Comparative Assessment of Phytochemicals and Antioxidant Potential of Four Ferns

By

V. Karthiya

Reg.No.15PBO003

A Dissertation Submitted to the

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore – 641-043.

In Partial Fulfillment of the Requirements for the Degree of

Degree of Master of Science in Botany

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A. Vijayalal 8/4/17

Signature of the

Head of Department

08/04/17

Signature of Supervisor

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Contents

List of Tables

List of Figures

List of Plates

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
दक्षिणी क्षेत्रीय केन्द्र / Southern Regional Centre
टी,एन.ए.यू कैम्पस / T.N.A.U. Campus
लाउली रोड / Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003
टेलीफोन / Phone: 0422-2432788, 2432123, 2432487
टेलीफक्स/ Telefax: 0422- 2432835
ई-मैल /E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.के./No.: BSIS/RC/5/23/2016/Tech. 1252 दिनांक/Date: 20th December 2016

सेवा में / To

Ms. V. Karthiya
II M. Sc. Botany
Department of Botany
Avinashilingam University
Coimbatore

महोदया/Madam,

The plant specimens brought by you for identification are identified as follows:

(1) *Adiantum raddianum* C. Presl (= *Adiantum cuneatum* G. Forst) - PTERIDACEAE
(2) *Pteridium aquilinum* (L.) Kuhn – PTERIDACEAE
(3) *Pteris quadriaurita* Retz. – PTERIDACEAE
(4) *Hemionitis arifolia* (Burm.f.) T. Moore (Basionym: *Asplenium arifolium* Burm.f.) - PTERIDACEAE

The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

GVS Murthy
20/12/16

(डॉ. जी.वी.एस. मूर्ति /Dr. G.V.S. Murthy)
वैज्ञानिक 'जी' एवं कार्यालय अध्यक्ष /
Scientist 'G' & Head of Office

वैज्ञानिक 'जी' एवंकार्यालयअध्यक्ष
SCIENTIST 'G' & Head of Office
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
दक्षिणी क्षेत्रीय केन्द्र
Southern Regional Centre
कोयम्बत्तूर / Coimbatore - 641 003.

1. INTRODUCTION

The Pteridophytes, also called reptile group of plants consists of the ferns and fern allies. These are one of the oldest and primitive plant groups on the earth and constitute a vast group of vascular cryptogams. They grow luxuriantly in moist, tropical and temperate forests and can adapt well in terrestrial habitat (Thomas, 2011). About 12,000 species of extant pteridophytes are recorded globally. India has a rich population of pteridophytes; most of the species appear in either the region or in the South Indian Mountains called the Western and Eastern Ghats (Gowrisnkar *et al.,* 2011). India represents more than 1,200 species of Pteridophytes in its tropical, subtropical and warm temperate regions belonging to 70 families which include 191 genera (Dixit and Vohra, 1984). In India almost 67 % of Pteridophytes are found in Eastern Himalayas and Northeast India.

Most of the aboriginal people are not well identified about the uses of Pteridophytes ever since it's not simply available like flowering plants. Among the 1,200 species of Pteridophytes 170 species have been found to be used as food, flavour, dye, medicine, bio-fertilizers, oil, fiber and bio-gas production (Manickam and Irudayaraj, 1992), and 66 were found to be used as a medicine in the different parts of the country. Among them 47 are endemic to India (Jenkins, 2008) and some of these species are placed under Rare, Endangered and Threatened (RET) category.

According to World Health Organization about 80% of the world population depends on the natural product (Kumar and Nagarajan, 2012) for their health due to minimal side effect, cost effective (Jagtap *et al.,* 2009) reduced toxicity and uncomplicated availability (Khan *et al*., 2009). Medicinal plants are rich sources of safe and effective medicines (Tiwari, 2008) and have been used by human being since ages in traditional medicine either in the form of plant extracts or pure compounds (Parekh and Chanda, 2007) due to their therapeutic potential and this search led to the discovery of novel drug candidates used against diverse diseases. Traditional medicinal information is important not only for its potential contribution to the drug development as well as people's healthcare (Pei, 2001).

Plants are a rich source of secondary metabolites with interesting biological activities. For few decades, phytochemicals (secondary plant metabolites), with unidentified pharmacological activities, have been comprehensively investigated as a source of medicinal agents (Krishnaraju *et al*., 2005).

Pteridophytes has been used as folk medicine, for more than 2000 years, and also been mentioned in ancient literature (Kumar and Roy, 1972; Watt, 1972). Theophrastus (327-287 B.C.) and Dioscorides (100 A.D.) have referred to medicinal attributes of certain ferns. Sushruta and Charaka (100 A.D.) mentioned medicinal uses of *Marsilea minuta* Linn. and *Adiantum capillus-veneris* Linn. in their Samhitas 5, 6. Pteridophytes are used in Homeopathic, Ayurvedic, and Unani medicines, and provided insecticides, antibiotics, food and ornamentation (Benniamin, 2011). They were found to be used to cure various diseases by the rural and tribal communities of India (Prathiha *et al*., 2011). Pteridophytes are not infected by microbial pathogens, which may be one of the significant aspects for the evolutionary achievement of pteridophytes and the information that they stay alive for more than 350 million years (Sharma and Vyas, 1985).

Experimental evidence suggests that free radicals and reactive oxygen species (ROS) can be involved in a high number of diseases such as cardiovascular diseases, cancer, neurodegenerative diseases, diabetes and aging (Richards and Sharma, 1991; Niwa, 1991 and Li *et al.,* 2010). Free radicals occur continuously in all cells as part of normal function. Oxygen free radicals can cause damage of biological tissue and lead to their injury. The mechanism of damage involves lipid peroxidation, which destroys cell structures, lipids, proteins and nucleic acids. They cause damage to cell membranes with the release of intracellular components, leading to further tissue damage (Poli *et al.,* 2004). Antioxidant enzymes and non-enzymatic defense system minimizes the harmful effect of ROS by various antioxidant mechanism.

Antioxidant supplements or food rich in medicinal plants are utilized to assist the human body in reducing harm by free radicals (Radha and Padma, 2011). Natural or phytochemical antioxidants are secondary metabolites in plants such as phenolic acids and flavonoids which are amongst the antioxidants produced by plants for their sustenance (Apak *et al.,* 2007). Recently, phenolics and flavonoids have been considered as great antioxidants and proved to be more effective than Vitamin C, E and carotenoids (Dai and Mumper, 2010).

The purpose of this study was to evaluate the phytochemical and antioxidant property of ethanol, acetone and aqueous extracts of four Pteridophytes from Western Ghats, Valparai. To our knowledge there were not much study regarding enzymatic and non-enzymatic studies and only few studies regarding free radical scavenging properties were reported.

The objectives of this present study are listed below:

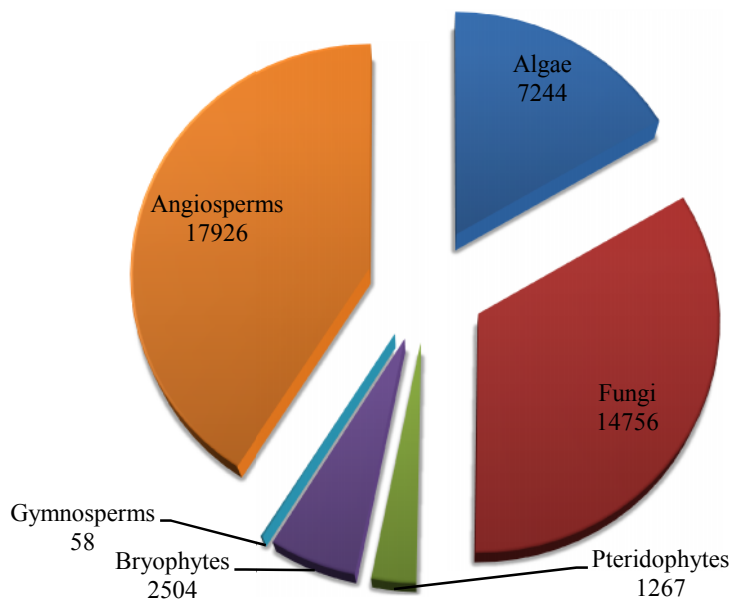
1. To study the phytochemicals present in the four ferns in ethanol, acetone and aqueous extracts.
2. To compare the qualitative analysis of phytochemical analysis of the four ferns in ethanol, acetone and aqueous extracts.
3. To analyse the quantitative phytochemical constituents in the four ferns in ethanol, acetone and aqueous extracts.
4. To evaluate the level of enzymatic and non enzymatic antioxidants in the four ferns and to compare it among them.
5. To study the free radical scavenging activity of the four ferns in ethanol, acetone and aqueous extract.

2. REVIEW OF LITERATURE

Pteridophytes are the most primitive seedless spore-bearing vascular plants having the oldest lineage of vascular plants, and the second-most speciose after angiosperms with approximately 12,000 species. About 250 million years ago the dominant part of earth's vegetation was pteridophytes, but now in present day has been largely replaced by the angiosperms (Dudani *et al.,* 2012). They also range greatly in size, leaf form and growth form. In India they have a varied habitat of tropical, sub-tropical, temperate and most deciduous forests.

Worldwide pteridophytic population accounts to 305 genera, comprising more than 13,000 species (Bir, 1992 and Chandra 2000). In India, about 191 genera comprising over 1250 species were recorded. According to detailed census, the Pteridophytic flora of India comprises of 67 families, 191 genera and more than 1,000 species (Dixit and Vohra, 1984) including 47 endemic Indian ferns, less than 10% of those reported previously and 414 species of Pteridophytes (219 At risk, of which 160 critically endangered, 82 Near-threatened and 113 Rare), constituting 41-43% of the total number of 950-1000 Pteridophytes. Chandra, (2000) recorded 34 families, 144 genera and more than 1100 species of ferns with about 235 endemic species from Indian region.

Fig.1 Status of Plant Species in India



In Tamil Nadu, the pteridophytes (ferns and fern allies) are represented by about 275 species in 44 families, of which 33 are endemic to the state and about 80 are recognised as threatened taxa (Manickam, 2007). Terrestrial species constitute more than 46% (about 90 species), whereas, lithophytes constitute 19% (67 species) of the pteridophyte flora of the state. Besides, there are several semi-aquatic ferns and fern allies and only five true aquatic ferns, such as *Azolla pinnata*, *Ceratopteris thalictroides* and *Salvinia molesta*, are found in Tamil Nadu (Arisdason and Lakshminarasimhan, 2016).

