid extracts obtained with different solvents were collected. The consistency, odour, colour, appearance of the extracts and their percentage yield were noted.
The extracts were then subjected to various qualitative test using reported methods, to determine the presence of various phytoconstituents such as alkaloids, glycosides, flavonoids, carbohydrates, amino acids, saponins, sterols and terpenoids, cardiac glycosides, coumarins, carotenoids, tannins, phenolic compounds, fixed oils and fats etc. (Table 4)

**Qualitative chemical identification of Rivea ornate.**[15-19]

The extracts were subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolic and tannins, phytosterols, fixed oils and fats, proteins amino acids, flavonoids, saponins, etc. using reported methods.

**Evaluation of antioxidant activity of Rivea ornata Roxb.**

Instruments: UV spectrophotometer (Shimadzu-UV-1601), Centrifuge Machine (Eltek-research centrifuge-TC-4100D).

**Chemicals:** All chemicals used for the study are purchased from SD-fine chemicals; India and all other reagent used were of analytical grade.

**4.18.1 DPPH radical scavenging activity 20-22**

Product extract and standard ascorbic acid solution (0.1 ml) of different concentrations viz. 10, 20, 40, 60, 80, 100μg/ml was added to 3 ml of a 0.004% methanol solution of DPPH. An equal amount of methanol and DPPH served as control. After 30 minutes incubation in the dark, absorbance was recorded at 517 nm, and the percentage inhibition activity was calculated from \[ \frac{(A0-A1)}{A0} \times 100 \], where A0 is the absorbance of the control, and A1 is the absorbance of the extract/standard. The antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration (in μg/ml) of extracts that inhibits the formation of DPPH radicals by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations. (Table 5) (Fig 5)

**4.18.2 Ferric Reducing Power determination.**[20-22]

Different concentrations of plant extract and standard ascorbic acid solution viz. 10, 20, 40, 60, 80, 100 μg/ml in 1ml of methanol were mixed with phosphate buffer (2.5ml, 0.2M pH 6.6) and potassium ferric cyanide \([K_3Fe(CN)_6]\) (2.5 ml, 1%). The mixture was incubated at 50oC for 20 min. A portion (2.5 ml) of tricholoro acetic acid (10%) was added to the mixture, which was then centrifuged at 3000 g (rpm) for 10 min at room temperature. The upper layer
of solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (FeCl3) (0.5 ml, 0.1%) and the absorbance of the reaction mixture indicated increased reducing power. The absorbance was measured at 700nm. All the tests were performed in triplicate and the graph was plotted with the average of three observations. (Table 6) (Fig 6)

RESULT AND DISCUSSION
PHARMACOGNOSTICAL STUDIES
Pharmacognostical evaluation is the first and foremost step to determine identity and to access the quality and purity of the crude drug. The selected plant is a crude drug therefore it was first subjected to pharmacognostical evaluation in reasonable details.

Identification and Authentication of Rivea ornata Roxb
Fresh and dry plants of Rivea ornata were collected from, Tirupati the month of May and its authentication was confirmed by Botanist, Dr. Madhava chetty, S.V. University Tirupati. Herbarium of the plant has been deposited at Department of Pharmacognosy, B. Pharmacy College, Rampura, Kakanpura, Dist. Panchmahal, Gujarat, India for future reference. VJS/SD-35. The plant was further subjected to morphological and microscopically examination to access the purity of the procured plant drug and various diagnostic features were recorded for the purpose of identification.

Macroscopic Evaluation
Stem & all branches are brown in color. Surface is smooth. Stem is non-woody. Stem is round in shape. Leaves ovate-cordate 3-5 in. diam. often broader than long, glabrous above, white silky-tomentose beneath while young; petiole 1-2 inch. Flowers large, white, in short, mostly 3-fid. Peduncles; bracts 1/2 in. Calyx deeply 5-lobed, persistent. Sepals 1/2 - 3/4 in., ovate-lanceolate, acute. Corolla salver-shaped; tube 2 in., cylindrical; limb 2 in. diam., white-silky without. Stamens 5, near the middle of the corolla-tube, included. Ovary superior, 4-celled, 4-ovuled, surrounded by an annular disk; style filiform; stigma 2-branched, linear-oblong, 1/10 in. Fruit 2/3 in. diam., indehiscent, globose, shining, yellow-brown, nearly dry.

