

Research Article



Phytochemical Evaluation and *In Vitro* Antioxidant Study of *Adiantum latifolium*

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ABSTRACT

Adiantum latifolium an important medicinal plant belonging to the family adiantaceae. The plant is scientifically proved to use as anti-inflammatory, analgesic, anti-infectious, and diuretic. In this study, the antioxidant property of extract of *Adiantum latifolium* leaves was evaluated using the antioxidant assay techniques like reducing power assay and phosphomolybdate assay. The extract of *Adiantum latifolium* was prepared after drying using cold extraction method and ethanol extraction. The extract prepared was phytochemically evaluated and the antioxidant study was carried out using phosphomolybdate assay and reducing power assay using ethanolic extract. Thus, reducing power assay concludes the antioxidant activity increase with increase in concentration and phosphomolybdate assay confirms the antioxidant activity. Thus, this study concludes the antioxidant activity of the leaves of *Adiantum latifolium* and further studies could be done to establish the same.

Keywords: *Adiantum latifolium* leaves extract, Cold extraction, Ethanol extraction, Reducing power assay, Phosphomolybdate assay.

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INTRODUCTION

In today's modern world, the risk of diseases due to oxidative stress is compounded by unhealthy lifestyle, exposure of chemicals, pollution, cigarette smoking, drugs, illness, and stress etc. Exogenous consumption of

anti-oxidants from plant, animal, and mineral sources have proved beneficial to human health and effective to reduce the incidence of free radical induced diseases. The antioxidants are also associated with reduction of free radical generation and improves antioxidant status in patients, thus it may be beneficial to recover normal function and treatment of such diseases. In recent years there has been increased interest in the therapeutic use of antioxidants in the treatment of disease associated with oxidative stress. Several studies reported that low antioxidant intake or low blood levels of antioxidants increase the risk of different diseases, in fact low dietary intake of fruits and vegetables double the risk of cancer. Therefore, wholesome antioxidant diet and natural antioxidant supplements as part of a healthy lifestyle are now being recognised to protect health from oxidative stress.

Adiantum latifolium is popularly called "maiden hair fern" because of the shiny black rachis of the leaves. It is one of the most widely distributed genera of the family

Pteridaceae. The plant *Adiantum latifolium*, is widely employed world wide as anti-inflammatory, analgesic, anti-infectious, and diuretic. Studies were undertaken to establish the antinociceptive and anti-inflammatory properties of the methanolic extract of *Adiantum latifolium* leaves and to investigate the mechanisms responsible for its effects^{2,3}.

MATERIALS AND METHODS

Collection, authentication and drying of plant material

Plant material of *Adiantum latifolium* was collected from surroundings of DPS, CPAS Puthuppally campus. The plant was authenticated by Dr. Rojimon P Thomas, Head of the Department of Botany, CMS College, Kottayam. The whole plant collected was washed for removing the dust and dirt. The leaves of *Adiantum latifolium* were shade dried separately for 15 days. The shade dried material was powdered using Mechanical grinder.

Extraction

Cold aqueous extraction

10g of air dried powder of leaves of *Adiantum latifolium* was weighed and soaked separately in 50ml cold water in a conical flask, stoppered with rubber cork and left uninterrupted for 24 hrs and then filtered off using muslin cloth into a conical flask and concentrated using water bath evaporation, where by the solvent was evaporated at its boiling temperature 100°C. The concentrated extract was again filtered using muslin cloth and was subjected to centrifugation at 5000 rpm for 5 minutes and the supernatant was obtained and stored at 4°C in a refrigerator for further use.

Ethanol extract 10g of air dried powder of leaves of *Adiantum latifolium* was weighed and was placed in 100ml



of organic solvent (ethanol) in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 hrs, it was filtered with the help of muslin cloth and centrifuged at 5000 rpm for 15 minutes. The supernatant was collected and the solvent was evaporated to make the final volume to one-fourth of the original volume, giving a concentration of 100 mg/ml and stored at room temperature in air tight container⁴.

