

## Phytochemical analysis of *Rhaphidophora australasica*

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### ABSTRACT

**Objective:** To subject *Rhaphidophora australasica* leaf extracts to phytochemical analysis. **Materials and Methods:** Phytochemical analysis was performed on extracts of Aqueous, ethyl acetate, acetone, chloroform and propane on *R. australasica* leaves by standard procedures and results were tabulated. **Results:** The presence of steroids was observed in all the fractions whereas proteins, triple sugars and amino acids were absent. **Conclusions:** These results could be used for identifying the medicinal values of this plant.

**KEY WORDS:** *Acanthaceae*, Acetone, Chloroform, Ethyl acetate, Propane, *Rhaphidophora australasica*

### INTRODUCTION

Antibiotics have saved the lives of millions of people and have contributed to the major gains in life expectancy over the past century. However, the clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens,<sup>[1]</sup> the recent appearance of strains with reduced susceptibility as well as undesirable side effects of certain antibiotics.<sup>[2]</sup> Infectious diseases caused by resistant microorganisms are associated with prolonged hospitalizations, increased cost, and greater risk for morbidity and mortality. Resistance is an especially vexing problem for people with impaired immunosystems, such as AIDS, cancer patients, and recipients of organ transplants.

The promiscuous use of antibiotics accounts for a major part of the community burden of antibiotic use and contributes dramatically to the rising prevalence of resistance among major human pathogens. Vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, *Mycobacterium tuberculosis*, and Gram-negative bacteria are recognized as the most difficult health-care-associated infections to control and treat. The development of extended-spectrum  $\beta$ -lactamases and carbapenemases that target Gram-negative bacteria has resulted in infections that can be extremely difficult to treat, leading to substantial increased illnesses and death rate.

The effect is pronounced in the third world as the costly replacement drugs for treating the highly resistant infectious diseases are unaffordable.<sup>[3]</sup> The resistance problem demands that a renewed effort should be made to screen various medicinal plants for their potential antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, phenolic compounds, steroids, resins, fatty acids, and gums which are capable of producing definite physiological action on body. Another driving factor that encouraged scientists to search for new antimicrobial substances from various sources including medicinal plants has been the rapid rate of plant species extinction. Medicinal plants are relied upon by 80% of the world's population, and in India, there is a rich tradition of using herbal medicine for the treatment of various infectious diseases, inflammations, injuries, and other diseases. Many of the plant materials used in traditional medicine are generally proved more effective and relatively cheaper than modern medicine<sup>[4]</sup> against certain ailments while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.<sup>[5]</sup>

### MATERIALS AND METHODS

#### Collection of Samples

The medicinal plants used for the experiment were leaves of *Rhaphidophora australasica* which were collected from the local medicinal farms.

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### Preparation of Extracts

500 g of aerial parts of dried powder of *R. australasica* was packed in a separate round bottom flask for sample extraction using different solvents namely ethanol, methanol, chloroform, ethyl acetate, and water. The extraction was conducted with 750 ml of each solvent for a period of 24 h. At the end of the extraction, the respective solvents were concentrated under reduced pressure and the crude extracts were stored in a refrigerator.

### Phytochemical Analysis

The extracts prepared were analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinone, cardiac glycosides, and reducing sugars based on the protocols available in the literature.<sup>[6-9]</sup>

#### Test for alkaloids

The extract of the crude dry powder of each solvent was evaporated to dryness in boiling water bath. The residues were dissolved in 2N hydrochloric acid. The mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer's reagent, the second portion was treated with equal amount of Dragendorff's reagent, and the third portion was treated with equal amount of Wagner's reagent. The cream precipitate, the orange precipitate, and brown precipitate indicated the presence of respective alkaloids.

#### Test for saponins

About 0.5 g of the plant extract was shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponin.

#### Test for tannins

About 0.5 g of extract was added in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

#### Test for steroids

2 ml of acetic anhydride was added to 0.5 g of methanol extract of each sample with 2 ml sulfuric acid. The color changed from violet to blue or green in some samples indicating the presence of steroids.

#### Test for flavonoids

2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, few drops of concentrated hydrochloric acid were added and the red color was observed for flavonoids and orange color for flavonoids.

#### Test for anthraquinones

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate was shaken with equal volume of 10% of ammonia solution. A pink violet or red color in the ammoniacal layer indicates the presence of anthraquinones.

#### Test for cardiac glycosides

0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulfuric acid. A brown ring obtained at the interface indicated the presence of a deoxysugar characteristic of cardiac glycosides.

#### Test for proteins

To 2ml of protein solution, 1ml of 40% NaOH solution and one to two drops of 1% CuSO<sub>4</sub> solution were added. A violet color indicated the presence of peptide linkage of the molecule.

#### Test for amino acids

2 ml of sample was added to 2 ml of ninhydrin reagent and kept in a water bath for 20 min. The appearance of purple color indicated the presence of amino acids in the sample.

#### Test for triterpenoids

5 ml of each extract was added to 2 ml of chloroform and 3 ml of concentrated H<sub>2</sub>SO<sub>4</sub> to form a monolayer of reddish brown coloration at the interface which confirms the presence of terpenoids.

#### Test for triple sugar

To 2 ml of extract, two drops of Molisch's reagent was added and shaken well. 2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately, which indicated the presence of carbohydrates.

**Table 1: The phytochemical analysis of *Rhaphidophora australasica***

Flavonoids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Triterpenoids	-	-	+	-	+
Saponins	+	+	+	-	-
Tannins	+	-	-	-	+
Triple sugar	+	-	-	-	-
Amino acid	-	-	-	-	+
Anthroquinones	+	-	-	-	+
Steroids	+	+	+	+	+
Proteins	-	-	-	-	-
Cardiac glycosides	+	-	+	+	+

“+”: Presence, “-”: Absence

## RESULTS AND DISCUSSION

Therefore, the present study was aimed to focus the various phytochemical constituents from various extracts of *R. australasica* that have been investigated.

Table 1 shows the phytochemical analysis of aqueous, ethyl acetate, acetone, chloroform, and propane extracts of *R. australasica*. Phytochemical screening of the crude extracts revealed the presence steroids in all the extracts. In case of triterpenoids, they were present only in acetone and propane extracts and absent in rest of the extracts. Tannins are present in aqueous and propane extract, whereas tannins were absent in other extracts. Proteins are absent in all the five extracts, whereas the amino acids were present only in the propane extract and absent in remaining all the extracts. Triple sugar was present only in aqueous extract and absent in all the five extracts. Anthraquinones were present in aqueous and propane extracts. Cardiac glycosides are present in acetone, chloroform, and propane extracts. Flavonoids and alkaloids were present in all the extracts. This knowledge could be used for identifying the various medicinal potentials of this plant.

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