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Enzyme Inhibitory, Antioxidant, Antifungal and Phytotoxic Properties of Pilea microphylla (Urticaceae)

T. M. K. P. Thennakoon[1]*, K. P. N. G. Kanuwana[1], H.A.R. P. Perera[2], R.V. Vidhyajini[2]

[1]Department of Indigenous Medical Resources, Faculty of Indigenous Health Sciences and Technology, Gampaha Wickramarachchi University of Indigenous Medicine, Kandy road, Yakkala. Sri Lanka.

[2]Department of Kaumarabhruthya and Sthree roga, Faculty of Indigenous Medicine, Gampaha Wickramarachchi University of Indigenous Medicine, Kandy road, Yakkala. Sri Lanka.

***Corresponding Author**
E-Mail Id: kpthennakoon96@gmail.com*

ABSTRACT

Pilea microphylla (P. microphylla), small shrubs that thrive in shaded environments, which belongs to the family Urticaceae. Currently this plant gained attention due to the medical properties it contains. The main objective of this study is to investigate the enzyme inhibitory, antioxidant and phytotoxic properties of Pilea microphylla. For that methanolic extracts were prepared from the P. microphylla and their inhibitory activities against key enzymes mainly α-amylase, acetylcholinesterase, and lipase, antioxidant potential, Antifungal and phytotoxic effects were evaluated using appropriate assays. The findings revealed that the methanolic extract did not demonstrate antifungal properties, cytotoxicity, or α-amylase inhibitory activity. The IC50 values obtained for the antioxidant, acetylcholinesterase inhibitory, and lipase inhibitory assays were 46.69 mg L-1, 132.531 mg L-1, and 25.148 mg L-1, respectively. Notably, the extract exhibited significant inhibitory activity on root growth in the test plant (50.12%), but its effect on shoot growth was less pronounced (8.84%). In conclusion, the study highlighted the bioactivity of P. microphylla methanolic extracts. While the extract did not exhibit antifungal or cytotoxic properties, it demonstrated moderate antioxidant activity and inhibitory effects on acetylcholinesterase and lipase enzymes. Additionally, the extract showed phytotoxic activity by inhibiting root growth. Further research and analysis of P. microphylla and its extracts may contribute to our understanding of its potential applications in various fields.

Keywords: *Pilea microphylla, antioxidant, α-amylase, acetyl cholinesterase, lipase, phytotoxicity*

INTRODUCTION

Pilea microphylla, commonly known as Angel wood, Joy powder plant, Artillery plant, or Brihantina, belongs to the largest genus in the Urticaceae family and one of the largest genera in the Urticales order. This genus encompasses over 600 species that are predominantly found in tropical, subtropical, and warm temperate regions. *Pilea microphylla* itself is a remarkable species that was discovered during a study of the alien urban flora of Palermo in June 2017 (Scafidi & Raimondo, 2018).[9]

Pilea microphylla is a versatile plant, with various growth forms including succulent herbs, epiphytes, and small shrubs. It is known to thrive in shaded environments, often adapting well to heavy shade conditions. The plant has gained attention not only for its ornamental value but also for its historical medicinal use by native Indians. Traditional medicinal practices

involve using the entire plant infusion as a diuretic and for relieving labor pains in women. *Pilea microphylla* has also been used for treating infertility, inflammation, urinary problems, diarrhea, stomach and intestinal ailments, and as an application for sores and bruises (Bhellum & Sania, 2016).[1,2]

TAXONOMY

Table 1: *Taxonomy of Pilea microphylla.*

Taxonomic Rank	Classification
Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Rosales
Family	Urticaceae
Genus	Pilea
Species	*Pilea microphylla*

MORPHOLOGY

Growth Habit

According to the University of Florida IFAS Extension, *Pilea microphylla* is a succulent herb with a low-growing habit, reaching heights of 0.5 to 1.5 feet and spreading 1 to 2 feet in width.

Shoots

The stems of *Pilea microphylla*, as described by the University of Florida IFAS Extension, are delicate, green, almost translucent, and succulent. The leaves are opposite to subopposite, obovate in shape, and typically have short petioles.

Most leaves display three primary veins originating from the leaf base. These evergreen leaves measure approximately 8.25-1.14 mm in length and 3.64–1.13 mm in width.

Roots

Pilea microphylla typically possesses fibrous roots, although occasionally a short taproot may be present.

Inflorescence

The University of Florida IFAS Extension notes that *Pilea microphylla* produces dense, branched clusters of small whitish to greenish flowers known as cymes. These inflorescences arise from the leaf axils.

Flowers

The flowers of *Pilea microphylla* are unisexual, with sepals measuring 4 mm in male flowers and 3 mm in female flowers. They lack petals but have four stamens. The plant exhibits year-round flowering.

Fruits

The University of Florida IFAS Extension describes the fruits of *Pilea microphylla* as light brown achenes, measuring less than 0.5 mm in length and having a smooth surface.

Fig. 1: *Pilea microphylla plant.*

Fig. 2: *Pilea microphylla* leaves

Fig. 3: *Pilea microphylla* flowers

AIM

This study was carried out to evaluate the bioactivity of *Pilea microphylla*. Methanolic extracts obtained from the plant was tested for phytotoxicity, cytotoxicity, antioxidant and antifungal properties. Lipase inhibitory assays and α-amylase assays were also carried out on the extracts obtained.