The phytochemical analysis

The leaf extract of *Adiantum latifolium* was analysed for the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins and terpenoids⁵.

Evaluation of *in vitro* antioxidant activity of ethanolic extracts of *Adiantum latifolium*

1. Reducing power assay

The total antioxidant activity can be measured by the reducing antioxidant power assay. The antioxidant compounds present in the sample form a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700 nm using UV-Spectrophotometer.

Preparation of standard and test solution

100 mg extract was dissolved in ethanol and made up to 50ml in a standard flask, the concentration of the test sample being 2mg/ml. Other concentrations of the samples were prepared by diluting 2.5ml, 5ml, 7.5ml and 10ml of the above solution and made up to 10ml with ethanol, each having a concentration of 0.5 mg/ml, 1mg/ml, 1.5 mg/ml and 2mg/ml respectively. Reference compound ascorbic acid also was prepared in the same concentrations under similar dilutions with water from standard ascorbic acid solution (2 mg/ml).

Working Procedure: To 1ml of the different concentrations of the extract (0.5mg/ml, 1mg/ml, 1.5mg/ml, 2mg/ml) mixed in distilled water then mix 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K₃ Fe (CN)₆]. The same concentrations of standard ascorbic acid, prepared in the similar way used as standard solution. All the solutions were incubated at 50°C for 20 minute. Then, the reaction was terminated by adding 2.5 ml of 10% trichloroacetic acid and the mixture was centrifuged at 650 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with the distilled water (2.5 ml) and 0.5 ml of 0.1% FeCl₃ was added. Blank solution was prepared in the same way without adding any extract. Then the absorbance was measured at 700 nm in a spectrophotometer against a blank sample^{4,6}.

2. Phosphomolybdate assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation.

Preparation of the standard and the test solution

10 mg of plant extract was dissolved in 1ml of DMSO. Then 100 µl, 50 µl, 10 µl was pipette out and made up to 10 ml using DMSO to give the desirable concentrations.

Standard ascorbic acid was prepared using same dilutions to get the same concentrations.

Working procedure

100 µl from each concentrations prepared sample was taken and 1ml of reagent solution was added to it and incubated in a boiling water bath at 95°C for 90 min. After 90 min, the absorbance of the solution was read at 695 nm. Ascorbic acid (10mg/ml) was used as the standard. The Phosphomolybdenum reduction potential (PRP) of the studied extracts were reported in percentage⁷⁻¹⁰.

RESULTS

Phytochemical screening

The results of the phytochemical screening of extracts of leaves of *Adiantum latifolium* revealed the presence of phytoconstituents such as flavonoids, saponins, carbohydrates, steroids, phenols.

Table 1: Results of phytochemical analysis

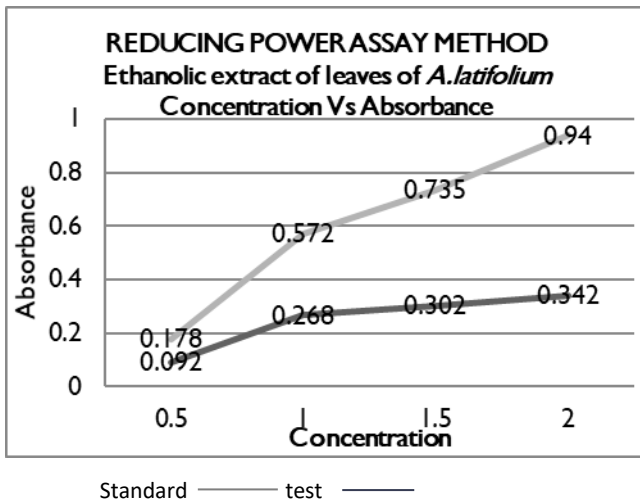
Chemical constituents	Ethanolic extract	Aqueous extract
Alkaloids	-ve	-ve
Saponins	+ve	+ve
Triterpenes	-ve	-ve
Carbohydrate	+ve	+ve
Steroids	+ve	+ve
Proteins	-ve	-ve
Flavonoids	+ve	+ve
Phenols	+ve	+ve
Cardiac glycosides	-ve	-ve

