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EVALUATION OF ANTIBACTERIAL ACTIVITY AND ANTIOXIDANTACTIVITY OF *NEPENTHES MIRANDA*

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ABSTRACT

The aim of the present study was to investigate the Antibacterial and Antioxidant activity of ethanol leaf and pitcher extract of *Nepenthes miranda*. The Antibacterial activity was evaluated against gram Negative bacteria (*Escherichia coli*) using agar well diffusion method. A well was prepared in the agar medium for the placing the Ethanolic leaf and pitcher extract of *Nepenthes* Miranda. Both leaf extract and pitcher extract doesn't exhibit zone of inhibition. The antioxidant activity was carried out using 96 well micro titer -DPPH based discoloration assay. Antioxidant activity of the pitcher and leaf extract were compared with the standard solution of Ascorbic acid.

KEYWORDS: *Nepenthes miranda*, Anti-oxidant, Anti-bacterial.

INTRODUCTION

In evolutionary history, carnivorous plants have evolved convergent five times. These plants are situated in poor-soil situations and have adapted their leaves to catch animals and outsource nutrients that are lacking in the soil. Recently, new advancements in digestive enzymes and leaf modification into traps have been discovered. Carnivorous plants give significant light on the relationship between ecology and evolution, as well as how environmental changes can lead to changes in shape and function. Concerns regarding climate change's possible impact on carnivorous plants have recently received a lot of attention, as have efforts to save carnivorous plants. Because carnivorous plants exhibit distinct adaptations, they have been used as a model todemonstrate the relationship between

ecological conditions and evolution.

MATERIALS AND METHODS

Plant material and collection

The leaf and pitcher of Nepenthes Miranda was gathered in March 2023 from Kallie, Kozhikode,Kerala. The plant was identified and auentified by our advisor, Dr. R.V. Celestin baboo, H.O.D., Department of Pharmacognosy, Jamia Salafiya Pharmacy College, Pulikkal, Malappuram.

Preparation of plant Extraction: The dried leaf and pitcher was powdered as a coarse powder. It was then subjected to cold extraction process using ethanol as solvent. The extraction procedure was carried out for two weeks. After 2 weeks it was filtered and concentrated to produce concentrated extract. The weight of the herb utilized and the weight of the extracted extract were used to compute the % yield of extract. The extract was kept in an airtight container for further studies.

EVALUATION OF ANTI-OXIDANT ACTIVITY

A 96-well micro titer plate-DPPH-based decolorization assay was used to carry out the Anti-oxidant activity of leaf and pitcher extract Nepenthes miranda. Ascorbic acid was used as the reference standard for anti-oxidant activities.

Procedure for anti-oxidant screening: Initially a Eppendorf tube (2ml) was taken to dilute the respective extracts. a micropipette with a volume of capacity 100 µL used to take the sample from the Eppendorf tube. The sample was added to the column of 96 well micro titer plates by use of micropipette. DPPH (24 mg in 100 ml methanol) was added to each column using a micropipette. Ascorbic acid (standard) was placed in a column, and DPPH was added in the way described above. The anti-oxidant activity of the column was assessed by decolorization and comparison with ascorbic acid.

EVALUATION OF ANTI-BACTERIAL ACTIVITY

The anti-bacterial activity of an alcoholic extract of "Nepenthes miranda" leaf and pitcher against the microorganism Escherichia coli was determined using the disc diffusion method and ciprofloxacin as the reference standard.

Test micro organism used: The plant extracts were tested against cultures of gram Negative bacteria like *Escherichia coli.*

Preparation of medium: In 1000 ml of distilled water, dissolve 38 g of Muller Hinton Agar. Boil for 1 minute with frequent agitation to completely dissolve the medium. Cool, autoclave at 121°C after 15 minutes. Fill sterile petri dishes halfway with chilled Muller Hinton Agar. Allow to cool to room temperature.

Screening of Antibacterial activity: Antibacterial activity was carried out using disc diffusion method. A whatsman filter paper is used for applications of the extract (it us wetted with alcoholic extract). About 0.1ml of inoculum was spread on the agar plate by spread plate technique. Filter paper disc is placed on to the medium along with the standard disc of ciprofloxacin (5µg) at the Centre of medium. The plates were incubated for 24 hrs. The zone of inhibitions were determined.