Microscopic Evaluation
Free hand transverse sections (T.S) of fresh leaves and petiole of Rivea ornata were taken and studied for their histological characters.
Transverse section of leaf of *Rivea ornata*

Type of leaf of *Rivea ornata* is dorsiventral leaf.

**Lamina** The upper epidermis consist of polygonal tabular cells, the cuticle is moderately thick and is striated, stomata are present which is anomocytic. In lamina portion cylindrical palisade cells and spongy parenchyma amongst which rosette of calcium oxalate present. The lower epidermis consist of tabular cells with numerous anomocytic stomata present. Sparsely scattered glandular trichomes occur on both surface. There are so many unicellular and multicellular trichomes are present.

**Midrib** Collenchymas present below upper epidermis and above lower epidermis. The collenchymatous cells are isodiametric with well developed angular thickening. Beneath the collenchymas on both side 2 to 5 layers of parenchyma are present. The vascular bundle consist of wide phloem and xylem which is bicollateral. In parenchyma rosette of calcium oxalate is present. (Fig. 1).

**3.1.3.2 Transverse section of petiole of *Rivea ornata***

The general structure of the transverse section of the petiole (plate 3A) appeared circular. The outermost layer is formed of one layer of epidermis with some hairs followed by 4 layers of collenchyma and parenchyma cells. The vascular bundles are arranged in a circle, some of the bundles are separated by uni or biseriate parenchyma cells. The phloem region is formed of primary and secondary phloem and they are followed by the xylem which is formed of secondary xylem vessels separated by xylem sclerenchyma and few primary xylem vessels separated by xylem parenchyma. The pith is a wide region of thickened parenchyma cells. (Fig. 2)

**3.1.3.4 Surface preparation of leaf of *Rivea ornata Roxb.***

In the surface view the lower epidermal cells exhibit wavy margin, but upper epidermal cells are polygonal with straight walls. Stomata are paracytic and present on both the surfaces but more common on lower surface. The unicellular covering trichomes with bulbous bases are present on both the surfaces (Figure 3).

**3.1.3.5 Powder study of leaf:**

1. Anomocytic stomata
2. Rosette of Calcium Oxalate Crystal
3. Xylem vessel
4. trichomes
5. Surface view Striated epidemis with mesophyl

Fig. 1. Photograph of TS of leaf of *Rivea ornata* Roxb.
Fig. 2 Photograph of TS of petiole of *Rivea ornata* Roxb.

Fig. 3 Photograph of surface preparation of *Rivea ornata* Roxb
5.1.4 Quantitative microscopy of Aerial part of *Rivea ornata Roxb*.

Quantitative microscopy of Aerial part of *Rivea ornata Roxb.* was done and stomatal index, vein-islet number, vein termination number and palisade ratio were determined which are given in (Table 1).

**Table 1. Quantitative microscopy of leaf of *Rivea ornata Roxb.***

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Determination</th>
<th>Value ( per sq. mm )</th>
</tr>
</thead>
<tbody>
<tr>
<td>i)</td>
<td>Stomatal index</td>
<td>23.25-26.35</td>
</tr>
<tr>
<td></td>
<td>Upper epidermis</td>
<td>22.15-25.45</td>
</tr>
<tr>
<td></td>
<td>Lower epidermis</td>
<td></td>
</tr>
<tr>
<td>ii)</td>
<td>Vein-islet number</td>
<td>50-56</td>
</tr>
<tr>
<td>iii)</td>
<td>Vein-termination number</td>
<td>65-70</td>
</tr>
<tr>
<td>iv)</td>
<td>Palisade ratio</td>
<td>10-12</td>
</tr>
</tbody>
</table>

5.2) Proximate Analysis

**Table 2. Study of different parameters obtained from proximate analysis of aerial parts of *Rivea ornata Roxb.***

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Determination</th>
<th>Percentage W/W</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total Ash</td>
<td>15.45</td>
</tr>
<tr>
<td>2.</td>
<td>Acid insoluble Ash</td>
<td>6.63</td>
</tr>
</tbody>
</table>
5.3) PHYTOCHEMICAL STUDIES

5.3.1) Preliminary Phytoprofile

The percentage of different chemical constituents in the crude drug can be detected by subjecting them to successive extraction using solvents in the order of increasing polarity. The extract obtained were then dried completely and kept in vacuum desiccators. They were then subjected to qualitative chemical tests in order to detect the various chemical constituents present in them.