Valparai hills of the Western Ghats that is rich biodiversity and indigenous population. It is located in the Western boundary range in Coimbatore district in the Southwest of Tamilnadu and lies between at 10.37 N 76.97 longitudes and E10.37:76.97 latitude form a portion of Tamilnadu. It has an average elevation of the hills ranges between 1193 meters (3914 feet) above (MSL) and about annual rainfall between 3523.3mm to 2882.7 mm, and temperature various between 23.6 ℃ to 19.9 ℃. The vegetation type of forest is moist deciduous forest, and evergreen forest present (Santhoshkumar and Nagarajan, 2014).

Traditional uses of Pteridophytes

Traditional medical knowledge is important not only for its potential contribution to drug development and market values, but also for the people's healthcare (Pei, 2001). Traditionally people used pteridophytes as medicine and anti bacterial agents. Recently there has been growing interest in exploiting the biological activities of flora and fauna owing to the natural origin, cost effectiveness and lesser side effects (Ahmad and Srivastava *et al*., 2008 and Chellaram and Edward *et al.,* 2009). About 61 species of Pteridophytes have been reported to have medicinal uses among the various tribal and non-tribal people in the Western Ghats and these plants were frequently used to treat stomach problems, poisonous bites, nervous disorders, cough, fever, asthma and diabetes (Benjamin and Manickam, 2007).

Pteridophytes are used in homeopathic, ayurvedic, tribal and unani medicines provide insecticides, antibiotics, food and ornamentation but habitat destruction by man has today endangered more than 10% of the fern species. Various researchers have reported that few ferns have medicinal uses and they have mentioned their numbers as follows: 27 species by Kirthikar *et al.,* (1935), 44 species by Chopra *et al.,* (1956), 11 species by Nadkarni (1954), 29 species by Nayar (1959) and 61 by Benjamin and Manickam (2007).

Economic Importance of Pteridophytes

Ferns don't have any 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 (Shrivastava *et al.,* 2014). Out of 1,000 species of pteridophytes that occur in India, 170 species have been found to be used as food, flavour, dye, medicine, bio-fertilizers, oil, fiber and bio-gas production (Manickam and Irudayaraj, 1992).

Medicinal properties of ferns

Pteridophytes have a vital role in the earth's biodiversity. Commercial and medicinal values of higher plants have been investigated carefully, unfortunately pteridophytes have been unnoticed. That's why there is lack of available information on literature regarding medicinally important except a few studies. (Caius, 1935. and Manandhar, 1996). Pteridophyte plants have medicinal value. (Singh *et al*., 2001).

Medicinal ferns are gaining importance in recent days by the fact that several medicinal ferns from India have been subjected to phytochemical analysis. The rare, endangered and medicinally important spleenworts, *Asplenium sp.* and *Psilotum* have been investigated phytochemically by Lal (1979), Khare and Shankar (1987) and Varma (1992). It has been confirmed that the spores of the brackish water fern *Achrosticum aureum* Linn. seem to be a potential allergen (Yesmeen and Devi 1987). An oil, which can be used as a potential antibiotic and anticancer chemotherapeutic agent has been extracted from various species *Ophioglossum.* Ethnobotanical observations on ferns and ferns allies of 2007 reported medicinal properties of pteridophytes from the western Ghats, India.

The ferns taken for the study were *Pteridium aquilinum*, *Hemionitis arifolia, Adiantum raddianum* and *Pteris quadriaurita.* The details of phytochemical, and antioxidant work done so far with regards to the ferns taken for the study has been reviewed here.

Pteridium aquilinum

Pteridium is a cosmopolitan genus that acts as aggressive and invasive plant, (Matos *et al.,* 2014) in many parts of the world, except in the Polar Regions (Marrs and Watt, 2006). It is considered as one of the five most abundant plants occupying a variety of habitats throughout the earth (Shahin *et al.,* 1999) which can produce leaves even on burnt areas

quickly. The term 'bracken' is used as a common name for the four *Pteridium s*pecies belonging to the Dennstaedtiaceae family (Marrs and Watt, 2006; Rasmussen *et al*., 2015). It appears to contain a wider variety of secondary plant compounds than all other ferns (Cooper-Diver, 1976). It overcomes its competitors or adjacent vegetation by shadowing the surrounding areas with its high canopy, which reduces the access to light.

Phytochemicals

A water soluble norsesquiterpene glycoside ptaquiloside, is the major carcinogen in bracken (Smith and Seawright, 1995). It has been researched and demonstrated that direct consumption of bracken fern by cattle causes diseases such as bovine enzootic haematuria (Rasmussen *et al.,* 2015; Somvanshi *et al.,* 2006). Allelopathic phytotoxins or secondary metabolites produced by them suppresses the growth of other plants in the near by vicinity (Madeja *et al*., 2009).

Previous phytochemical investigations of this plant have led to the purification of polysaccharide (Xu *et al.,* 2009) and the isolation of 5-hydroxypyrrolidin-2-one (Chen *et al.,* 2008), a variety of proanthocyanidins (Markham, 1988), sesquiterpene glycoside ptaquiloside (Niwa *et al.,* 1983; Hirono *et al.,* 1984) and flavonol glycosides (Wang *et al.,* 1973; Imperato, 1995, 1996; Imperato and Minutiello, 1997). It contains flavanoids, which have antibiotic and antibacterial properties (Pieroni and Quave, 2005; Hassan *et al.,* 2007) investigated a novel homoflavanol derivative with a cyclopentene-polyol ester substituent on B ring (1) together with two known flavonoids kaempferol (2) and quercetin (3) from *P. aquilinum.*

The carcinogenic compound in bracken, ptaquiloside can damage DNA, blood cells and destroys thiamine (Vitamin B1). This in turn causes beriberi, a disease linked to nutritional deficiency. Hydrogen cyanide is released by the young fronds of bracken when eaten by mammals or insects. The ferns like *Pteridium aquilinum* were toxic in nature and subsequent doses of the same extract for longer therapy might be attributed to toxicity. The clinical trials of the powdered extract of whole plant, as a single dose for in-vitro study (100 mg /ml) attempted to study its anthelmintic property showed no side effects and symptoms of toxicity.

Anti-oxidant activity has been reported for a water-soluble polysaccharide from *Pteridium aquilinum* (Xu *et al*., 2009). The plant is used as an anti-fungal agent in the traditional medicinal system of Tanzania; the use has been validated through scientific studies

(Hamza *et al.*, 2006). The plant is also used as a folk phytotherapeutic in the Tyrrhenian part of the Basilicata region of southern Italy (Guarrera *et al*., 2005).

Uses

The species is traditionally used by the Chinese people to heal jaundice with damp-heat pathogen, rheumatoid arthritis and hypertension. Studies has confirmed some useful functions of starch from bracken rhizome, such as purging heat, detoxification, preventing cancer, and enhancing health (Liu *et al.,* 2006). Wild braken (*Pteridium aquilinum* (L) Kuhn. *Var. latiusculum*) has been described as one of the most common ferns in China. The use of its fronds as food appears to be widespread (Xu *et al*., 2009). Fronds were employed in antirheumatic mattress. The aerial parts of the plant were used as litter for domestic animals and to make brooms to clean ovens from ashes and sticks (Salerno *et al.,* 2005). It is used to treat worms and to relieve stomach cramps and increases urine flow (Rout *et al.,* 2009). A veterinary use of the plant was reported by Viegi and coworkers (Viegi *et al.,* 2003): in particular, a decoction of leaves and roots of *Pteridium aquilinum* was used for the expulsion of the placenta in cows. Other uses (antibacterial and diuretic properties) of the leaf decoction were reported (Mustafa *et al.,* 2012). An antihypertensive activity was attributed to the fronds of the plant (Carrio and Valles*,* 2012). Bracken contain many nutrients, such as carbohydrates, protein, fat, vitamins, carotenoids, and trace mineral (Wang and Wu, 2013).

Hemionitis arifolia

H. arifolia (Burm) Moore belonging to family hemionitidaceae is endemic (Rukmini *et al.,* 2015) and were normally distributed in higher and lower elevations (Murty *et al*., 2011). It has been used in burns, menstrual disorders, antifertility and anti-flatulence. *H. arifolia* fronds are simple, rhizome short, sub erect, clothed with narrow brown scales. The fern is both an epiphyte and grows on trees as well as a terrestrial plant. It is also used in folk medicinal practices to treat diabetes mellitus in certain remote villages of Trivandrum district, Kerala state. This fern is also used to treat aches and burns. Leaf extract is applied to centipede bite and wounds and about 10g of root powder is taken orally with water in empty stomach twice a day for 10 days for treatment of hypertension (Nair *et al*., 2006; Rao *et al.,* 2007; Rout *et al*., 2009 and Sahayaraj *et al*., 2009).

Phytochemicals

Rukmini *et al*., (2015) through GC-MS analysis reported ten major chemical constituents (4- methyl & Mesityl oxide, 4- Hydroxy -4- methyl & diaceton, 1,3- dimethyl &

m- xylene & m-xylo, 14- Ethylene, 3,7,11,15 –tetramethyl-R, n-Hexadecanoic acid, Phytol, oleic acid, stearic acid and 4- stigmasten-3-one.

Uses

Hemionitis arifolia is used to treat cuts, wounds and menstrual disorders and Leucostegia immerse for treating boils and constipation (Benjamin and Manickam, 2007). Frond juice has been used to cure burns and a folklore anti-diabetes fern, was evaluated for its hypoglycaemic and anti-diabetic properties using rats (Mannan *et al.,* 2008) and (Nair *et al*., 2006). It is also used to control Hypertension (Rout *et al.,* 2009).

Adiantum raddianum

Adiantum linn. belonging to Adiantaceae family is one of the most common and widely distributed species. The genus *Adiantum* is commonly called as maiden - hair fern and it is derived from the Greek word "*adiantos*," which means unwetted. These ferns are aptly named because the leaflets repel water; when their fronds are plunged into water, they emerge dry. However, during the survey it was observed that different species of *Adiantum* are being reported under the various vernacular names as avenca in Brazil; hansraj/ hanmspadi in India; maiden hair fern in English (Singh *et al.,* 2013). *Adiantum* has about 23 species in India widely distributed in the Indian subcontinent. Maximum number (15 species; 78%) of species occurs on the mountains of south India. The plant selected for study is *Adiantum raddianum* C. Presl Adiantaceae; its synonym is *Adiantum cuneatum* Langsd & Fisch. The plant is a common terrestrial or lithophytic herb. The plant grows in evergreen and semi-evergreen forests at high altitudes (Easa and Nandhakumar, 2003).

Phytochemicals

A number of phytoconstituents were reported in aforesaid *Adiantum* species like β-sitosterol, stigmasterol, compesterol (Marino *et al*., 1989), oleanane compounds, astragalin, isoquercitine, rutin (Yoko & Masso, 1969), triterpenoids, keto alcohol, α-carotene monoepoxide, leucopelargonidin, kaempferol, and quercitin glucosides (Rangaswami & Iyer, 1967).

