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PHYTOCHEMICAL ANALYSIS OF *ADIANTUM* AND *PTERIS* FERNS & ITS ROLE AS ANTIOXIDANT

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ABSTRACT

A fern is any one or more of a group of about 12,000 species commonly known as Cryptogamic embryophytic vascular plants. They have stem roots and leaves like other vascular plants. Ferns reproduce via spores but with neither seeds nor flowers, Ferns are not of major economic importance, but some are grown or gathered for food, as ornamental plants, for remediating contaminated soils, ability to remove some chemical pollutants from the environment. Some are significant weeds. They also play a role in mythology, medicine, and art. *Adiantum* and *Pteris* plants have a great medicinal value as it has been reported to have versatile phytochemical constituents including flavonoid, phenols, saponins, tannins and saponins. The plants contain high phenol & flavonoid which indicates that the sample has antioxidant effects.

Key Words: Phytochemical, Flavonoids, Saponins, Tannins, Phenols.

Oxidation refers to transfer of electrons from a substance to an oxidizing agent. Oxidation reactions result in free radicals, which immediately start chain reactions that result in damage to the living cells. Metabolism in majority of complex living organisms requires oxygen for its survival. But oxygen is a highly reactive molecule damages living organisms by producing reactive oxygen species. Super oxide anion, hydrogen peroxide, peroxyl radicals, reactive hydroxyl radicals are the most common reactive oxygen species and nitric oxide, peroxy nitrite are the nitrogen derived free radicals.

An antioxidant slows or prevents the oxidation of the molecules, terminate these chain reactions, removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. Considered as reducing agents such as thiols, ascorbic acid, polyphenols. Antioxidants are classified into two broad divisions, whether they are soluble in water (hydrophilic) react with oxidants in the cell cytosol and the blood plasma,or in lipids (hydrophobic) protect cell membranes from lipid peroxidation. In general, the plants and animals maintain complex systems of multiple types of antioxidants, such as- glutathione, vitamin C, and vitamin E as well as enzymes such as Catalase, Superoxide dismutase and various peroxidases (Mangesh Khond *et al.,* 2009, Bandow *et al*., 2003),.

Low levels of antioxidants or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill the living cells. to investigate the in vivo antioxidant potential methanolic extraction of *Adiantum* and *Pteris* , the study of their phytochemical compositions, antioxidant activities including 1, 1-Diphenyl -2-picrylhydrazyl (DPPH) radical scavenging and reducing power activity. The medicinal plants are used in traditional treatments to cure various diseases for thousands of years. The aim of this study was to identify antioxidant efficacy through presence of scavenging activity

MATERIAL AND METHODS

Extraction

The plant materials were collected from the locality of Jagdalpur and Bhilai. The leaves were initially separated from the main plants body and rinsed with distilled water, and dried under shade paper towel in laboratory then homogenized into fine particles and stored in air tight bottles and were used for all the extraction process.

Preparation of extracts: Extraction of aqueous component

- **Cold aqueous extraction**

10g of each stem and leaves air dried powder was weighed and soaked separately in 50ml

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cold water in a conical flask stopper with rubber cork and left uninterrupted for 24 hrs and then filtered off using sterile filter paper (Whatman No: 1) into a sterile conical flak and subjected to water bath evaporation, where the solvent was evaporated at its boiling temperature 100°C. The extract was got with the help of muslin cloth and was subjected to centrifugation at 5000X rpm for 5 minutes and the supernatant was obtained and stored at 4°C for further use

Methanol extract

10g of each leaf air dried powder was weight and was placed in 100ml of organic solvent (methanol) in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 hrs after 24 hrs it was filtered with the help of muslin cloth and centrifuged at 5000X rpm for 15 minutes. The supernatant was collected and the solvent was evaporated to make the final volume of one-fourth of the original volume, giving a concentration of 40 µg/0.1ml stored at 40°C in air tight bottles for further studies .

Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). The yellow coloured precipitate was indicated that presence of alkaloids.

Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered it. The filtrates sample was used to test for the presence of carbohydrates.

Fehling's Test

Filtrates samples were hydrolysed with the dilute HCl, and neutralized with alkali then heated with Fehling's A and B solutions. The red coloured precipitate indicated that presence of reducing sugars.

Detection of glycosides

Sample extracts were hydrolysed with the dilute HCl, and then subjected to test for glycosides.

Borntrager's test

To 2 ml of filtrate hydrolysate,3 ml of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Pink color indicated the presence of glycosides.

- **Detection of steroids and terpenoids**

In 1 ml of methanol plant extract 1ml of chloroform was added and 2-3 ml of acetic anhydride was mixed then 1-2 drops of concentrated H_2SO_4 was added. Then dark green colouration of the solution indicated that the presence of steroids and pink or red colouration of the solution indicated that presence of terpenoid.

- **Detection of saponins**

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes only. Then formation of 1 cm layer of foam indicated that presence of saponins.

Foam Test

0.5g of plant extracts was shaken with 2 ml of water. The foam was produced persists for 10 minutes it was indicated that the presence of saponins.

- **Detection of phenols**

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. The bluish black colour was indicated that presence of phenols

- **Detection of proteins and amino acids**

Xanthoproteic Test: The extracts were treated with few drops of concentrated nitric acid. Then the formation of yellow colour indicated that presence of proteins.

- **Detection of flavonoids**

In Methanol extract 10% NaOH was added and dilute HCl was added to that solution. The change of colour from yellow to colourless provides the positive result.

- **Assay of free radical scavenging activity By DPPH (1, 1-Diphenyl -2-picrylhydrazyl) method**

The antioxidant activities were determined using DPPH, (Sigma-Aldrich, Germany;

M.W.394.32M) as a free radical. 1µg/ml solution of plant extract in methanol was prepared & 6×10^{-5}mol/L DPPH was prepared in methanol. 0.1 ml of plant sample extracts was added to 3.9 ml of DPPH solution. Then the decrease in absorbance at 517nm was recorded at 1 min interval up to 15 minute or until the reaction is reached a level. Firstly, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control. Ascorbic acid (Merck; M.W.176.13) was used as a standard. The experiment was carried out in triplate. Then the free radical scavenging activity was calculated by the following formula:

Percentage (%) DPPH radical scavenging activity =

[(Absorbance of control -Absorbance of test Sample)] / (Absorbance of control) × 100

Assay of reducing power

The reductive capability of the extract was quantified by the method of (Oyaizu., 1986).1 ml of extract (100, 200 and 300 µg/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 [K_3 Fe (CN) $_6$]. Similar concentrations of standard routine were used as standard. The mixture was incubated at 50°C for 20 minute. Then, the reaction was terminated by adding 2.5 ml of 10% trichloroacetic acid. The upper layer of solution (2.5 ml) was mixed with the distilled water (2.5 ml) and 0.5 ml of 0.1% $FeCl_3$ was added. Blank reagent is prepared as above without adding any extract. Then the absorbance was measured at 700 nm in a spectrophotometer against a blank sample. Result was found that increased absorbance of the reaction mixture indicated greater reducing power (Chopra, *et al.,* 2002)

