Phytochemical Screening and Antibacterial Activity of an Epiphytic Orchid *Rynchostylis retusa* (L.) Blume. Collected from Kannur District, Kerala, India

Ruthisha PK*
Department of Botany, Sir Syed College, Taliparamba, Kerala - 670142, India

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**ABSTRACT**

Treatment with medicinal plants consists of the ancient tradition of India and which has been continued for years. Our ancestors collected rare plants from forests and mountains for treatment purpose. At the same time, they give deep concern for the conservation of the species on the earth. Their knowledge and services are not profitable but valuable. The study is focused on the phytochemical and antimicrobial activity of the medicinal plant *Rynchostylis retusa* of the family Orchidaceae. The plant is growing in sacred groves and local gardens of Payyanur of Kannur district, Kerala, India and used for traditional healing practices. The Orchidaceae family includes many plants having medicinal properties, but most of them were not studied or exploited yet. Qualitative analysis of primary metabolites and secondary metabolites indicates the presence of phytochemicals in *Rynchostylis retusa*. The total alkaloid, carbohydrate, protein, phenolic compounds and flavonoids were seen in significant amount in this plant. *In vitro* antibacterial (agar well diffusion) activity was done by gram-negative *Pseudomonas aeruginosa* (ATCC 27853), gram-positive *Streptococcus mutans* (MTCC 890), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and antifungal activity by *Aspergillus niger* (ATCC 16404). The plant extracts exhibited potent antimicrobial activity against these strains. The studies with the medicinal plant *R. retusa* make clear that the plant is rich in active components and phytochemicals. The plant contains a favourable amount of phytochemicals and this makes them cure various diseases. The plant also shows antioxidant and antibacterial activities.

**INTRODUCTION**

Medicinal plants are those plants that have medicinal values and are widely used for medicinal purposes. Medicinal plants incorporate different herbs, shrubs, and trees. These plants are an important source of active agents. In plants, these compounds help them to protect from insects, bacteria, fungi and plant eating herbivorous animals and also have biological utilities in plants. These chemical compounds were more than 12000 in number at the whole plant kingdom known to science yet. They work on the human body in exactly the same way as pharmacological medications do. Comparing to modern medicine, herbal medicine is more benefi-
cial and not having any markable side effects. Orchid plants were living in various ecological conditions and their habit also differed based upon the environment. They grow in epiphytic, saprophytic and terrestrial conditions. Among them, for structural and nutritional support, epiphytic orchids are completely relying upon trees or other plants. They are not absorbing nutrients from the host plant, so they are not parasitic plants (Dematte and Dematte, 1996; Jonathan and Raju, 2005). They absorb nutrients from debris accumulated on the bark of the host plant and water from rain and mist. Epiphytic roots have aerial roots which store and absorb water and they do not have the benefit of assimilation of water from the soil. Water is absorbed by velamen tissue found in the roots and they consist of many layers of dead cells. Thus epiphytic orchids can able to stand drought (Batchelor and American Orchid Society Bulletin, West Palm Beach, 1981; Dematte and Dematte, 1996).

The plant Rhynchostylis retusa is an epiphytic orchid species with a monopodial growth pattern. The plant is widely distributed in Teak plantations and on perennial trees. During the flowering season, beautiful white flowers with pink patches appeared in the racemose inflorescence. The non-pseudobulbous epiphytic herb consisting of 5-25 cm long stout stem and 25 cm long strap-shaped leaves. The leaves are coriaceous, linear and unequally lobed at the apex. Densely flowered racemose inflorescence about 60 cm long and violet and pink-tinted flowers and 1.5-2 cm broad. Petals and sepals are similar. The dorsal sepal is ovate, lateral sepals obliquely and broadly ovate. Lip 3 lobed and clawed. Lateral lobes obscure, midlobe elongated, inflexed and cuneiform in appearance.

**Medicinal uses**

In traditional medicine, the extract of fresh leaves was used to cure ear pain, rheumatic disease, blood dysentery, external inflammations and skin diseases. Many medicinal preparations with different parts of this plant are used to treat asthma, tuberculosis, epilepsy, vertigo, palpitation, kidney stone and menstrual disorders (Hossain, 2011; Jonathan and Raju, 2005). Leaf juice of this plant and aerial roots are used to cure ear pain and used for ear cleaning (Basumatary et al., 2004).