Uses

The traditional uses of *Adiantum* species known to be for respiratory problems such as cough cold, fever, pneumonia, and mucous formation (Chopra *et al.,* 1956). All the species are commonly grown as ornamental plants and majority of them are also used in traditional medicines to cure various diseases like cough, fever, skin diseases, catarrhal affection, throat

infection, bronchial disorders, dysentery, ulcer, epilepsy, leprosy, biliousness, inflammation, tumors of spleen, liver and other viscera, cold, headache, piles, hair growth etc. It is also considered as tonic and diuretic (Paul and Jeeva *et al*., 2012).

Eight different species of the genus *Adiantum* are medicinal, but *Adiantum raddianum* is not reported as medicinal (Nayar, 1959). Ethnomedicinally, the genus is important and popularly known as "Hansraj" in Ayurvedic system of medicine. It has been used in cold, tumors of spleen, liver and other viscera, skin diseases, bronchitis and inflammatory diseases. It is ails considered as toxic and diuretic (Singh *et al.*, 2008). *Adiantum raddianum* C. Presl are sold in the market by the trade names "Hansraj" and "Paroshan".

Pteris quadriaurita

Pteris quadriaurita Retz. belonging to the family Pteridaceae, is a common terrestrial herb growing in semi-shaded localities having completely dry soils in plains and Ghats at lower altitudes.

Uses

Pteris quadriaurita used as antihelmintic (Nayar, 1959). Decoction of fresh rhizome and fronds of *Pteris quadriaurita* are given in chronic disorders arising from obstructions of viscera and spleen. (Chopra *et al.,* 1992). Plants used as antihelmintic. Decoction of fresh rhizome and fronds were given in chronic disorders arising from obstructions of viscera and spleen. Leaf juice (10ml) along with fresh Date palm (*Phoenix sylvestris*) toddy (200 ml) for five days internal use, early in the morning before sunrise and in the evening after sunset in empty stomach, normalizes menstrual cycle (Padhy and Dash, 2015).

Phytochemical studies

In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents Krishnaraju *et al*., 2005. Plants gifted with numerous phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and additional metabolites, are rich source of available antioxidant activity (Zheng and Wang, 2001; Cai *et al*., 2003).

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.

Antioxidants

Antioxidants are substances that remove, prevent or delay oxidative damage to a target molecule. Therefore, an antioxidant may act to control the level of free radicals to counteract oxidative damage (Rafieian-Kopaie and Baradaran, 2013). Antioxidants can be classified in to three main types : first line defence antioxidants (SOD, GAT, GTx, glutathione reductase and some minerals like Se, Mn, Cu, Zn), second line defence antioxidants (GSH, Vit.C, uric acid, alb umin, bil irubin, vitamin. E , carbotenoids flavonoids etc.) and third line defence antioxidants (complex group of enzymes for repair of damaged DNA, damaged protein, oxidised lipid and peroxides and also to stop chain propagation of peroxyl lipid radicals (Gupta and Sharma, 2006).

A lot of evidence on protective and curative effects of medicinal plants on various complications have been reported. Some of these effects include antimicrobial (Bahmani *et al.,* 2012), anti-cancer (Shirzad *et al.,* 2012), antidiabetic, anti-atherosclerosis (Rafieian-Kopaie *et al.,* 2011 and Nasri, 2013), immune-modulatory (Shirzad *et al.*, 2009), and even renoprotection or hepato-protective effects (Rafieian-Kopaie and Baradaran, 2013; Rafieian-Kopaie and Nasri, 2012; Nasri, 2013; Baradaran and Rafieian-Kopaie, 2013 and Rafieian-Kopaie and Nasri, 2013). Polyphenols are useful phytochemicals, which provide health benefits such as antioxidants (Hit *et al*., 2016).

Antioxidant properties of Pteridophytes

Oxidative stress has been identified as the root cause of the development and progression of several diseases (Kasote *et al*., 2015).

Studies have shown that many antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities. (Sala *et al*., 2002 and Rice-Evans *et al*., 1995). The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing . Ashokkumar *et al*., 2008. Veerapur *et al*., 2009. and in recent years.

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saponins. The plants contain high phenol & flavonoid which indicates that the sample has antioxidant effects.

The phytochemical analysis and antimicrobial activity of *A. lunulatum* and *H. arifolia* is confirmed and this can be further investigated to separate and used in the pharmaceutical application for the development of new drugs (Moorthy *et al.,* 2014).

Studies have revealed that lots of these antioxidant compounds have anti-inflammatory, antitumor, antimutagenic, anticarcinogenic, antibacterial, antiatherosclerotic, as well as antiviral activities. (Sala *et al.,* 2002 and Rice-Evans *et al.,* 1995). The eating of natural antioxidants has been related with reduced hazards of cancer, diabetes, cardiovascular disease and other diseases associated with ageing. (Ashokkumar *et al*., 2008 and Veerapur *et al*., 2009).

Free radical Scavenging

Free radicals have been implicated in many diseases such as cancer, atherosclerosis, diabetes neurodegenerative disorders and aging. Previous research reports suggest that higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases particularly cardiovascular diseases and cancer.

In modern years, there has been a universal trend on the way to the use of the natural phytochemicals existing in berry crops, herbs, oil seeds, fruits and vegetables beans, teas etc. (Kitts *et al.,* 2000; Muselík *et al.,* 2007 and Wang *et al.,* 2000). In modern centuries, secondary plant metabolites (phytochemicals), up to that time unknown pharmacological activities have been widely studied as a source of therapeutic agents Krishnaraju *et al.,* 2005. Therefore, it is predicted that phytochemicals with adequate antibacterial value will be used for the handling of bacterial infections Balandrin *et al.,* 1985. In view of the rich diversity of Indian medicinal plants as well as Pteridophytes, it is predictable that, the screening of plant extract for antibacterial activity might be helpful for humans and plants diseases Sharma *et al*.,1985.

Herbal drugs and natural products have been known to human being for many years and they have used as a soure of different therapy and treatment of many diseases (Singh, 1999).

For the treatment of infectious diseases, different medicinal plants have been mentioned by many phytotherapy manuals because of Numerous plants have been investigated for treatment of urinary tract infections, gastrointestinal disorders, and

respiratory and cutaneous diseases (Somchit *et al*., 2003). Antioxidant properties and free radical scavenging activity are found naturally in plants which is helpful for the mankind to develop natural drugs.

3. MATERIALS AND METHODS

3.1 Collection of plant material:

Plant materials were collected at respective places as given in the table.1. Collected ferns were authenticated by Botanical survey of India, Coimbatore, Tamil nadu, India.

Table 1. List of plant materials taken for this study

S.No	Plant Name	Family	Place
1	*Pteridium aquilinum*	polypodiaceae	Stanmore, Valparai, Coimbatore, Tamil Nadu, India.
2	*Hemionitis arifolia*	Hemionitidaceae	Pudhukadu, Valparai, Coimbatore, Tamil Nadu, India.
3	*Adiantum raddianum*	Adiantaceae	Pudhukadu, Valparai, Coimbatore, Tamil Nadu, India.
5	*Pteris quadriaurita*	Pteridaceae	Monica estate, Valparai, Coimbatore, Tamil Nadu, India.

Leaves were rinsed with distilled water. Afterwards, the samples were dried under shade, ground and sieved for extraction process.

3.2 Solvent extraction

3.2.1 Aqueous extraction - [Farombi *et.al,* 2003]

Powdered samples (10g each) was weighed and soaked separately in 50ml cold water in a conical flask stoppered with rubber cork and kept in orbital shaker for 24 hours. It was then filtered off using sterile filter paper (What Man No: 1) into a sterile conical flask.

3.2.2 Ethanol Extract - Maceration

Powdered samples (10 g each) were weighed. The extracts were prepared in same as in aqueous extraction and stored at 4º C in air tight containers.

3.2.3 Acetone Extract - Maceration

Powdered samples (10 g each) were weighed. The extracts were prepared in same manner as in aqueous extraction and stored at 4º C in air tight containers.

Plate – 1

Classification	
Kingdom	Plantae
Division	Pteridophyta
Class	Pteridopsida
Order	Polypodiales
Family	polypodiaceae
Genus	***Pteridium***
Species	***aquilinum***

Plate -2

Classification	
Kingdom	Plantae
Division	Pteridophyta
Class	Pteridopsida
Order	polypodiales
Family	Hemionitidaceae
Genus	***Hemionitis***
Species	***arifolia***

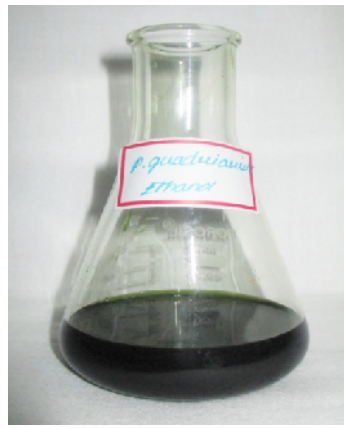
Plate 3

Classification	
Kingdom	Plantae
Division	Pteridophyta
Class	Pteridopsida
Order	Polypodiales
Family	Adiantaceae
Genus	***Adiantum***
Species	***Raddianum***

Plate 4

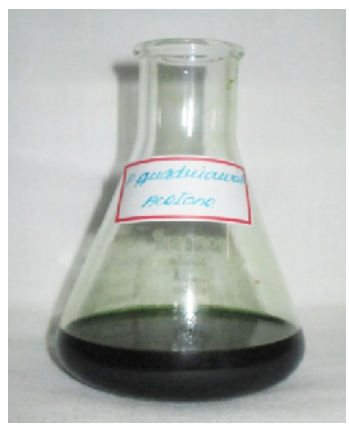
Classification	
Kingdom	Plantae
Division	Pteridophyta
Class	pteridopsida
Order	polypodiales
Family	Pteridaceae
Genus	***Pteris***
Species	***quadriaurita***

Plate 5. Powder of *Pteris quadriaurita* and it's Ethanol, Acetone and Aqueous Extract