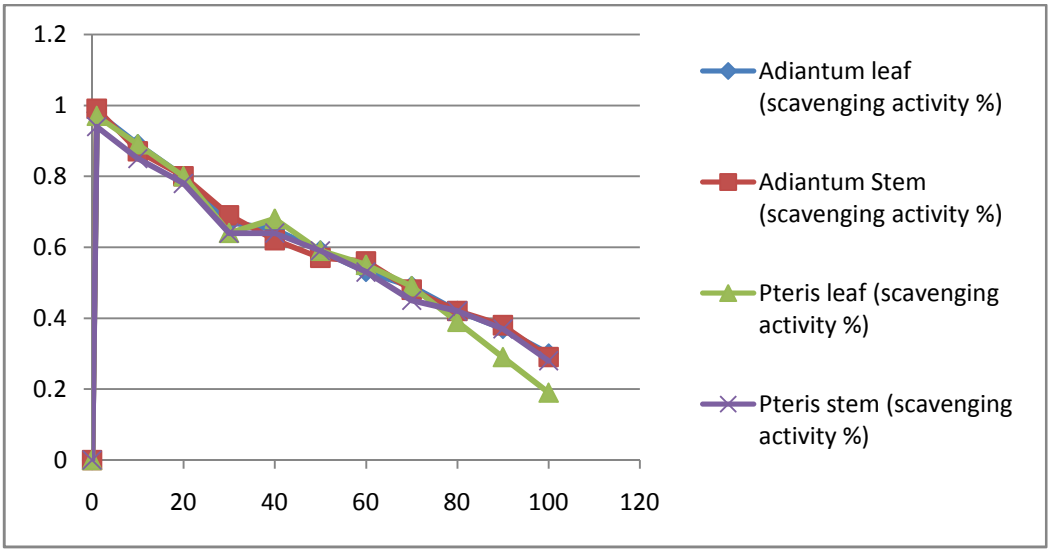
Table 1: Results of Phytochemical Analysis

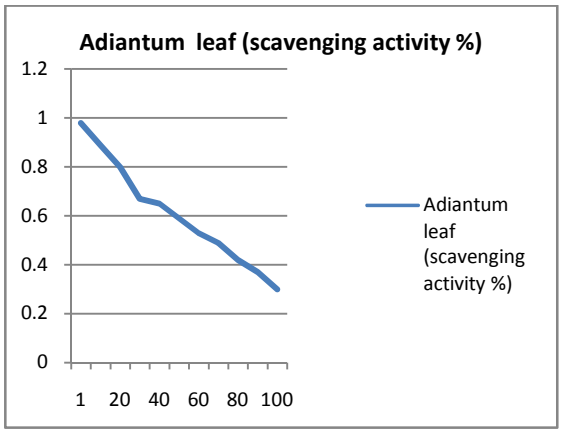
	Phytochemicals	***Adiantum* leaf**	***Adiantum* stem**	***Pteris* leaf**	***Pteris* stem**
1	**Tannins**	**+ve**	**+ve**	**+ve**	**+ve**
2	**Saponin**	**+ve**	**+ve**	**+ve**	**+ve**
3	**Flavonoids**	**+ve**	**+ve**	**+ve**	**+ve**
4	**Cardiac glycosides**	**--ve**	**-ve**	**--ve**	**-ve**
5	**Steroids**	**+ve**	**+ve**	**-ve**	**-ve**
6	**Terpenoids**	**+ve**	**+ve**	**+ve**	**+ve**
7	**Carbohydrates**	**-ve**	**-ve**	**-ve**	**-ve**
8	**Phenols**	**+ve**	**-ve**	**+ve**	**-ve**
9	**Proteins**	**-ve**	**+ve**	**-ve**	**+ve**
10	**Alkaloids**	**-ve**	**-ve**	**-ve**	**-ve**

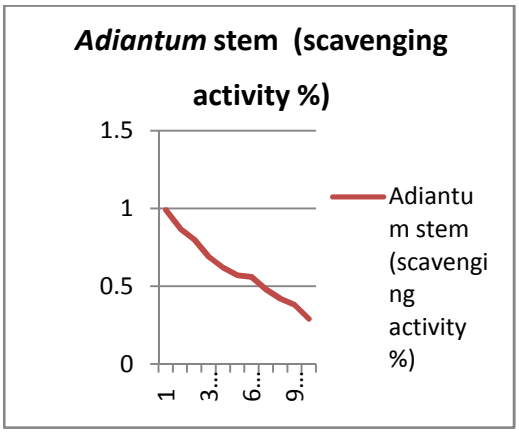
µg/ml	***Adiantum leaf* (scavenging activity %)**	***Adiantum Stem* (scavengingactivity %)**	***Pteris leaf* (scavenging activity %)**	***Pteris stem* (scavenging activity %)**
0	0	0	0	0
1	0.98	0.99	0.97	0.94
10	0.89	0.87	0.89	0.85
20	0.80	0.80	0.80	0.78
30	0.67	0.69	0.64	0.64
40	0.65	0.62	0.68	0.64
50	0.59	0.57	0.59	0.59

