

RESEARCH ARTICLE

Evaluation of 2,2-diphenyl-1-picrylhydrazyl Scavenging Activity and Phytochemical Analysis of *Mukia Maderaspatana* (L.) M. Roem.

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ABSTRACT

Mukia maderaspatana belongs to the family Cucurbitaceae is an important plant described in Ayurveda. This plant is used for the treatment of a number of ailments such as urinary disorder and cardiac problems. The leaf of *M. maderaspatana* was extracted with different organic solvents in increasing order of polarity. The results of the preliminary investigation revealed the presence of alkaloids, steroids, flavonoids, terpenoids, tannins, glycosides, and saponins. Antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The results of antioxidant activity indicate that the methanolic and petroleum ether extracts of the leaf of *M. maderaspatana* possess significant scavenging activity against DPPH (ethanolic solvent and methanolic solvent of 300 µg/ml each). This study revealed that the methanolic extracts of *M. maderaspatana* have demonstrated significant antioxidant activity.

Keywords: Cucurbitaceae, leaf extract, *Mukia maderaspatana*, nuclear magnetic resonance, phytochemistry.

INTRODUCTION

Plant is man's friend in survival, giving him food and medicine from the days beyond drawn of civilization.^[3] Plant continues to be a major source of medicine, as they have throughout human history.^[16] For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Nowadays, the use of phytochemicals for pharmaceutical purpose has gradually increased many countries. According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from develop countries use traditional medicine, which has compounds derived from medicinal plants. Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. These are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern

drug design.^[20] The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds.^[7] Correlation between the phytoconstituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well.^[17] *Mukia maderaspatana* Linn. belongs to the family name is Cucurbitaceae. It is a prostate herb or tendril climber found throughout India. *M. maderaspatana* is a medicinal plant. It occurs in wild areas as well as cultivated in Kitchen gardens. It is traditionally used as a leafy vegetable and to cure several ailments in South India. It is used in vertigo and biliousness. The present work was aimed to assess the preliminary phytochemical analysis and antioxidant activity of *M. maderaspatana*.

MATERIALS AND METHODS**Collection of plant sample**

The fresh leaves of *M. maderaspatana* will be collected from Madukkarai, Coimbatore District, Tamil Nadu. The plants will be identify and authenticate at the herbarium of Botanical Survey of India, Coimbatore, Tamil Nadu. The fresh

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leaves are washed with tap water and allow to shade dry at room temperature. The dried leaves are powdered by electrical blender.

Preparation of plant extracts

About 30 g of powdered *M. maderaspatana* leaf was successively extracted using 300 ml of methanol and petroleum ether using the Soxhlet extractor for 8–10 h.^[8] The extract was filtered through Whatman No.1 filter paper to remove all undissolved matter including cellular materials and other constitutions that are insoluble in the extraction solvent.

Preliminary phytochemical studies

The methanol and petroleum ether extracts were subjected to preliminary phytochemical tests to determine the group of secondary metabolites present in the powdered *M. maderaspatana* was followed by Harborne.^[9]

ANTIOXIDANT ANALYSIS OF *MUKIA MADERASPATANA*

DPPH radical scavenging activity

The antioxidant activity of the methanolic and ethanolic extraction of *M. maderaspatana* was measured the method described by Brand-Williams *et al.*^[4]

RESULTS

Preliminary phytochemical studies on *M. maderaspatana* were carried out to find out the presence of phytochemical constituents. *M. maderaspatana* is a prostrate or climbing scabrid herb. Tendrils are simple, which are distributed in throughout India. The plants were also screened for antioxidant responses. In this phytochemical evaluation, initially, physical constants were evaluated for its presence as well as for its quantity. The petroleum ether and methanolic extracts were found to contain flavonoids, saponins, glycosides, steroids, and phenolic compounds.

The plant material was subjected to phytochemical analysis separately for observing the presence of alkaloids, flavonoids, terpenoids, phenolic

compounds, glycosides, saponins, steroids, and tannin [Table 1]. All results observed were in leaves of *M. maderaspatana*. Flavonoids are found in optimum concentration in the present study. Flavonoids are pharmacologically active substances. Saponins are steroid glycosides. It may be steroid glycosides or may be terpene glycosides. The combination of hydrophilic triterpene with a hydrophilic sugar gives saponins. In general, saponins are toxic, but many experiments showed that consumption of saponins in lower concentration by human beings may be beneficial in reducing heart diseases. In the present investigation, because of the presence of saponins, the leaves of *M. maderaspatana* in methanolic solvent may have some medicinal property. Glycosides were also present in *M. maderaspatana*. The present study reveal can optimum precipitation of glycosides. Hence, the plant may be tested for antistress, antidiabetic, and anti-inflammatory properties as is evident from the works of above-mentioned authors. Phenolic compounds were also detected in both solvents. They show a high degree of precipitation of phenolic compounds. Due to these phenolic compounds, the susceptibility of the plant may greater even in high temperatures. The phytochemical and antioxidant activity of both extracts of *M. maderaspatana* that using the diagnostic feature one can identify these two solvents for further investigation.