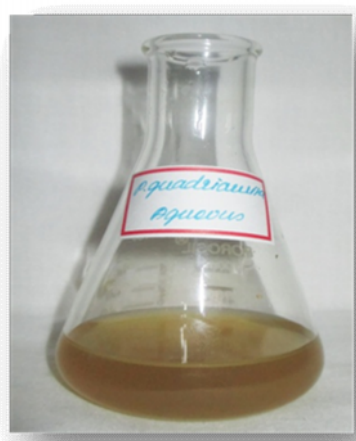


Ethanol Extract

Plant powder

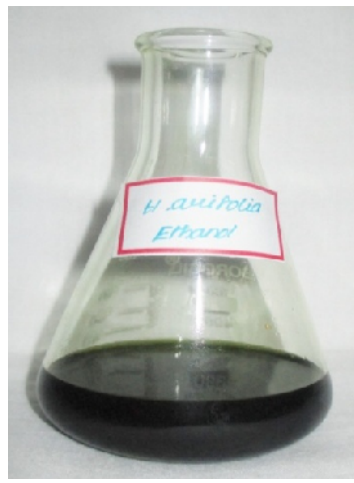


Acetone Extract



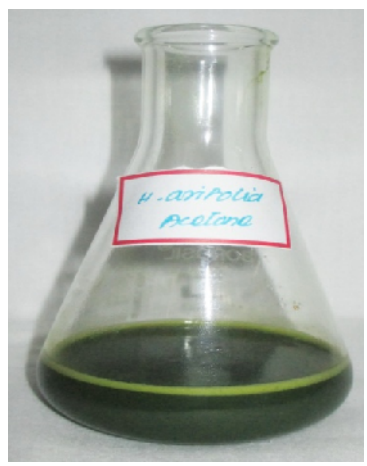
Aqueous Extract

Plate 6. Powder of *Hemionitis arifolia* and it's Ethanol, Acetone and Aqueous Extract

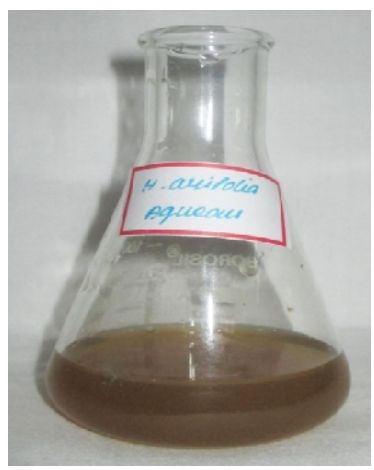


Ethanol Extract

Plant powder

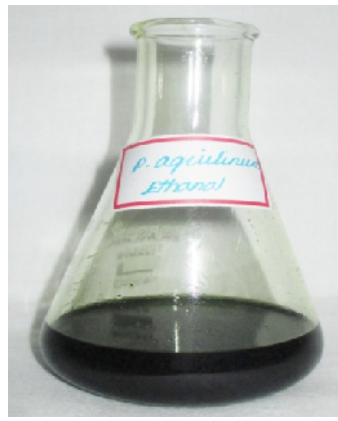


Acetone Extract



Aqueous Extract

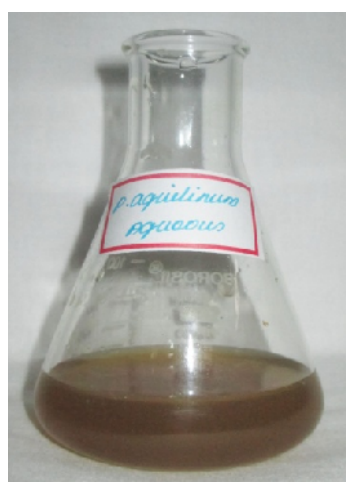
Plate 7. Powder of *Pteridium aquilinum* and it's Ethanol, Acetone and Aqueous Extract



Ethanol Extract

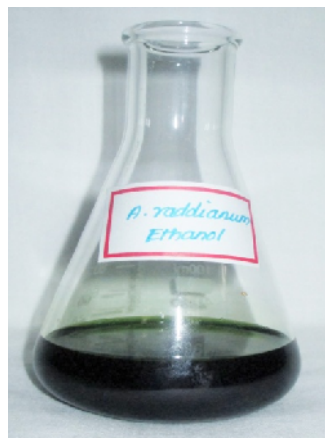
Plant powder

Acetone Extract



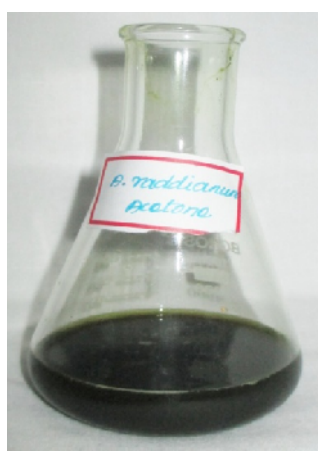
Aqueous Extract

Plate 8. Powder of *Adiantum raddianum* and it's Ethanol, Acetone and Aqueous Extract

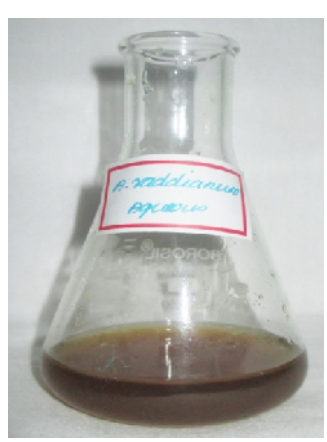


Ethanol Extract

Plant powder



Acetone Extract



Aqueous Extract

3.3 Physiochemical parameters

3.3.1 Determination of moisture content (AOAC, 1990)

A quantity of 5 g of sample was dried in the oven at 105˚C and moisture content was calculated as percentage.

$$\text{Moisture Content} = \frac{W - D}{W} \times 100$$

Where,

W = Wet weight

D = Dry weight

3.3.2 Determination of solubility percentage (Kokate, 1994)

Ethanol

- ✓ About 5 g of powdered material was weighed and macerated with 100 ml of 90% ethanol in a closed flask for 24 hours shaking frequently during the first 6 hours and kept undisturbed for 18 hours.
- ✓ Thereafter, it was filtered rapidly taking precautions against loss of the solvent.
- ✓ About 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed, swallowed dish dried at 105˚ C for 6 hrs and cooled in a desiccator and weighed.
- ✓ The content of extractable matter (% w/w) air-dried material was calculated as follows.

$$\text{soluble extractives (\% w/w)} = \frac{\text{(Weight of residue) x 5 x 100}}{\text{Weight of the sample}}$$

Acetone and Water

The procedure adopted for the solubility percentage of the plant powder in ethanol is used with acetone and water instead of alcohol to get the water solubility percentage.

3.4 Pharmacognostic study

3.4.1 Fluorescence analysis (Kokoshi *et al.,* 1958)

The fluorescence characters of leaf powders were observed under ordinary and ultra violet light at 360 nm using different chemical reagents.

3.5 Phytochemical Screenings

The extracts obtained in the successive extraction process of the four ferns were subjected to various preliminary phytochemical screening for the identification of

phytoconstituents present. Quantitative analysis was carried out to do comparative study among them.

3.5.1 Qualitative Tests

3.5.1.1 Test for Carbohydrates

Molisch's test:

To a small amount of the extract few drops of Molisch's reagent was added followed by the addition of conc. H_2SO_4 along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 ml of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates.

3.5.1.2 Test for Reducing Sugar

Benedict's Test

Extract (2 ml) were treated with 2 ml of Benedict's reagent and heated in a water bath for 3 minutes. Presence of green, red or yellow ppt indicates the presence of reducing sugar.

3.5.1.3 Test for Proteins

Millon's test:

Extract (3 ml) was mixed with 5 ml of millon's reagent. White precipitate is formed. On warming precipitate turn's brick red or the precipitate dissolves giving red colored solution.

Biuret test:

To extract (3 ml) few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple colour. On addition of alkali, it becomes dark violet.

3.5.1.4 Vitamin C

DNPH test:

Sample was treated with Dinitrophenyl hydrazine and sulphuric acid. Formation of yellow precipitate indicates the presence of vitamin C**.**

3.5.1.5 Test for Alkaloids

Mayer's test:

Sample (2 ml) was treated with few drops of Mayer's reagent. Appearance of white precipitate indicated the presence of alkaloids.

Wagner's test:

Sample (2 ml) was mixed with few drops of Wagner's reagent. Appearance of reddish brown precipitate indicated the presence of alkaloids.

Hager's test:

Sample solution and few drops of Hager's reagent were added. Appearance of yellow precipitate indicated the presence of alkaloids.

3.5.1.6 Tests for Flavonoids

Shinoda test:

Sample extract was treated with 5 ml of 95% ethanol, few drops of concentrated Hydrochloric acid and 0.5 g of magnesium turnings were also added. Pink colour was observed. Addition of increasing amount of sodium hydroxide to the residue shown yellow coloration, this decolorized after addition of acid indicates the presence of flavones.

Flavanones:

Sample extract (1 ml) was taken and 10 % of sodium hydroxide was added. Yellow to orange colour formation indicates the presence of flavanones.

Alkaline test:

Sample extract (1 ml) was treated with few drops of sodium hydroxide. Yellow colour is formed which turns to be colourless after adding a few drops of diluted acid.

3.5.1.7 Test for Glycosides:

Legal's test:

Sample extract was mixed with few drops of pyridine and 2 drops of 2 % sodium nitroprusside was added. To the reaction mixture 0.5 ml of 20 % sodium hydroxide was added. Appearance of pink to red color indicated the presence of glycosides.

Bromine water test

Sample (1 ml) was treated with 3 drops of bromine water and the formation of yellow precipitate indicates the presence of glycosides.

3.5.1.8 Test for cardiac Glycosides

Keller-killani test:

Five ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1 ml of conc. sulphuric acid, a brown ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

3.5.1.9 Test for anthroquinone

Borntragor's test

Extract (0.5 ml) was added with 5-10 ml of dilute hydrochloric acid and boiled on water bath for 10 minutes. Solution was filtered and filtrate was extracted with benzene and mixed with ammonia solution. Red color was obtained in ammonia layer that indicated the presence of anthraquinone glycosides.

3.5.1.10 Test for Terpenoids

Extracts were treated with $CHCl_3$ (0.5 ml) and 1ml of Conc. H_2SO_4. Formation of reddish brown precipitate shows the presence of terpenoids.

3.5.1.11 Test for Saponin

Foam test:

To 1 ml of the extract 5 ml distilled water was added and shaken vigorously. Formation of foam indicated presence of saponins.

3.5.1.12 Test for phenols

Extract (2 ml) was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour. To 1 ml of the extract, 2 ml of distilled water, 3 drops of 10% aqueous ferric chloride ($FeCl_3$) and 3 drops of potassium Ferro cyanide were added. Formation of blue or green color showed the presence of polyphenols.

Extract (2 ml) was treated with 3ml of 10 % lead acetate. Formation of precipitate indicates the presence of phenols.

3.5.1.13 Test for Tannins(Trease and Evans, 1989)

To 1ml of extract solution, 4 ml of water and 1-2 drops of 10 % ferric chloride solution was added. Blue colour indicates gallic tannins and green black catecholic tannins.

3.5.1.14 Test for Catechin

Match stick was dipped in plant extract, dried and then moistened with concentrated HCl. Warm near flame, a red or pink wood is produced which shows the presence of catechin.