60	0.53	0.56	0.55	0.53
70	0.49	0.48	0.49	0.45
80	0.42	0.42	0.39	0.42
90	0.37	0.38	0.29	0.37
100	0.30	0.29	0.19	0.28

Table 2: Result of DPPH ASSAY







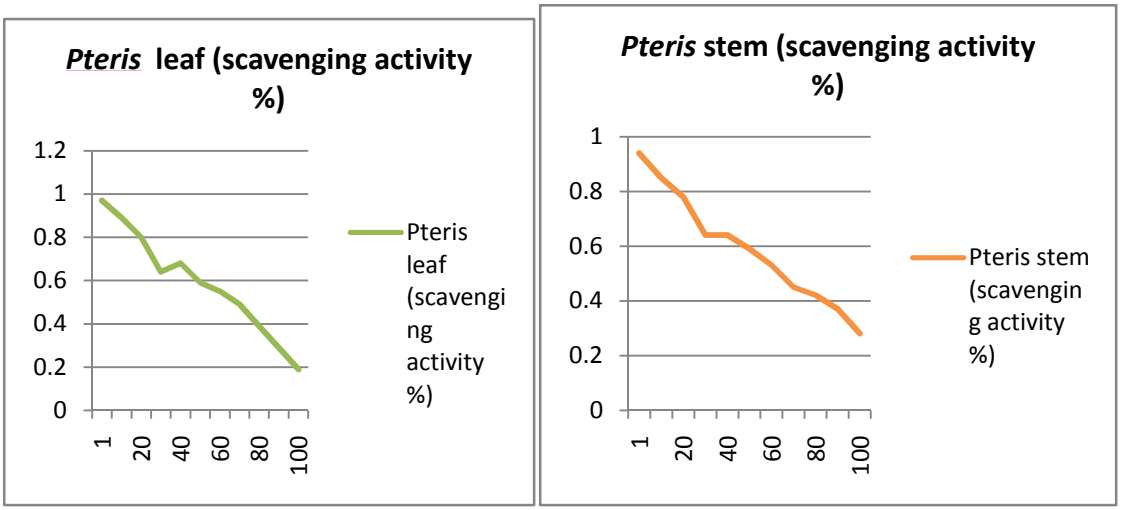
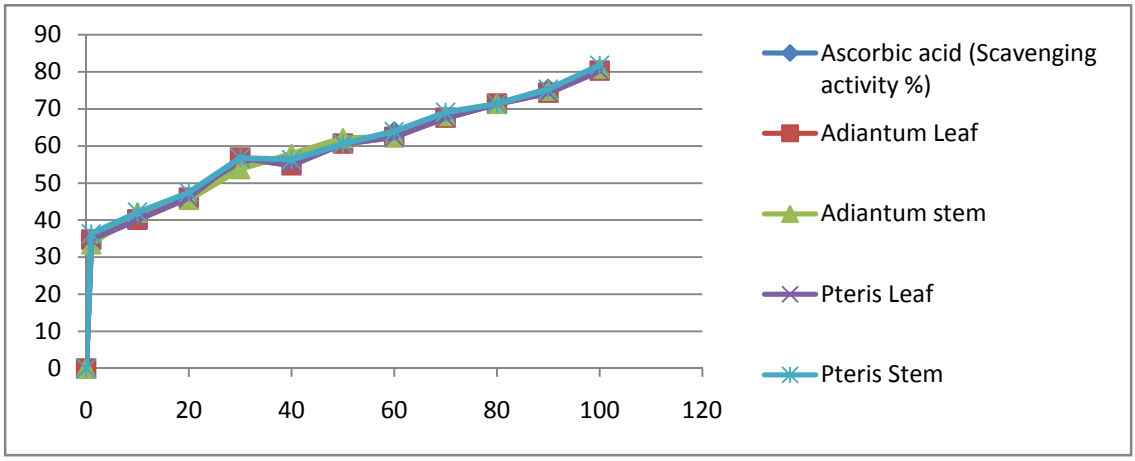
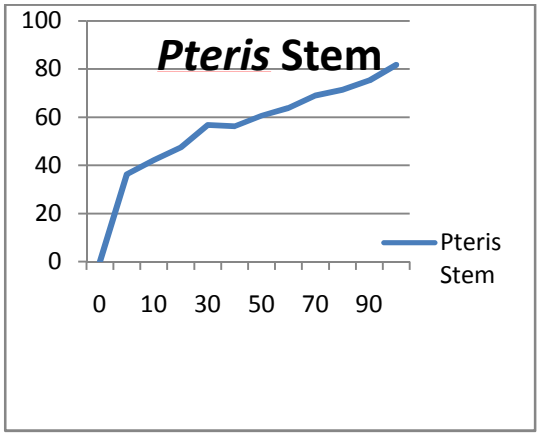
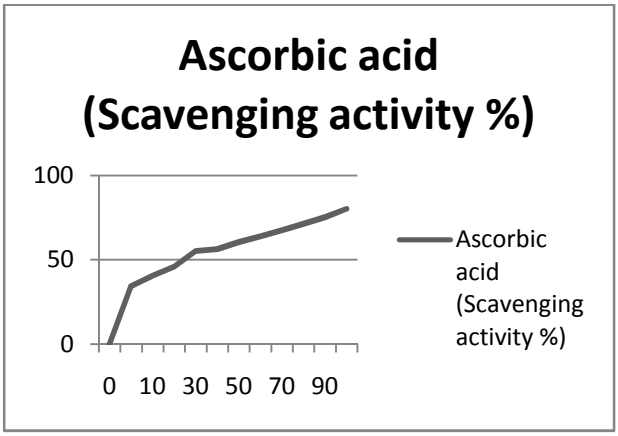