Antioxidant assay

1. Reducing power assay

Table 2: Results of reducing power assay

Concentration (mg/ml)	Absorbance of Test	Absorbance of Standard (Ascorbic acid)
0.5	0.092	0.178
1	0.268	0.572
1.5	0.302	0.735
2	0.342	0.940

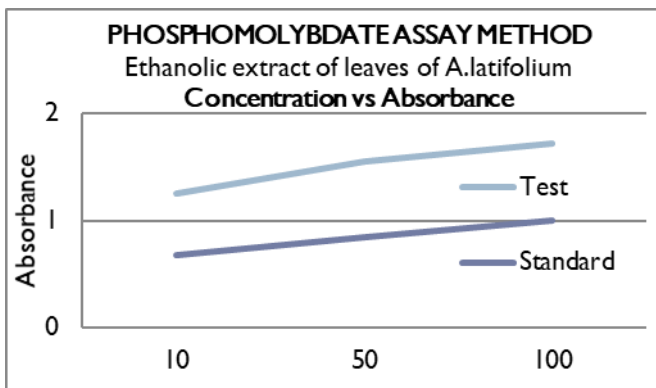


Graph 1: Reducing power of the extract from *Adiantum latifolium*

1. Phosphomolybdate assay

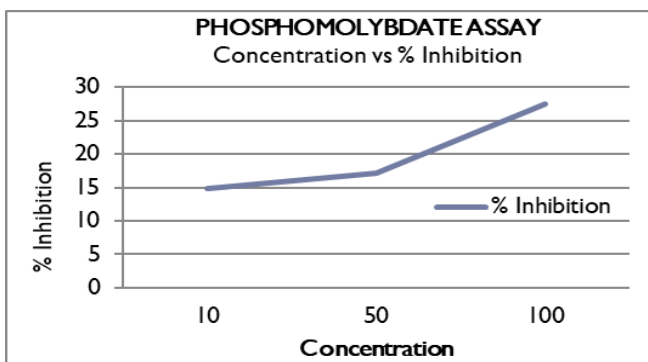
Table 3: Results of Phosphomolybdate Assay.

Concentration (µg/ml)	Absorbance of Standard	Absorbance of Test	% Inhibition
10	0.674	0.574	14.83
50	0.848	0.702	17.21
100	0.997	0.722	27.58



Graph 2: Phosphomolybdate assay of ethanollic extract of *Adiantum latifolium*

$$\text{Percentage inhibition} = \frac{\text{Absorbance of Standard} - \text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 100$$



Graph 3: Percentage inhibition graph of phosphomolybdate assay

CONCLUSIONS

Natural antioxidants that are present in herbs are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. It contains free radical scavengers like flavonoids and phenolic compounds. In this study, we have evaluated the free radical scavenging activity of ethanollic extract of *Adiantum latifolium*. *Adiantum latifolium* is one of the most interesting plants in the family Adiantaceae known for its diversified habits, gregarious nature. *Adiantum latifolium* is a highly potential medicinal plant in Ayurveda. The leaves are useful in traditional medicine as anxiolytic, analgesic and anti-inflammatory. In the present study, aqueous and ethanollic extracts of leaves of *Adiantum latifolium* were used. The preliminary phytochemical screening of extracts showed the presence of flavonoids, saponins, phenols, carbohydrates, steroids. The ethanollic extract was used for antioxidant studies. The study was carried out using two tests - reducing power assay and phosphomolybdate assay. The reducing power assay determines the total antioxidant activity by the comparative analysis with that of standard and the study concluded that the antioxidant activity increases with increase in concentration and they are comparable with that of standard at each concentration. Phosphomolybdate assay confirms the antioxidant activity by formation of phosphomolybdenum complex. The antioxidant activity was calculated using percentage inhibition, where the percentage inhibition increased the antioxidant activity also increased. The maximum inhibition was shown at the concentration of 100 µ/ ml.

Thus, this study concludes the antioxidant activity of the leaves of *Adiantum latifolium* and further studies could be done to establish the same.

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