3.5.1.15 Test for Phlobatanins:

0.5 g extract was dissolved in distilled water and filtered. The filtrate was boiled with 2M HCl solution. Formation of red precipitate showed the presence of phlobatanins.

3.5.1.16 Test for Quinones

To 1ml of test solution Alcoholic KOH solution was added separately. Quinones were indicated by colour ranging from red to blue.

3.5.1.17 Test for Sterols

Extract (2 ml) was treated with 2 ml of trichloroacetic acid. On heating the colour changes from red to violet. This indicates the presence of sterols.

3.5.1.18 Test for Phytosterols

Salkowski's test:

0.2g of the extract was mixed with 2 ml of chloroform (CHCl3) and concentrated 6M H2SO4 (3ml) was carefully added forming a layer. A reddish brown coloration of the interface indicated the presence of phytosterols.

3.5.1.19 Test for Coumarins

The aqueous leaf extract (5 ml) was evaporated to dryness in a water bath. Distilled water (3 ml) was added and the mixture heated on a water bath to boil and the mixture cooled under running water. The solution (0.5 ml, 10%) was added. Both test tubes were observed

under Ultra Violet light and presence of coumarins was indicated by (blue or green) fluorescence in test tube containing ammonia solution.

Take few drops of distilled water in a test tube and add 1gm of plant powder. The paper soaked in NaOH was covered over the test tube is diluted and boiled. Coumarins is indicated by yellow fluorescence and examined under UV light.

Data collected

The change of colour was observed when the test reagent was added to the prepared sample for the phytochemical test. The result was recorded as present (+) or absent (-) depending on the outcome of the test.

3.5.2 Quantitative test

The biochemical parameters analyzed were

1. Carbohydrates
2. Proteins

3.5.2.1 Biochemicals parameters

3.5.2.1.1 Total Carbohydrates: (Hedge and Hofreiter, 1962)

Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone, a green coloured product with an absorption maximum at 630nm.

Materials

- 2.5N HCl
- Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice cold 95 % H_2SO_4 prepared fresh before use.

Standard glucose (Stock): Dissolved 100 mg in 100 ml of water.

Working standard – 10 ml of a stock solution was diluted to 100 ml distilled water. After adding few drops of toluenetored in refrigerator after adding a few drops of toluene.

Procedure

- ✓ About 100 mg of the sample was taken in a boiling tube and it was hydrolysed by keeping it in a boiling water bath for three hours with 5ml of 2.5N HCl and cooled to room temperature.
- ✓ Then it was neutralized with solid sodium carbonate until the effervescence created.
- ✓ The volume was made up to 100 ml and centrifuged.
- ✓ The supernatant was collected and 0.5 and 1 ml aliquots were taken for analysis.
- ✓ The standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and '0' served as blank.
- ✓ The volume was made up to 1 ml in all the tubes including the sample test tubes by adding distilled water.
- ✓ Then, 4 ml of anthrone reagent was added and heated for eight minutes in a boiling water bath.
- ✓ Then it was cooled rapidly and the green colour developed was read at 630 nm.
- ✓ A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
- ✓ From the graph, the amount of carbohydrates present in the sample tube was calculated.

Calculation

Amount of carbohydrates present in 100mg of the sample is calculated by

$$\frac{\text{mg of glucose}}{\text{volume of test sample}} \times 100$$

3.5.2.1.2 Estimation of protein (Lowry *et al*., 1951)

Principle

The blue colour developed by phosphomolybdic phosphotungstic components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured in the Lowry's method.

Materials:

- ➢ Reagent A - 2 % sodium carbonate in 0.1 sodium hydroxide

- Reagent B - 0.5 % copper sulphate ($CuSO_4.5H_2O$) in 1% potassium sodium tartarate.
- Reagent C - Alkaline copper solution : Mixed 50 ml of A and 1 ml of B prior to use.
- Reagent D - Folin-Ciocalteau Reagent.

Stock standard : 50 mg of bovine serum albumin (Fraction V) was weighed and dissolved in distilled water and the volume was made up to 50 ml in a standard flask.

Working standard: About 10ml of the stock solution was diluted to 50 ml with distilled water in a standard flask. One ml of this solution contain 200 µg proteins.

Procedure

Extraction of protein from sample

Extraction is usually carried out with buffers used for the enzyme assay. About 50mg of the weighed sample was ground well with pestle and mortar in 5- 10 ml of the buffered centrifuged. The sample was used for protein estimation.

Estimation of protein

- ✓ About 0.2, 0.4, 0.6, 0.8, and 1 ml of working standard were pipette into a series of test tubes and 0.1 ml and 0.2 ml of the sample extract in two other test tubes.
- ✓ The volume was made up to 1 ml in all test tubes. A tube with 1 ml of water severed as the blank.
- ✓ About 5 ml of reagent C was added to each tube including the blank, mixed well and allowed to stand for 10 minutes.
- ✓ Then 0.5 ml of reagent D was added. Mixed well and incubated at room temperature in the dark for 30 min. Blue colour developed was read at 660 nm.
- ✓ A standard graph was drawn and the amount of protein present in the sample was calculated.

Calculation

The amount of protein present in the sample was expressed in

$$\text{mg/g or 100g} = \frac{\text{mg of protein}}{\text{volume of the test standard}} \times \text{concentration of the standard}$$

3.5.2.2 Phytochemical Parameters

- Alkaloids
- Phenol
- Flavonoid
- Tannin

3.5.2.2.1 Estimation of Total Phenol content (Malick and Singh, 1980)

Principle

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue coloured complex (molybdenum blue), which can be estimated spectrophotometrically at 650 nm.

Materials

- Ethanol (80 %)
- Folin-ciocalteau reagent
- Na_2CO_3 (20 %)

Stock standard: Gallic acid (100µg/ml in water)

Working standard: Dilute 10 times of stock

Procedure

- ✓ Grind 0.5 g of the sample with a pestle and mortar in 10 times volume of 80 % ethanol.
- ✓ Centrifuge the homogenate at 10,000 rpm for 20 minutes. Re-extraction is done and the supernatants were pooled and evaporated to dryness.
- ✓ Dissolve the residue in a known volume of distilled water (5 ml).
- ✓ Pipette out different aliquots (0.2 to 2 ml) into test tubes.
- ✓ Make up the volume in each tube to 3 ml with distilled water.
- ✓ Add 0.5ml of Folin-Ciocalteau reagent.
- ✓ After 3 minutes, add 2 ml of 20 percent Na_2CO_3 solution to each tube.

- ✓ Mix thoroughly, place the tube in boiling water for exactly 1 minutes, cool and measure the absorbance at 650nm against a reagent blank.
- ✓ Prepare a standard curve using different concentrations of gallic acid.

Calculation

From the standard curve the concentration of phenols in the sample were observed and express as GAE mg of phenols/g of material.

3.5.2.2.2 Determination of total flavonoid content (Grubesic *et al*., 2005)

Principle

The content of flavonols was determined by using rutin as a reference compound. This method was based on the formation of complex with maximum absorption at 440 nm.

Reagents:

1. Aluminium chloride solution (20 mg/ml)
2. Sodium acetate (30 mg/ml)

Procedure

- ✓ About 1 ml of each extract was mixed with 1 ml aluminium chloride and 3 ml sodium acetate.
- ✓ After 2.5 hrs the samples were read at 440 nm absorbance.
- ✓ The absorption of standard rutin solution in methanol was measured under the same conditions.
- ✓ A duplicate was carried out for all the determinations.

Calculation:

The amount of flavonoids in plant extracts in rutin equivalents was calculated by the following formula.

$$X = (A - m_0) / (A_0 - m)$$

Where,

X = flavonoid content (µg/ml) in rutin equivalents

A = absorption of plant extraction solution,

A_0 = absorption of standard rutin solution,

m = weight of plant extract (μg)

m_0 = weight of rutin in the solution (μg)

3.5.2.2.3 Estimation of Tannin (Folin and Ciocalteu Method, 1927)

Principle

The principle behind this method is reduction of Phosphotungstomolybdic acid in alkaline solution to produce coloured complex.

Materials

1. Folin- Ciocalteu reagent
2. Sodium carbonate
3. Tannic acid

Procedure

- ✓ To 0.1 ml of the sample extract 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent, 1 ml of 35% sodium carbonate solution were added and diluted to 10 ml with distilled water.
- ✓ The mixture was shaken well, kept at room temperature for 30 min and was measured at 725 nm.
- ✓ Blank was prepared with water instead of the sample.
- ✓ A set of standard solutions of Tannic acid is treated in the same manner as described earlier and read against a blank.

Calculation

The results of Tannin are expressed in terms of Tannic acid in mg/g tissue TAE of extract.

3.6. Antioxidants assays

3.6.1. Enzymatic assays

The enzymatic antioxidant analysed in this present study were Catalase, Peroxidase, Polyphenol oxidase and Ascorbate oxiadase.

3.5.1.2. Peroxidase (Reddy *et al*., 1995)

Principle

In the presence of the hydrogen donor pyrogallol or dianisidine, peroxidase converts H_2O_2 to H_2O and O_2. The oxidation of pyrogallol or dianisidine to a coloured product called purpurogalli can be followed spectrophotometrically at 430nm.

Reagents

- Pyrogallol : 0.05 M in 0.1M phosphate buffer (pH 6.5)
- H_2O_2: 1% in 0.1M phosphate buffer, pH 6.5

Procedure

- ✓ About 3 ml of pyrogallol solution, 0.1 ml of the extract was added and the spectrophotometer was adjusted to read zero at 430 nm.
- ✓ To the test cuvette, 0.5 ml of H_2O_2 was added and mixed.
- ✓ The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer.

Calculation

One unit of peroxidase is defined as the change in absorbance at 430 nm/minute.

3.5.1.3. Polyphenol Oxidase (PPO) (Esterbauer *et al*., 1977)

Principle

Polyphenol oxidase was copper protein of wide occurrence in nature, which catalase the aerobic oxidation of certain phenolic substrate to quinines, which are auto oxidized to dark brown pigments generally known as melanins. The polyphenol oxidase (PPO) comprises catechol oxidase and laccase.

Reagents

- Tris-HCl (50 mM, pH 7.2).
- Sorbitol (0.4 M).
- Sodium chloride (10 mM).
- Phosphate buffer (0.1 M, pH 6.5).
- Catechol (0.01 M).

Procedure

- ✓ Into a cuvette, 0.2 ml of the sample extract was added to the reaction mixture containing, 2.5 ml of phosphate buffer and 0.3 ml of catechol solution.
- ✓ The change in the absorbance was recorded every 30 sec up to 5 minute.