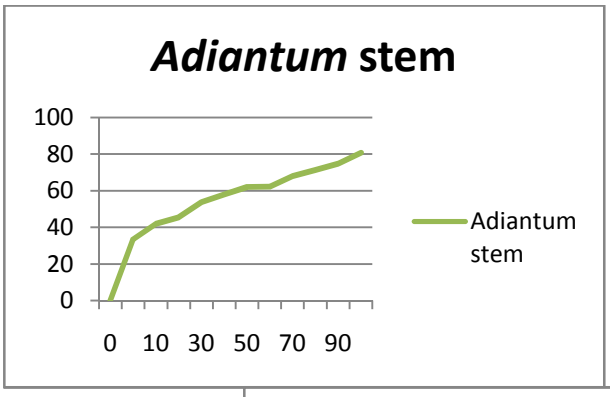
Table 3: Scavenging activity % of ascorbic acid and other parts of the plants

µg/ml	Ascorbic acid (Scavenging activity %)	*Adiantum* Leaf	*Adiantum* stem	*Pteris* Leaf	*Pteris* Stem
1	34.26	34.76	33.44	34.76	36.31
10	40.63	40.13	41.99	40.13	42.13
20	45.98	45.98	45.49	45.98	47.48
30	55.26	56.77	53.76	56.77	56.76
40	56.30	54.80	57.79	54.80	56.30
50	60.59	60.60	62.09	60.60	60.59
60	63.88	62.38	62.32	62.38	63.88
70	67.57	67.57	68.07	67.57	69.07
80	71.37	71.37	71.35	71.37	71.37
90	75.29	74.29	74.82	74.29	75.29
100	80.25	80.25	80.78	80.25	81.78


90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
Ascorbic acid (Scavenging activity %)
Adiantum Leaf
Adiantum stem
Pteris Leaf
Pteris Stem