RESULTS

Anti-oxidant activity

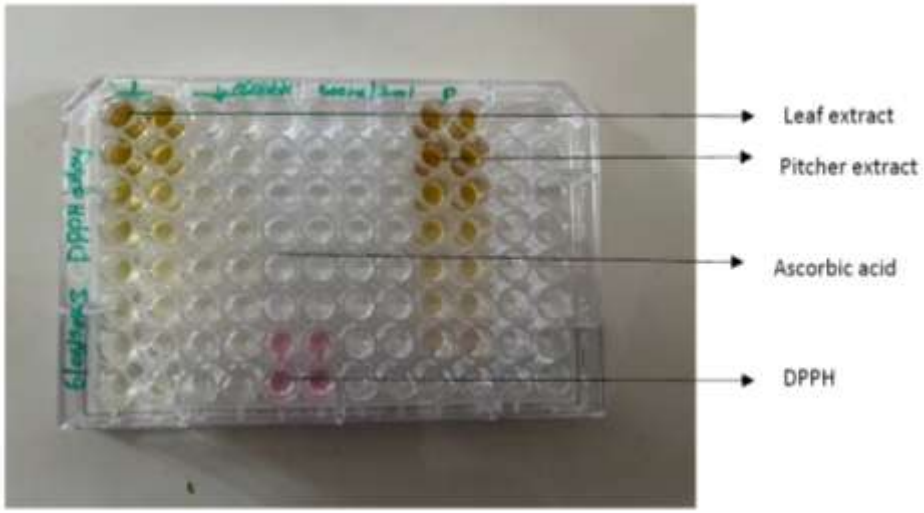

Fig. 2: Result of anti-oxidant activityAnti-bacterial activity.

Anti-bacterial activity

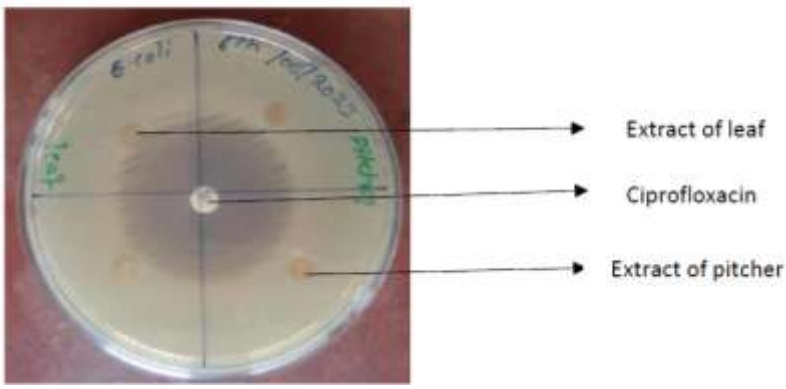

Fig 3: Result of Antibacterial activity.

Phytochemical screening

Chemical Test	Leaf Extract	Pitcher Extract	DIGESTIVE FLUID
Alkaloid	+	+	--
Carbohydrate	-	-	-
Lipid	-	-	-
Flavonoid	+	-	-
Tannin	-	-	-
Saponin	-	-	-

Fig: Result of Phytochemical Analysis of Leaf, Pitcher Extract and Digestive Fluid of *NepenthesMiranda.*

CONCLUSION

- We chose a significant Carnivorous plant, Nepenthes miranda, for the current study based on a review of the literature.
- We used a scientific approach to examine the Pharmacognostic, Phytochemical, Anti-oxidant, and Anti-bacterial activity of Nepenthes miranda.
- The plant was collected, and its Pharmacognostical characteristics, such as macroscopy and microscopy, were analyzed, paving the way for the plant's authenticity and identification.
- The extracts' antioxidant activity was measured using the 96 well-microtiter plate method with DPPH reagent, and their antibacterial activity was examined using the disc diffusion method against the bacteria E. coli. It has no discernible growth inhibition against the chosen bacteria.
- The current plant analysis concludes that the leaf components of *Nepenthes Miranda* possess antioxidant activity.

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