According to Kumari et al. (2012) the whole plant preparations of R. retusa is used to cure rheumatic disease, tuberculosis, epilepsy, blood dysentery, menstrual disorders, gout, asthma, skin diseases and external inflammations. The plant is used to treat throat inflammation and also used as an emollient (Shanavaskhan et al., 2012). Roots of the plant used to cure malarial fever (Tiwari et al., 2012; Radhika and Murthy, 2013). Dried flowers are used as an insect repellent and to induce vomiting and juice of roots applied to cuts and wounds (Subedi et al., 2013).

**MATERIALS AND METHODS**

**Preparation of Extracts**

The whole plant extracts of Rhynchostylis retusa were collected from Kasargod District, Kerala, India. The plants were identified in department of PG studies and research in botany, Sir Syed College, Tali-paramba. The collected plants were washed thoroughly with tap water followed by distilled water for the removal of dust and soil particles, cut into pieces and shade dried at room temperature for 15 days, then coarsely powdered using Panasonic Mixer and used for extraction. The powder (20 gm) was extracted with petroleum ether, chloroform, ethanol in a Soxhlet apparatus (3840; Borosil Glassworks Ltd., Mumbai, India) and finally, the dried powder was macerated using water with constant stirring for 48 h using the orbital shaker (Rivotek; Riviera Glass Pvt., Ltd., Mumbai, India) and the extract was filtered. The extracts were concentrated using a rotary evaporator and stored at -20°C in the deep freezer (RQV-300; plus, REMI electro technik Ltd., Thane, Maharashtra, India) for further analysis. Quantitative analysis was done by dissolving 1 mg extract in 1 ml respective solvent. The antimicrobial study was done with a well diffusion method by dissolving 1 mg extract in 1 ml DMSO.

**Extract Recovery Percentage**

The amount of extract recovered after successive extraction was weighed and the percentage yield was calculated by the following formula,

\[ \text{Extract Recovery Percentage} = \frac{\text{Amount of extract}}{\text{Amount of plant sample (G)}} \times 100 \]

**Phytochemical Screening**

For preliminary phytochemical screening, plant extracts of the whole plants of Rhynchostylis retusa were subjected to various qualitative chemical tests to determine the presence of phytoconstituents like glycosides, tannins, phytosterols, proteins, amino acids, carbohydrates, flavonoids, phenolic compounds, oils and fats, and saponins (Raaman, 2006). The total carbohydrate content of the selected plants was done by the anthrone method Morris (1948). Total protein content was estimated by (Lowry et al., 1951) and alkaloid content was estimated using the method Harborne (1973). The total phenolics of the different plant extracts were determined according to the method described by Makkar (2003). The
flavonoid contents of all the extracts were quantified according to the method described by Zhishen et al. (1999).

**Antibacterial Activity Test**

The antibacterial activity was carried out on the chloroform and ethanol extracts of *Rhynchostylis retusa* against *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus mutans* (MTCC 890), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). Antifungal activity was tested against *Aspergillus niger* (ATCC 16404). The agar well diffusion method was employed for the determination of antimicrobial activities of the selected plants. A suspension of the tested bacteria (0.1 mL of 1 × 108 cells/mL) was spread on the solid media plates. Wells of approximately 10 mm was bored using a well cutter and sample of 25, 50, and 100 µg concentrations were added. After being incubated at 37°C for 2 h, the plates were incubated at 37°C for 24 h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993) in centimetres. Streptomycin was used as a positive control with the tested bacteria. In order to access the biological significance and ability of the sample, the antifungal activity was determined by Agar well diffusion method. Potato Dextrose agar plates were prepared and overnight grown species of fungus, *Aspergillus niger*, was swabbed. Wells of approximately 10 mm was bored using a well cutter and samples of different concentration were added; the zone of inhibition was measured after overnight incubation and compared with that of standard antifungal (Clotrimazole).