Pteris Stem
100
80
60
40
20
0
0 10 30 50 70 90
Pteris Stem


Ascorbic acid (Scavenging activity %)
100
50
0
0 10 30 50 70 90
Ascorbic acid (Scavenging activity %)


Adiantum stem
100
80
60
40
20
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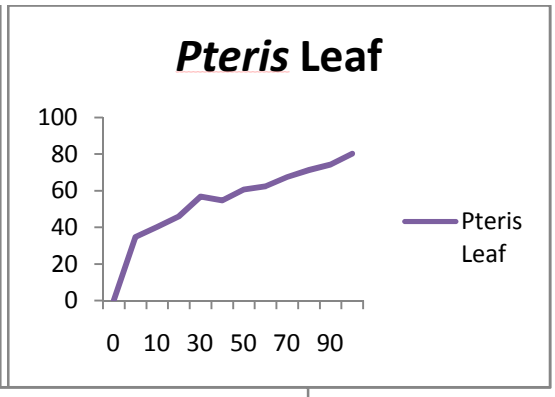
0 10 30 50 70 90
Adiantum stem


Pteris Leaf
100
80
60
40
20
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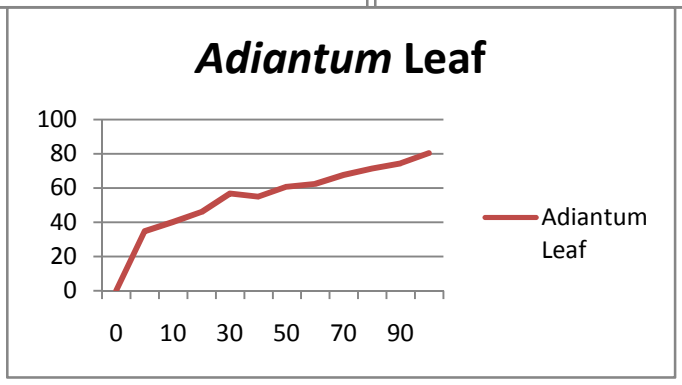
Pteris Leaf


Adiantum Leaf
100
80
60
40
20
0
0 10 30 50 70 90
Adiantum Leaf

DISCUSSION

Phytochemicals screening of aqueous and methanolic extract of *Adiantum* and *Pteris* leaf and stem showed the tannins, saponins, flavonoids & terpenoids steroids terpenoid .DPPH is a radical that has been used widely to evaluate the antioxidant activity of various natural products. In this study, DPPH scavenging activity has been found in plant extract due to decolourization of purple colour to yellow. In phytochemical analysis the result obtained the phenols, proteins, saponins, tannins and flavonoids are present, and they have been shown to have multiple biological functions, including antioxidant activity. In DPPH the absorbance is decreases due to presence of antioxidant activity. Due to decrease of absorbance the purple colour was turns to yellow. It was reported by (Yadav *et al.,* 2011) that DPPH absorbance is reduced by antioxidant compound or free radicals spices to become stable diagnostic molecules resulting colour change from purple to yellow that can indicates that hydrogen denoting ability of extract sample fern. There is a significant increase in absorbance of the reaction mixture indicates the reducing power. In this experiment leaf has a more reducing power than the stem as shown in graph. The results obtained clearly indicates that leaf and stem of fern have a significant potential to use as a natural antioxidant agent. The overall results of this study indicates that the various extract concentration from fern leaf have interesting antioxidative properties (Kaushik, *et al.,* 2010) and these plant samples could be utilize as potential source of natural antioxidant in the food or in pharmaceuticals industry.

CONCLUSION

This work analysed the phytochemical constitution and antioxidant activity of leaf and stem of fern. These plants have a great medicinal value as it has been reported to have versatile phytochemical constituents including flavonoid, phenols, saponins, tannins and saponins etc. Antioxidant activity of *Adiantum* and *Pteris* extracts has been found by means of free radical scavenging assays, reducing power assay. The plants contain high phenol & flavonoid which indicates that the sample has antioxidant effects.

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