Calculation

One unit is equal to the changes in absorbance at 495nm/minute

The activity of PPO can be calculated using the formula

Enzyme units in the sample = K × (ΔA/minute)

where,

K for catechol oxidase = 0.272

K for laccase = 0.242

3.5.1.4. Ascorbate oxidase (Vines and Oberbacher, 1965)

Principle

$$\text{Ascorbic acid} + {}^{1}/_{2}O_2 \xrightarrow{\text{ascorbate oxidase}} \text{dehydro ascorbic acid} + O_2$$

The disappearance of ascorbic acid is measured at 245 nm by spectrophotometer

Reagents

- ➢ M Phosphate buffer (pH 6.5)
- ➢ Ascorbic acid

Procedure

- ✓ In a test tube, 3ml of ascorbic acid (8.8 mg in 300ml phosphate buffer) mixed with 0.1 ml of the extract was taken.
- ✓ The absorbance at 265 nm was measured after every 30 seconds for a period of 5 minutes.
- ✓ The ascorbate oxidase activity is expressed in terms of units/g. One enzyme unit is equivalent to 0.01 OD changes per minute.

Calculation

One enzyme unit is equal to 0.01 OD changes/minute

3.5.2. Non-enzymatic assays

3.5.2.1. Ascorbic acid (Roe and Keuther, 1943)

Principle

Ascorbate is converted into dehydroascorbate on treatment with activated charcoal, which reacts with 2, 4-dinitrophenyl hydrazine to form osazones. These osazones produce an orange coloured solution when dissolved in sulphuric acid, whose absorbance can be measured spectrophotometrically at 540nm.

Reagents

- TCA (4%)
- 2,4-dinitrophenyl hydrazine reagent (2%) in 9 N H2SO4
- Thiourea (10%)
- Sulphuric acid (85%)
- Ascorbic acid (100 mg of Ascorbic acid in 100 ml of 4% TCA)

Procedure

- ✓ Ascorbic acid solution of 1 ml were taken and added with 0.1 ml of plant extract.
- ✓ The reaction mixture was made up to 2.0 ml with 4% TCA.
- ✓ To this, 0.5 ml of DNPH reagent was added followed by 2 drops of 10% thiourea solution.
- ✓ The tubes were incubated at 37°C for 3 h.
- ✓ The osazone formed was dissolved by the addition of 2.5 ml of 85% sulphuric acid.
- ✓ DNPH reagent and thiourea were added to the blank after the addition of sulphuric acid.
- ✓ After cooling the tubes, the absorbance was read spectrophotometrically at 540 nm.

Calculation

The concentration of ascorbate in the sample was calculated and expressed in terms of mg/g tissue.

3.5.2.2. Total polyphenol (Malick and Singh, 1980)

Principle

Phenols react with phosphomolybdic acid in Folin - ciocalteau reagent in alkaline medium and produce blue coloured complex (molybdenum blue), which is read in a spectrophotometer at 650nm.

Reagents

- Diluted Folin - Ciocalteau reagent (1:10 dilution)
- 20% Sodium carbonate
- Ethanol

Stock solution: 100 mg of catechol was made up with 100 ml distilled water

Working standard: 10 ml of stock standard was diluted to 100 ml. 1.0 ml of this contains 100 µg of catechol.

Procedure

- ✓ A working standard of 0.5 – 2.5 ml catechol solution corresponding to 50 – 250 µg of catechol were pipetted out into a series of test tubes including samples and standard.
- ✓ The volume was made upto 2.5 ml with water. To all the tubes added 0.5ml of diluted Folin – Ciocalteau reagent.
- ✓ After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added to each tube and mixed thoroughly.
- ✓ The tubes were placed in a boiling water bath for exactly one minute. Cooled and measured at 650nm against a reagent blank.

Calculation

The results were expressed as mg / g tissue.

3.5.2.3. Reduced Glutathione (GSH) (Boyne and Ellman., 1979)

Principle

Reduced glutathione (GSH) is measured by its reaction with 5,5-dithio- 2 - nitrobenzoic acid (DTNB) (Ellman's reaction) to give a compound that absorbs at 412nm.

Reagents

1. TCA (5% and 25%)
2. Sodium phosphate buffer (0.2 M phosphate buffer)
3. DTNB (0.6 mM in 0.2 M phosphate buffer)
4. Standard GSH (10 n moles/ml in 5% TCA)

Procedure

- ✓ About 0.1 ml of plant extract was made up to 1.0ml with 0.2 M sodium phosphate buffer (pH 8.0).
- ✓ Standard GSH corresponding to concentrations ranging between 2 and 10 n moles were also prepared.
- ✓ To the above mixture 2 ml of freshly prepared DTNB solution was added and developed intensity of the yellow colour was measured in a spectrophotometer. (at 412 nm after 10 minutes).

Calculation

The values are expressed in n moles/g tissue.

3.5.3. Free radical scavenging activity

3.5.3.1. DPPH radical scavenging activity (Mensor *et al.*,2001)

Principle

DPPH radical reacts with an antioxidant compound that can donate hydrogen, and gets reduced. DPPH, when acted upon by an antioxidant, is converted into diphenylpicryl hydrazine. This can be identified by the conversion of purple to light yellow colour.

Reagents

1. DPPH – 2,2-diphenyl-2-picryl hydrazyl hydrate (0.3mM in methanol)

2. Methanol

Procedure

The extracts (20µl) were added to 0.5ml of methanolic solution of DPPH and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the extracts, served as the positive control.

After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer.

Calculation

The radical scavenging activity was calculated as follows

$$\text{Scavenging activity \%} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

3.5.3.2. Hydrogen Peroxide Scavenging Activity (Ruch *et al,* 1989)

Principle

The principle of this method is that there is a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2.

Reagents used

1. H_2O_2
2. 0.1 M Phosphate Buffer (pH- 7.4)
3. Stock Standard H_2O_2

Procedure

A solution of 45 mM H_2O_2 was prepared in 0.1M phosphate buffer (pH-7.4). Sample at 30µg/ml concentration in 3.4 ml phosphate buffer was added to 6 ml of H_2O_2 solution (45mM) and absorbance of the reaction mixture was recorded at 230 nm. A blank solution contained the sodium phosphate buffer without H_2O_2.

Calculation

The percentage of H_2O_2 scavenging by extract and standard components was calculated using the following formula,

$$H_2O_2 \text{ scavenging activity (\%)} = \frac{\text{Absorbance of control-Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

4. RESULT AND DISCUSSION

In this present study phytochemical study was done for the four ferns (*P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*), along with antioxidant activity studies. The results obtained for various parameters are discussed with earlier work in same fern or other pteridophytes.

4.1 Physiochemical Parameters

4.1.1 Moisture content

Present investigation revealed that *P. quadriaurita* had the maximum moisture content (4.09 %) when compared with all the four ferns, and the minimum was found to be present in *H. arifolia* (3.35 %).

Awe and Amobi (2015) while investigating the phytochemicals and antibacterial property of *P. aquilinum* reported its moisture content as 9.26% which means that the leaf is low in moisture. Moisture content in the ferns that we have taken for this study showed to be less when compared with the study mentioned earlier.

4.1.2 Solubility test

Solubility test results revealed that *H. arifolia* (11.6 %) and *P. quadriaurita* (4.43 %) showed the maximum and minimum solubility in ethanol. Among the four ferns solubility percentage in acetone showed that *A. raddianum* had maximum (3.88 %) and *P. quadriaurita* had minimum (1.6 %) values. Aqueous extract of the four ferns had the lowest percentage, in which *H. arifolia* had the lowest (6.72 %) and highest was noted in *P. quadriaurita* (11.45 %).

Table: 2. Physiochemical parameters of the four medicinal plants

Physiochemical parameters	Moisture content (%)	Solubility (in %)		
		Ethanol	Acetone	Aqueous
P. quadriaurita	4.09	4.43	1.6	11.45
H. arifolia	3.35	11.6	2.23	6.72
P. aquilinum	3.83	8.26	3.54	10.38
A. raddianum	3.64	6.38	3.88	8.67

Present result is in conformity with the finding of Singh *et al*., (2013) they reported that for four *Adiantum* species (*A. capillus-veneris* L, *A. lunulatum* Burm. F, *A. peruvianum* Klotzsch and *A. venustum* D.Don) their alcohol soluble percentage were 3.20%, 4.35%, 5.61%, 9.42% and their water soluble percentage were 8.865, 7.21%, 18.07%, and 11.04% respectively.

4.2 Fluorescence analysis

Many phytocompounds fluoresce when suitably illuminated. The fluorescence colour is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyte over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples. The fluorescence property of all the four ferns (*P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*) powders as such with chemical reagents, under day light and UV light are summarized in Table 3. Kala et al., (2011) previously applied fluorescence characters as a tool to characterize the different medicinal plants of South India.

4.3 Preliminary Phytochemical screening

Preliminary screening tests may be useful and lead to the detection of bioactive principles and drug discovery. Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds (Sutha *et al*., 2014). Different chemical compounds such as alkaloid, terpenoid, coumarin, tannin, saponin, flavonoid, quinone, anthraquinone, phenol, catechin, phlobatannins, sterols, phytosterols and glycosides were tested in leaf extracts of four ferns.

The results of the phytochemical screening of ethanol, acetone and aqueous leaf extracts of ferns (*P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*) indicated the presence of different types of active constituents such as flavonoids, phenolics, proteins, carbohydrates, saponins, tannins, glycosides, steroids and triterpenoids. The common compound present in all the four ferns irrespective of the solvent used for extraction were carbohydrate, reducing sugar, vitamin c, flavonoids, phenol and tannins. Among the three extracts in the four ferns, ethanol

Table 3. Behaviours of plant powder of *P.aquilinum, H.arifolia, A.raddianum* and *P.quadriaurita* under day and UV light.