**RESULTS AND DISCUSSION**

**Extract yield percentage of the plant extracts**

The medicinal orchid *Rhynchostylis retusa* shows maximum extract yield in hot water extract (13.3%) and shows the least yield in petroleum ether extract (5.7%). Ethanol extract has an extract yield percentage of 10.6 and chloroform has a yield of 7.1%.

**Phytochemical Screening**

The presence or absence of selected metabolites by colour formation with respective chemicals was determined by qualitative chemical tests. Primary metabolites like Carbohydrate, Protein, Amino acids and secondary metabolites such as Alkaloid, Flavonoid, Tannin, Terpenoid, Saponin, Phenolic compounds, Glycosides, Cardiac Glycosides, Fixed oils and Fats in petroleum ether, chloroform, ethanol and hot water extracts were present in the selected plant.

The preliminary phytochemical analysis shows maximum positive results in chloroform and ethanol extracts. Among 12 phytochemicals studied, ethanol extract shows positive results in 11 extracts like Carbohydrate, Protein, Amino acid, Alkaloid, Flavonoid, Tannin, Terpenoid, Phenol Glycosides, Cardiac Glycosides, Fixed oils and Fats and negative result in the case of Saponin. In the case of chloroform extract, the plant shows positive results in Carbohydrate, Protein, Amino acid, Alkaloid, Flavonoid, Tannin, Terpenoid, Phenol, Fixed oils and Fats and negative result in the case of Saponin, Glycosides and Cardiac Glycosides. Water extract shows positive results in the case of phytochemicals Carbohydrate, Flavonol, Phenol, Fixed oils and Fats, Saponins and negative result in Protein, Amino acid, Alkaloid, Tannin, Terpenoid, Glycosides and Cardiac Glycosides. Carbohydrate, Alkaloid, Flavonoid, Tannin, Phenol shows positive results in preliminary phytochemical analysis of petroleum ether extract and shows negative results in Glycosides, Cardiac Glycosides, Fixed oils and Fats Protein, Amino acid, Terpenoid and Saponins.

The result of the quantitative analysis of alkaloid shows an alkaloid content of 0.052±0.002mg/g in whole plant extract of *Rhynchostylis retusa*. Carbohydrate content in whole plant extract of *Rhynchostylis retusa* is 91.69±70mg/g. Quantification of total flavonoid shows a flavonoid content of 250.88±2.0mg/g in ethanol extract and it was the highest value and chloroform extract shows 120.63±1.5 mg/g, and it was the least. Water extract has 149.82±4.8mg/g and petroleum ether ether extract shows a result of 157.20±4.0mg/g. Total phenolic content in chloroform extract shows an amount of 57.11±1.8mg/g and water extract has 57.41±71mg/g, petroleum ether ether extract has 57.15±1.5mg/g and ethanol extract shows an amount of 110.35±58mg/g.

**Antimicrobial activity**

The chloroform and ethanol extracts of *Rhynchostylis retusa* was evaluated for in-vitro antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, *S. mutans*, and antifungal activity against *A. niger*. The chloroform extract of the plant showed a maximum zone of inhibition against *P. aeruginosa* (1.8 cm), *E. Coli* (1.5 cm), *S. mutans* (1.2 cm), *S. aureus* (1cm) and the fungus *A. niger* (1.5 cm). The ethanol extract of the plant showed a maximum zone of inhibition against *P. aeruginosa* (2 cm), *S. aureus* (1.3cm), *S. mutans* (1 cm), *E. coli* (1 cm) and the fungus *A. niger* (1.2 cm). The zone of inhibition exhibited by the plant extracts was shown in Table 1, Table 2, Table 3, Table 4, Table 5 and Figure 1.
### Table 1: Extract yield of *Rhynchostylis retusa* whole plant in different solvents

| S. No. | Solvent      | Yield % |
|--------|--------------|---------|
| 1      | Petroleum ether | 5.7     |
| 2      | Chloroform    | 7.1     |
| 3      | Ethanol       | 10.6    |
| 4      | Water         | 13.3    |