MATERIAL AND METHODOLOGY

The crude plant extract was then screened for antifungal activity against *Cladosporium* (Karunanayake et al., 2011), cytotoxicity activity against brine shrimps (Gadir, 2012),[4,5] antioxidant activity against DPPH (2,2'-diphenyl-1-picrylhydrazyl) (Napagoda et al., 2015),[6] α-amylase enzyme inhibitory activity (Nickvar et al., 2008), acetylchlolinesterase inhibitory activity (Ellman *et al*., 1961),[3] lipase inhibitory activity and phytotoxicity activity against

lettuce seed germination (Piyasena et al., 2015)[7,8] and readings were taken.

Pilea micropylla plants were collected. All plant parts were cleaned and washed thoroughly with running tap water. And the sample was air dried until all the water evaporated. The sample was then grinded and the dry weight was taken (8.10 g). The powdered sample was then extracted with methanol. Extraction was filtered through cotton wool and the filtrate was concentrated in a rotary evaporator and the crude extract was obtained. The dry weight of the crude extract was measured (510 mg).[10]

Phytotoxicity assay using lettuce seed germination

Concentration series of the crude extracts were prepared with 1% DMSO in distilled water. Lettuce seeds were surface sterilized by washing thoroughly with tap water. Sterilized seeds were socked in extracts for about 5 min. 2 mL of each extract was added to the Petri dishes (90 mm) lined with filter paper discs. Then 10 seeds were placed in the respective Petri dish which wetted with respective extracts. The Petri plates were wrapped with parafilm and incubated at room temperature for about 5 days (Piyasena et al., 2015). After 5 days, root and the shoot lengths were recorded. Experiments were carried out in triplicates, while 1% DMSO in distilled water served as the control. Percentage inhibition of root and shoot growth was plotted against the concentration of extracts. Abscisic acid was used as the positive control.

According to Piyasena *et al.*, 2015 the percentage inhibition of root and shoot elongation of extracts of *E. crassipes* against lettuce seed germination expressed as follows.

$$\text{Phytotoxicity\%} = \frac{\text{Control shoot/root length} - \text{Sample shoot/root length}}{\text{Control shoot/root length}} \text{ X } 100$$

Brine shrimp lethality activity (Krishnaraja et al., 2005)

The extracts and the pure compounds were subjected to brine shrimp lethality to access the possible cytotoxic activity against living normal cells. Concentrations of 62.5ppm, 125ppm,250ppm,500ppm and 1000 ppm for crude extracts were studied. The cytotoxic activity was reported as the concentration required to kill 50% of brine shrimps (LC_{50}).

Cytotoxicity activity of extracts of *E. crassipes* against brine shrimps (Krishnaraju et al., 2005).

$$\text{Mortality\%} = \frac{\text{No.of shrimps died after 24hours}}{\text{No.of shrimps added}} \text{ X } 100$$

DPPH radical-scavenging assay

The DPPH free radical scavenging capability of *E. crassipes*, extract was determined according to the method described with slight modifications (Napagoda et al., 2016). An aliquot of 1.5 mL of extract methanol of *E. crassipes* at different concentrations (62.5, 125,250,500 and 1000 ppm) was mixed with 500 µL of 1.2mg mM DPPH (dissolved in ethanol until 5 mL). The mixture was vigorously shaken and left to stand at room temperature for 30 min in a dark room. Absorbance was read at 515 nm using UV-vis spectrophotometer. Ascorbic acid was used as standard. Inhibition of DPPH radical scavenging

activity in percent (1%) was calculated according to the equation of 1% = [(Ablank Asample)/ Ablank] × 100 where - Asample is the absorbance of the sample, and Ablank is the absorbance of blank solution (containing all reagents except the test sample). ICs value was determined from the plotted graph of scavenging activity against the concentrations of the dragon fruit samples, which is defined as total antioxidant necessary to decrease the initial DPPH radical by 50%. Triplicate measurements were carried out and IC_{50} was calculated based on the percentage of DPPH radicals scavenged.

$$\% \text{ DPPH activity} = \frac{Ac-As}{Ac} \text{ X } 100$$

Ac = Absorbance of Control As = Absorbance of sample

The α-amylase inhibitory activity was calculated as percentage inhibition.

$$\% \text{ Inhibition} = \frac{Ac-As}{Ac} \text{ X } 100$$

Ac = Absorbance of Control As = Absorbance of sample

The lipase inhibitory activity was calculated as percentage inhibition (Saana at el., 2012).

$$\% \text{ Inhibition} = \frac{Ac-As}{Ac} \text{ X } 100$$

Ac = Absorbance of Control As = Absorbance of sample

Statistical analysis was performed using the Microsoft Excel Package (version 2010). All the results were in triplicate determinations and were expressed as Mean at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

The MeOH extract of *Pilea microphylla* exhibited phytotoxic effects. The phytotoxic effect was prominent in root inhibition (50.12%), but was not prominent in shoot inhibition (8.84%).

The sample gave LC50 -276.55 mg L-1 for brine shrimp mortality assay. In antioxidant assay the sample gave IC50 46.69 mg L-1. For α-amylase enzyme inhibitory assay and lipase inhibitory assay the IC50 values were 1073.87 mg L-1 and 25.148 mg L-1 respectively. IC 50 value for acetylchlolinesterase inhibitory assay was 132.531 mg L^{-1}.

Antifungal activity was not observed for the sample.

CONCLUSION

Pilea microphylla plant exhibits different types of bioactive properties. Here the plant was screened for antifungal, antioxidant, cytotoxic, α-amylase enzyme inhibitory, acetylchlolinesterase inhibitory, lipase enzyme inhibitory and phytotoxic activities. Phytotoxic, antioxidant, acetylchlolinesterase inhibitory and lipase enzyme inhibitory properties were prominently exhibited by the plant.

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