S.No	Treatment of powder	Colour under day light				Colour under UV light			
		P.aquilinum	***H.arifolia***	***A.raddianum***	***P.quadriaurita***	***P.aquilinum***	***H.arifolia***	***A.raddianum***	***P.quadriaurita***
1	Powder as such	Pickle green	Olive green	Seaweed green	Moss green	Moss green	Olive green	Pine green	Crocodile green
2	Ammonia	Pear green	Moss green	Pickle green	Moss green	Parakeet green	Seaweed green	Fern green	Sage green
3	Iodine	Juniper green	Juniper green	Juniper green	Honey orange	Seaweed green	Seaweed green	Pine green	Seaweed green
4	Fecl3	Olive green	Pickle green	Moss green	Crocodile green	Seaweed green	Pine green	Seaweed green	Pine green
5	H_2SO4	Olive green	Olive green	Crocodile green	Pickle green	Crocodile green	Moss green	Mint green	Crocodile green
6	Ethanol	Juniper green	Emerald green	Shamrock green	Pine green	Rouge pink	Strawberry pink	Hot pink	Flamingo pink
7	Benzene	Moss green	Juniper green	Juniper green	Juniper green	Fuscia pink	Magenta pink	Fuscia pink	Seaform green
8	Acetic acid	Pear green	Pear green	Emerald green	Juniper green	Punch pink	Punch pink	Punch pink	Ballet slipper pink
9	Chloroform	Moss green	Pickle green	Crocodile green	Seaweed green	Hot pink	Magenta pink	Strawberry pink	Hot pink
10	Petroleum ether	Juniper green	Moss green	Pickle green	Seaweed green	Pickle green	Fern green	Mint green	Basil green
11	Hcl	Emerald green	Seaweed green	Olive green	Pickle green	Fern green	Pine green	Seaform green	Moss green
12	HNO_3	Tiger orange	Squash orange	Marmalade orange	Cider orange	Amber orange	Bronze orange	Olive green	Clay orange
13	Acetone	Moss green	Basil green	Crocodile green	Juniper green	Hot pink	Hot pink	Basil green	Hot pink
14	NaOH	Shamrock green	Seaweed green	Fern green	Pear green	Fern green	Fern green	Sage green	Fern green

was seen to have more phytochemicals (out of 19 phytochemicals tested 11 were present) when compared to acetone and aqueous extracts. Alkaloids was absent in ethanol and acetone extracts in all the four ferns. The presence or absence of the phyto-constituents depends upon the solvent medium used for extraction and the physiological property of individual taxa (Mithraja *et al*., 2012).

In the present investigation *P. quadriaurita* showed presence of carbohydrate, reducing sugar, protein, vitamin c, flavonoids, glycosides, phenols and tannins in all the three extracts. Alkaloid was absent in all the three extracts. Phytochemical analysis of active extracts of *P. quadriaurita* done by Thomas (2011) indicated the presence of flavonoids and phenols. Alkaloids were not detected in any of the extracts. The qualitative analysis of *Pteris* species showed flavonoids, alkaloids and phenolic compounds (Devi *et al.*, 2016). Gracelin *et al*., (2013) conducted qualitative and quantitative phytochemical analysis in five pteris fern species (*P. confusa, P. vittata,* P. *argyreae*, P. *biaurita*, P. *multiaurita*).

Aqueous extracts of *H. arifolia* exhibited presence of phenol, tannins, coumarin carbohydrate and steroids and absence of flavonoids in aqueous extract (Britto *et al.,* 2012). The ethanol extract of *H. arifolia* contained phenol, saponins, tannins, xanthoproteins, coumarin and carbohydrates and the petroleum ether extract contain phenol, saponins, tannins, coumarin and carbohydrate (Mithraja *et al.,* 2012). Phytochemical screening of *H. arifolia* in eight extracts (acetone, ethanol, chloroform, ethyl acetate, petroleum ether, methanol, hexone and dichloromethane respectively) was studied by Bindu *et al.,* (2012). Among them ethanol extract showed presence of carbohydrates, reducing sugar, cardiac glycosides. Kumudhavalli and Jaykar, (2012) evaluated the petroleum ether, chloroform, acetone, ethanol and aqueous extracts of *H. arifolia* for preliminary phytochemical screening. Similar results were obserbed by Moorthy *et al.,* (2014), where *H. arifolia* contained phenols, cardiac glycosides, saponins, flavanoids, steroids, coumarin, xanthoproteins, tannins, carbohydrates in aqueous, ethanol, and petroleum ether extracts. Similar results were observed by Kumar *et al.,* (2015) in evaluation of phytoconstituents of *H. arifolia* with 95% and 50% ethanol extracts.

Phytochemical studies in *H. arifolia* leaves revealed presence of carbohydrates, glycosides, saponins, flavonoids, phenolics, tannins, phytosterols and fixed oils was identified (Kumar *et al*., 2015). worked with six ferns, among which *H. arifolia* showed only

Table. 4. Phytochemical investigation of ferns in Ethanol, Acetone and Aqueous extracts

S.No	Name of the Phytochemicals		Name of the test	Ethanol				Acetone				Aqueous			
				1	2	3	4	1	2	3	4	1	2	3	4
1	Carbohydrates		Molisch's	+	+	+	+	+	+	+	+	+	+	+	+
2	Reducing Sugar		Benedicts test	+	+	+	+	+	+	+	+	+	+	+	+
3	Proteins		Millon's test	-	-	-	-	-	-	-	-	-	-	-	-
			Biuret test	-	-	-	-	-	-	-	-	-	-	-	-
			Tannic acid test	+	+	+	+	+	+	+	+	+	+	+	+
4	Vitamin C		DNPH test	+	+	+	+	+	+	+	+	+	+	+	+
5	Alkaloids		Mayer's	-	-	-	-	-	-	-	-	-	-	-	-
			Wagner's	-	+	-	-	-	-	-	-	+	+	+	+
			Hager's test	-	-	-	-	-	-	-	-	+	+	+	+
6	Flavonoids	Flavones	Shinodia test	-	-	-	-	-	-	-	-	-	-	-	-
		Flavanones	NaOH test	+	+	+	+	+	+	+	+	+	+	+	+
		Alkaline test		-	-	-	-	-	-	-	-	-	+	-	-
7	Glycosides		Bromine water test	-	+	-	-	-	-	-	-	-	+	-	-
			Legal's test	+	+	-	-	+	+	+	+	+	+	+	+
8	Cardiac glycosides		Keller-killani test	+	+	+	+	+	+	+	+	-	-	-	-
9	Anthraquinone		Borntragors test	-	-	-	-	-	-	-	-	+	-	+	+
10	Terpenoids		Salkowki's test	+	+	+	+	-	-	-	-	+	-	+	+
11	Saponins			-	-	+	-	-	-	-	-	+	+	+	+
12	Phenols		Ferric chloride test	+	+	+	+	+	+	+	+	+	+	+	+
13	Tannin	Gallic tannin		+	+	+	+	+	+	+	+	+	+	+	+
14	Catechin			-	-	-	-	-	-	-	-	+	+	+	+
15	Phlobatanins			-	+	+	-	-	+	+	-	+	-	+	+
16	Quinones			-	-	-	-	-	-	-	-	+	-	+	+
17	sterols			-	-	-	-	-	-	-	-	+	+	+	+
18	Phytosterols		Salkowki's test	+	+	+	+	-	+	-	-	+	-	+	+
19	Coumarins			+	+	+	+	+	+	+	+	-	-	-	-

Key:

1 – *P. quadriaurita* 2. *H. arifolia* + = Present

3 – *P. aquilinum* 4. *A. raddianum* - = Absent

positive for tannin and phenol. And he observed that aqueous extract performed well next to ethanolic extract which showed positive among the phytochemicals in all six ferns. Aqueous extract of *H. arifolia* observed that tannins, saponins, flavonoids, quinones, gardioglycosides, terpinoids, phenols, coumarins, and betacyanin were present, while glycosides, steroids, alkaloids and anthicyanin were absent. According to Rukmini *et al*., (2015) phytochemical screening of *H. arifolia* showed the presence of alkaloids, flavonoids, tannins, saponins along with phenols in all the extracts investigated.

Phytochemical study of *P. aquilinum* by Kardong *et al.,* (2013) revealed presence of saponin, terpenoids, flavanoid, phenol and cardiac glycoside while anthraquinone and steroids were absent. The preliminary study of *Pteridium aquilinum* was done by Selvaraj *et al.,* (2005) and the results revealed that of ethanol, chloroform, hexane extracts and crude phytocdysteroidal fraction contained steroids and it was absence only the aqueous fraction. Reducing sugar was observed only in chloroform, ethanol, crude phytocdysteroidal and aqueous showed carbohydrates. Alkaloids were not observed in any of the extracts and the fraction analysed. Except chloroform and phytocdysteroidal, all other extracts contain phenolic compounds. Saponin was showed in the analysed extracts except hexane and phytocdysteroidal. Tannins and flavonoids were reported in ethanol and aqueous extracts. From the presence of above phytochemicals selvaraj *et al*., (2005) concluded that *P. aquilinum* may show therapeutic activities. This results are comparable to earlier studies on plants showed that, flavonoids is likely to be accountable for pharmacological and biochemical actions viz., anti-oxidant, anti-allergic, anti-inflammatory, hepatoprotective, anti-carcinogenic, antiviral and anti-thrombotic activities.

Study done by Thomas (2014) in *A. raddianum* revealed the presence of flavanoids and phenols in all the four extracts and absence was alkaloids in all the four extract (petroleum ether, acetone, methanol and aqueous) and sterols, steroids, phenols, polyphenol were present only in petroleum ether extract but absent in other extracts. Phytochemicals screening of aqueous and methanolic extract of *Adiantum* and *Pteris* leaf and stem showed the tannins, saponins, flavonoids & terpenoids steroids terpenoid. The plants contain high phenol & flavonoid which indicates that the sample has antioxidant effects. 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.

4.4 Quantitative test

4.4.1 Biochemical parameters

Biochemical parameters are affected by seasonal variations, which may be due to the habitat conditions, the growing stage of plant and the microclimatic conditions at the time of collection (Kapila *et al*., 2014).

4.4.1.1 Total Carbohydrate

Present study revealed that the carbohydrate present in all the ferns didn't show much of variation, only slight differences were recorded. Among the four ferns (*P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*) in ethanol extract highest to lowest values were found in *P. quadriaurita, H. arifolia* and *A. raddianum* and *P. aquilinum* as 43.51 mg/100 mg, 43.4 mg/100mg, 43.21 mg/100 mg and 42.82 mg/100 mg respectively. Ma *et al.,* (2009) reported that carbohydrate was 99.5% in purified polysaccharide of *P. aquilinum*.

4.4.1.2 Total Protein

Total protein estimation in all the four ferns in ethanol, acetone and aqueous extracts revealed that the quantity of protein also seemed to be like carbohydrates with less variation among them. The protein content ranged from 104.26 – 102.96 mg/100g. Quantification of bioactive compounds by jaishee and chakraborty, (2014) in *Pteris biaurita* revealed 37.09 mg/g of total protein.