Table 3. Preliminary Phytoprofile of aerial parts of *Rivea ornata* Roxb.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solvent</th>
<th>Color and consistency after drying</th>
<th>Average value (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Petroleum ether (60-80ºc)</td>
<td>Yellowish, solid mass</td>
<td>2.50</td>
</tr>
<tr>
<td>2.</td>
<td>Ethyl acetate</td>
<td>Greenish, sticky mass</td>
<td>3.21</td>
</tr>
<tr>
<td>3.</td>
<td>Chloroform</td>
<td>Greenish, sticky mass</td>
<td>2.13</td>
</tr>
<tr>
<td>4.</td>
<td>Methanol</td>
<td>Greenish yellow, sticky mass</td>
<td>6.21</td>
</tr>
<tr>
<td>5.</td>
<td>Water</td>
<td>Dark Brown solid mass</td>
<td>9.61</td>
</tr>
</tbody>
</table>

5.3.2) Tests for preliminary Phytochemical screening of powder of aerial part of *Rivea ornata* Roxb.:

- Qualitative chemical examination of various successive extracts of powder indicated the presence of carbohydrates, steroids, Triterpenoid glycosides, alkaloid, phytosterols, steroids, mucilage.
- Phytosterols were detected by Libermann Burchard test and salkowaski reaction, carbohydrates by molisch’s, Fehling’s and Benedict’s test, saponin by foam test, flavonoids by shinoda test, tannins and phenolics by Lead acetate test.

Table 4. Test for Preliminary Phytochemical screening of aerial parts of *Rivea ornata* Roxb.

<table>
<thead>
<tr>
<th>SR NO</th>
<th>Tests of phytoconstituents</th>
<th>P. ether extract</th>
<th>Ethyl acetate extract</th>
<th>Chloroform extract</th>
<th>Methanol Extract</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tests for alkaloids a) Mayer’s reagent b) Dragendorff’s reagent c) Hager’s reagent d) Wagner’s reagent</td>
<td>*</td>
<td>*</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Tests for flavonoids a) Shinoda test b) Fluorescence test c) FeCl3 test d) Lead acetate test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>--------------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Tests for saponins a) Froth test b) Hemolytic zone</td>
<td>+ve +ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Tests for carbohydrates a) Molisch’s test b) Fehling’s solution test c) Benedict’s test:</td>
<td>+ve +ve +ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Tests for cardiac glycoside a) Legal’s test b) Keller Killiani’s test c) Baljet test</td>
<td>-ve -ve -ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Tests for fixed oil and fat a) Spot test b) Saponification test</td>
<td>+ve +ve +ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Tests for sterols and triterpenoids a) Libermann-burchard’s test b) Salkowski reaction</td>
<td>+ve +ve +ve +ve +ve +ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Tests for anthraquinone glycosides a) Borntrager’s test b) Modifying borntrager’s test</td>
<td>-ve -ve -ve -ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Tests for phenolic compounds a) Test with FeCl3 b) Test with folin-ciocalteu reagent</td>
<td>+ve +ve +ve +ve +ve +ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Tests for coumarins a) With ammonia b) With hydroxylamine hydrochloride</td>
<td>-ve -ve -ve -ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Tests for tannins a) Test with gelatin b) Reaction with lead acetate</td>
<td>+ve +ve +ve +ve +ve +ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* indicates positive reaction, -ve indicates negative reaction.
Antioxidant activity of the plant *Rivea ornata* Roxb.

The antioxidant activity of the alcoholic extract of the plant *Rivea ornata* Roxb. was carried out by *in vitro* antioxidant models. In the models tested, the antioxidant activity of the formulation was studied in relation to Ascorbic acid, a known antioxidant.