### Table 2: Preliminary phytochemical studies of *Rhynchostylis retusa* whole-plant extracts

| S. No. | Phytochemicals   | Pet. ether | Chloroform | Ethanol | Water |
|--------|------------------|------------|------------|---------|-------|
| 1      | Carbohydrate     | +          | +          | +       | +     |
| 2      | Protein          | -          | +          | +       | -     |
| 3      | Amino acid       | -          | +          | +       | -     |
| 4      | Alkaloid         | +          | +          | +       | -     |
| 5      | Flavonoid        | +          | +          | +       | +     |
| 6      | Tannin           | +          | +          | +       | -     |
| 7      | Terpenoids       | -          | +          | +       | -     |
| 8      | Saponin          | -          | -          | -       | +     |
| 9      | Phenol           | +          | +          | +       | +     |
| 10     | Glycosides       | -          | -          | +       | -     |
| 11     | Cardiac Glycosides | -      | -          | +       | -     |
| 12     | Fixed oils and Fats | -    | +          | +       | +     |

### Table 3: Quantification of phytochemicals in *Rhynchostylis retusa* whole plant extracts

| Phytochemicals | Amount(mg/g) |
|----------------|--------------|
| Alkaloid       | 0.05±0.002   |
| Carbohydrate   | 91.69±0.7    |
| Protein        | 329.28±2.3   |

### Table 4: Quantification of phenolic compounds and flavonoids

| Phytochemicals | Petroleum ether Solvents mg/g | Petroleum ether | Chloroform | Ethanol | Water |
|----------------|-------------------------------|----------------|------------|---------|-------|
| Phenol         | 57.15±1.5                    | 57.11±1.8      | 110.35±.58 | 57.41±.71|
| Flavonoid      | 157.20±4.0                   | 120.63±1.5     | 250.88±2.0 | 149.82±4.8|

### Table 5: Zone of inhibition of *R. retusa* chloroform and ethanol extract

| Sample         | Conc (μg) | E.coli | P. aeruginosa | S. aureus | S. mutans | A. niger |
|----------------|-----------|--------|---------------|-----------|-----------|---------|
| Chloroform     | 25        | -      | 1             | -         | -         | -       |
|                | 50        | 1.2    | 1.4           | -         | 1         | 1.2     |
|                | 100       | 1.5    | 1.8           | 1         | 1.2       | 1.5     |
| Ethanol        | 25        | -      | -             | 1         | -         | -       |
|                | 50        | -      | 1.3           | 1         | -         | -       |
|                | 100       | 1      | 2             | 1.3       | 1         | 1.2     |
| Streptomycin   | 100       | 3      | 3.7           | 3         | 2.7       | -       |
| Clotrimazole   | 100       | -      | -             | -         | -         | 1.9     |
Figure 1: Antimicrobial activity of ethanol and chloroform extracts of *R. retusa*
CONCLUSION

The studies with the medicinal plant *R. retusa* make clear that the plant is rich in active components and phytochemicals. The plant contains a favourable amount of phytochemicals and this makes them cure various diseases. The plant also shows antioxidant and antibacterial activities. Antibacterial activities of chloroform and ethanol extracts of the selected plants were tested against four human pathogenic Gram-positive and Gram-negative multidrug resistant bacteria and fungi (*E. coli*, *P. aeruginosa*, *S. aureus*, *S. mutans*, and *A. niger*). The chloroform extract of *R. retusa* shows maximum inhibition against *P. aeruginosa*, a gram-negative rod-shaped bacterium that causes numerous diseases in humans, including pneumonia. The ethanol extract of the plant also shows maximum inhibition against the bacteria *P. aeruginosa*; the zone of inhibition is 2 cm. An inhibition area of 1.2 cm is obtained against *A. niger*. Treatment with medicinal plants consists of the ancient tradition of India and which has been continued for years. Our ancestors collected rare plants from forests and mountains for treatment purpose. At the same time, they give deep concern for the conservation of the species on the earth. Their knowledge and services are not profitable but valuable. There is no doubt that the ignorance of the modern world with the traditional medicine methods, their doubts about them destroyed our tremendous legacy. Urbanisation killed the sources of medicinal plants in the wild and our rich knowledge of medicinal plants depleting from generation to generation.

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Conflict of Interest

The authors declare that they have no conflict of Interest for this study.

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