DPPH scavenging activity of *M. maderaspatana*

The antioxidant activities in leaf of *M. maderaspatana* methanol and ethanolic extracts were assessed by DPPH activity. The DPPH activity of different concentrations of

Table 1: Preliminary phytochemical analysis of *Mukia maderaspatana*

Name of secondary metabolite	Petroleum ether solvent	Methanolic solvent
Alkaloids	–	–
Flavonoids	++	++
Saponins	–	++
Glycosides	++	+
Steroids	++	+
Phenols	++	+
Tannins	–	–

++: More present, +: Present, -: Absent

methanol and ethanolic extracts (100–300 µg/ml) along with standard ascorbic acid was presented in Table 2. With the increasing concentrations, positive scavenging activity was noted. The percentage of scavenging activity is increasing with the increasing concentration in both extracts. Among the five different concentrations (100–300 µg/ml) of both extracts tested, the higher percentage of inhibition (61.2 ± 0.26) was observed in 300 µg/ml of ethanol extract followed by (75 ± 0.67) 300 µg/ml of methanol extract against the standard ascorbic acid (79 ± 0.28) followed by percentage of inhibition (56.7 ± 0.27) 250 µg/ml of ethanol extract and (70 ± 0.65) of methanol extract observed in 250 µg/ml against the standard ascorbic acid (74 ± 0.44) 250 µg/ml. From the result, when compare the scavenging activity percentage of ethanol and methanol, the methanol extract shows higher activity than ethanol extract.

DPPH free radicals have the ability to take electron from the antioxidants that is why it is used for the antioxidants scavenging assays of the medicinal plants for its estimation. Table 2 summarizes the percentage scavenging activity in ethanol and methanol leaf extracts of *M. maderaspatana*.

DISCUSSION

M. maderaspatana traditionally used as a leafy vegetable and to cure several ailments in South India. It is used to treat cough, cold, constipation, vertigo, burning sensation, dyspepsia, flatulence, and dental pain.^[17] Extensive literature survey has shown that there are no scientific reports available on nutrient composition of *M. maderaspatana* L. Furthermore, earlier work focused on antimicrobial activity of aerial parts in chloroform, hexane, ethyl acetate, and methanol. Hence, the present study is carried out with the aim to explore phytochemical constitution in water, ethanol, ethyl acetate, acetone, and hexane extract of leaf parts, nutrient

potential in relation to its ethnomedicinal uses, and potential antibacterial activity against few bacterial strains. The results of phytochemical screening test performed on crude leaf extracts of *M. maderaspatana* plant are summarized in Table 1. Phytochemical analysis of petroleum ether and methanol extracts of *M. maderaspatana* leaf extract revealed the presence of flavonoids, glycosides, steroids, phenolic compounds, and saponins. The presence of these substances in the investigated plant accounts for its usefulness as medicinal plant. This information obtained is used to facilitate quantitative estimation and qualitative separation of constituents from the leaves. In addition to the phytochemical screening of the plant extract, we have checked the anthelmintic activities, and the extract showed the prominent activity toward aquatic leech; *Lymnatis nilotica*.^[6] Arunprasath and Gomathinayagam^[1] reported that maximum amount of all the compounds such as alkaloids, flavonoid, glycosides, steroids, phenols, tannins, saponins, and resins in leaves was present in methanol extract than the petroleum ether extract.

The measurement of the scavenging of DPPH radical allows one to determine exclusively the intrinsic ability of substance to donate hydrogen atom or electrons to this reactive species in a homogeneous system. The method is based on the reduction of methanolic DPPH solution because the presence of antioxidant substances having hydrogen-donating groups such as phenols and flavonoid compounds due to the formation of non-radical DPPH-H form.^[13] The SC50 values for DPPH assay of the samples have been given in Table 3. The ethanol and methanol extracts of *M. maderaspatana* have proved to be active antioxidants. The mechanism of the reaction between antioxidant compounds and DPPH depends on the structural conformation of these compounds. It has been reported that the free radical scavenging activity of flavonoids is

Table 2: Antioxidant-DPPH activity of *Mukia maderaspatana* leaf extract in different concentrations

Sample	% of inhibition					Comparison of activity
	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml	300 µg/ml	Ethanol > methanol
Ethanolic extract	47.6 ± 0.44	49.6 ± 0.63	52.3 ± 0.37	56.7 ± 0.27	61.2 ± 0.26	
Methanolic extract	58.4 ± 0.49	65 ± 0.46	67 ± 0.33	70 ± 0.65	75 ± 0.67	
Ascorbic acid	58.9 ± 0.55	69.6 ± 0.42	70 ± 0.43	74 ± 0.44	79 ± 0.28	

DPPH: 2,2-diphenyl-1-picrylhydrazyl

dependent on the presence of free OH groups, especially 3-OH.^[15,8,16] In the present study, the antioxidant activity of the methanol, chloroform, and ethyl acetate extracts may be attributed to the collective antioxidant effects of the phenolic compounds, and these results are in full agreement with previous studies on many plant species.^[12,1,6] The bioactive compounds obtained from medicinal plants have been used to treat various ailments caused by microorganisms. The most important of their bioactive principles are alkaloids, phenolic compounds, flavonoids, and tannins that may be evolved in plants as self-defense against pest and pathogens.^[19] DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of the plant extract.^[2] Scavenging of DPPH radical is related to the inhibition of lipid peroxidation.^[18] DPPH is usually used as a substance to evaluate the antioxidant activity.^[5] Antioxidants either transfer an electron or a hydrogen atom to DPPH, thus neutralizing its free radical character.^[14] DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action.^[10] The DPPH assay has been largely used as a quick, reliable, and reproducible parameter to search the *in vitro* general antioxidant activity of pure compounds as well as plant extracts.^[11] The reducing capacity of compounds could serve as indicator of potential antioxidant property.^[12]

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