**DPPH Method**

**Table 5: 1,1-Diphenyl-2-picryl hydrazyl (DPPH) radicals scavenging activity**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Concentration µg/ml</th>
<th>% inhibition</th>
<th>Alcoholic ext</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>43.84</td>
<td>45.56</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>50.2</td>
<td>55.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>58.5</td>
<td>60.33</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>60.59</td>
<td>47.65</td>
<td></td>
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<tr>
<td>6</td>
<td>80</td>
<td>61.96</td>
<td>70.22</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>65</td>
<td>75.15</td>
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<tr>
<td>8</td>
<td>IC 50 value</td>
<td>21.2</td>
<td>11.33</td>
<td></td>
</tr>
</tbody>
</table>

Result indicated the significant decrease in the concentration of DPPH radicals due to the scavenging ability of Alcohol extract of plant *Rivea ornata* Roxb. and Ascorbic acid, as a reference standard. Maximum inhibition of extract and Ascorbic acid was exhibited 65.0% and 75.15% inhibition respectively in 100µg/ml. The IC 50 values in DPPH radical scavenging model were 11.33µg/ml and 21.2 for Ascorbic acid and alcohol extract of plant *Rivea ornata* Roxb. respectively.
Ferric reducing antioxidant power

Table 6. Ferric reducing antioxidant activity

<table>
<thead>
<tr>
<th>Concentration (mcg/ml)</th>
<th>Ferric reducing power</th>
<th>Absorbance</th>
<th>Ascorbic acid</th>
<th>Alcoholic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>0.05</td>
<td>0.024</td>
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<tr>
<td>20</td>
<td></td>
<td>0.08</td>
<td>0.048</td>
<td></td>
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<tr>
<td>40</td>
<td></td>
<td>0.11</td>
<td>0.079</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>0.152</td>
<td>0.12</td>
<td></td>
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<td>80</td>
<td></td>
<td>0.195</td>
<td>0.155</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>0.252</td>
<td>0.195</td>
<td></td>
</tr>
</tbody>
</table>

![Graph showing Ferric reducing antioxidant activity](image)

Fig.6 Ferric reducing antioxidant activity Abs. Vs conc.

Result illustrates that Alcoholic extract of the plant *Rivea ornata* Roxb. had ferric reducing capacity and also comparable to Ascorbic acid.

CONCLUSION

*Rivea ornata* Roxb. (syn. Phang), family Convolvulaceae, is a woody climber, occurring in south India in Tripura. *Rivea ornata* Roxb. leaves contain total ash (13.80 %), acid insoluble ash (7.52 %), water soluble ash (6.21 %), alcohol soluble extractive value (6.58 %), water soluble extractive value (7.69 %) and moisture content (82.65 %). Petroleum ether ((60–80ºc) extract of *Rivea ornata* Roxb. was yellowish mass (2.53 %w/w), Toluene extract was green sticky mass (3.12 %w/w), Chloroform extract was greenish yellow sticky mass (2.13 %w/w), Methanol extract was greenish brown sticky mass (3.21 %w/w) and Water extract was reddish brown sticky mass (8.24 %w/w).
Qualitative chemical examination of various extracts of *Rivea ornata Roxb.* was carried out which revealed presence of phytoconstituents like carbohydrates, phytosterols, phenolic compounds, alkaloid, triterpenoids, fixed oil and tannins.

The reducing power of *Rivea ornata Roxb* extracts increased steadily with increasing concentrations and varied significantly with different concentrations. The methanol and ethyl acetate extracts appeared to possess the highest significant reducing activity among the extracts. The stronger reducing power in the methanol and ethyl acetate extracts was probably due to the concentration of antioxidant compounds like flavonoids and phenolics in the extract. In conclusion, antioxidant study of *Rivea ornata Roxb* suggested that *Rivea ornata Roxb* is a potential source of natural antioxidants. However, further investigations on *in vivo* antioxidant activities are highly recommended. It is also needed to determine phytoconstituents, which are responsible for the antioxidant activity.

**REFERENCE**