4.4.2 Physiochemical Properties

Plant phenolics, tannins and flavonoids represent major groups of plant constituents that work predominantly as powerful antioxidants or scavenger of free radicals. They play beneficial role in human health and cure/prevent ailments such as inflammatory disorders, cancer, cardiovascular diseases and diabetes which occur due to the deregulation of free radicals generation in the cells (Middleton *et al.,* 2000; Chouhan and Singh, 2011). Table 6 represents the tannin, total phenolic and flavonoid contents in different extracts of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum.*

Table. 5. Quantitative test for Biochemical parameters – Carbohydrate and Protein

Plant Name	Total Carbohydrate (mg / 100 g)			Total Protein (mg / 100 g)		
	Ethanol	**Acetone**	**Aqueous**	**Ethanol**	**Acetone**	**Aqueous**
P. quadriaurita	43.51±0.06	42.40±0.08	43.36±0.12	104.14±0.08	104.01±0.15	103.95±0.08
H. arifolia	43.4±0.08	42.90±0.09	42.86±0.10	103.40±0.10	103.46±0.05	103.09±0.07
P. aquilinum	42.82±0.10	43.05±0.10	43.89±0.12	102.96±0.10	104.26±0.08	104.14±0.10
A. raddianum	43.21±0.15	43.21±0.06	43.17±0.08	103.83±0.23	103.95±0.05	103.89±0.06
SEd	0.086			0.08819		
CD (p<0.05)	0.177			0.18202		

Values are mean ± SD of three samples in each group

* - Significant at 5% level (p<0.05)

Fig.2 Comparision of Total Carbohydrate in Ethanol, Acetone and Aqueous extract of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*

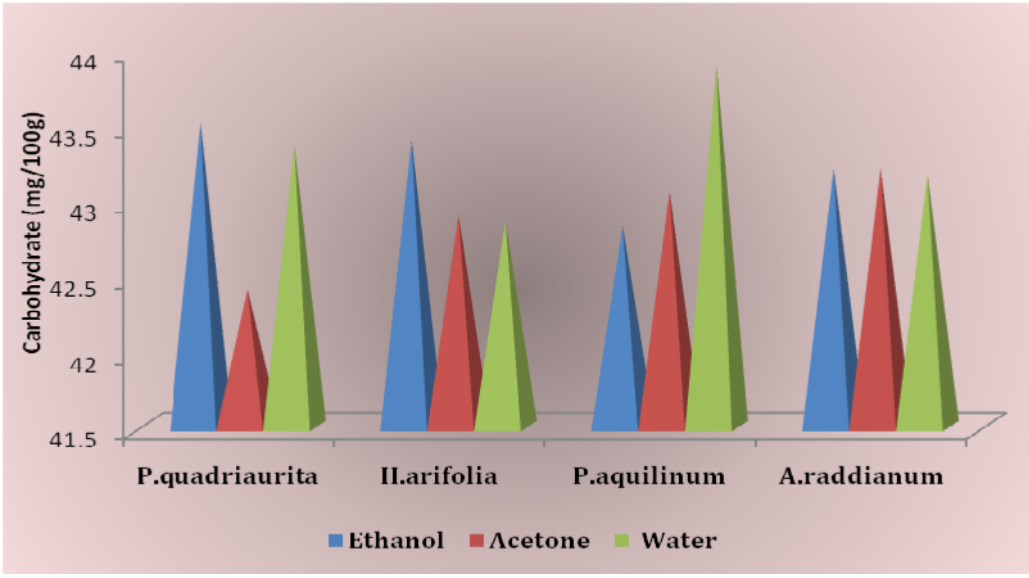
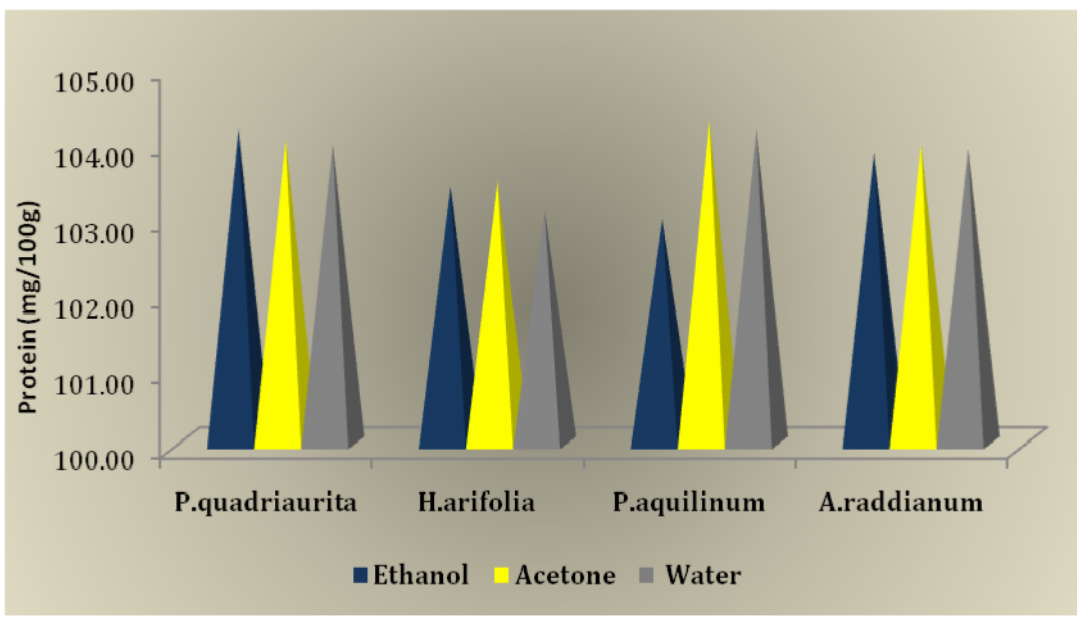


Fig.3 Comparision of Protein content in Ethanol, Acetone and Aqueous extract of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*



4.4.2.1 Total Flavonoids (TF)

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. The examination of the total flavonoids-quercetin was used as standard. In this study, the total flavonoid content was measured by aluminium colorimetric method and expressed as mg quercetin equivalents per gram of dry extract (µg QE/ ml of extract).

Concerning the total flavonoids content, the highest value was recorded in ethanol extract for *A. raddianum* (251.97 µg QE/g of extract) followed by *H. arifolia* (156 µg QE/g of extract), *P. aquilinium* (136.5 µg QE/g of extract) whereas in *P. quadriaurita* (134.77 µg QE/g of extract). On the other hand, Aqueous extract showed the lowest total flavonoids content in the four studied plants with the values of 183.67, 112.83, 100.73 and 99.1 µg QE/ml of extract for *A. raddianum*, *H. arifolia, P. aquilinium* and *P. quadriaurita* respectively.

Methanolic extract of *H. arifolia* was reported to have 58.68 mg QE/ml flavonoid content by Udayabhanu *et al*., (2014) and 0.32 mg/g of flavanoid was reported by Rukmini *et al.,* (2015). According to Jaishee and Chakraborty, (2014) *Pteris biaurita* had 14.539 mg/g flavonoid. Gracelin *et al.,* (2013) reported that flavonoids 12.15 mg, 14.20, 13.25, 17.55 and 9.5 mg in *P. confusa, P. vittata, P. argyreae, P. biaurita* and *P. multiaurita* respectively. Singh *et al.,* (2015) found that total flavonoid content of six road samples of *Pteris vittata* had a large variation ranging from 0.811 mg to 0.219 mg.

4.4.2.2 Total Phenolic Content:

In the present investigation the ethanolic, acetone and aqueous extracts of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* were examined for its total phenolic content using folin-ciocalteau reagent method. The reduction of Folin Ciocalteu reagent by phenolic ion will change its solution colour into blue. The reduction of complex will increase when the extract contain more phenolic compounds. Thus, the colour will be darker and the absorbance will be higher. Total phenolics content were expressed as mg gallic acid equivalents per gram of dry extract (µg GAE/ ml of extract). Results obtained in the present study revealed that the level of these phenolic compounds in the various plant extracts was considerable.

Table. 6. Quantitative test for the phytochemical – Flavonoid, Phenol and Tannin

Plant Name	Total Flavonoid			Total Phenol			Total Tannin		
	Ethanol	Acetone	Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone	Aqueous
P. quadriaurita	134.77±0.85	100.73±0.80	108.6 ±1.11	122.73±0.64	107.93±0.55	169.00±1.00	88.77±0.47	71.33±1.59	41.63±0.59
H. arifolia	156 ± 1.07	137.43±0.65	112.83 ± 1.44	178.13±0.50	175.1±1.01	155.37±1.15	136.47±1.53	131.63±0.76	64.83±2.21
P. aquilinum	136.5 ± 1.0	102 ± 1.39	99.1 ± 0.71	177.57±0.78	171.67±0.75	90.4±0.91	132.13±1.03	105.07±1.93	99.17±0.51
A. raddianum	251.97±1.5	242.67±0.91	183.76 ± 0.32	179.43±0.90	130.37±1.25	161.70±0.75	145.53±0.50	89±1.50	96.17±2.25
SEd	0.84108			0.71828			5.34819		
CD (p<0.05)	1.73590*			1.48246*			1.67719*		

Flavonoids expressed as µg Quercetin equivalent/ ml of extract

Phenolics expressed as µg Gallic acid equivalent/ ml of extract

Values are mean ± SD of three samples in each group

* - Significant at 5% level (p<0.05)

As seen in Table. 6, the highest were observed in ethanolic extract of *A. raddianum* with value of 179.43 µg GAE/ml whereas the lowest content was signaled for Aqueous extract of *P. aquilinum* with value of 90.4 µg GAE/ml. Among the three extracts ethanol extract showed highest values in all the four ferns. The ranking of the plants on the basis of flavonoid content in all the three extract was *A. raddianum* > *H. arifolia* > *P. aquilinum* > *P. quadriaurita*.

Similar works were reported regarding the amount of phenolic content in various ferns. Udayabhanu *et al.,* (2014) observed phenolic content in *H. arifolia* as 65mg/GAE/g. Quantification of bioactive compounds in *Pteris biaurira* by jaishee and chakraborty, (2014) revealed 10.399 mg/g dwt of total phenol. Singh *et al.,* (2008) analyzed four species of *Adiantum* i.e *A. capillus-veneris, A. peruvianum, A. venustum* and *A. caudatum* for total phenolic constituents present in them which resulted in 0.81% (w/w), 0.83% (w/w),0.71% (w/w) and 0.52%(w/w) respectively (as galic acid equivalent). Gracelin *et al*., (2013) reported that phenol of 9.33mg, 10.45, 8, 13.25 and 7mg in *P. confusa, P. vittata, P. argyreae, P. biaurita , P. multiaurita* respectively was present. According to Kaur *et al*., (2014) in *Pteris vittata* total phenolic content of PME (100µg/ml) was found to be 10.64mg GAE/g. Singh *et al*., (2015) reported that total phenolic content of six road samples of *Pteris vittata* showed large variation ranges from 0.21875 mg to 1.687. Simillar results were obtained from *Pteris biaurita* by jaishee and chakraborty, (2014) which revealed 10.399 mg/g dwt of total phenol. It appears that phenolic compounds extracted depend on plant species and the solvent used. Furthermore, environmental factors such as climate, soil, irrigation, temperature range, exposure to diseases and pests, the harvest season, the way of drying and storage or even the part of the plant tissue to be used are parameters that should be taken into consideration. The extraction procedure appears also to affect total phenolic content and antioxidant activity of each plant (Oukil et al., 2011; Skotti et al., 2014).

4.4.2.3 Total Tannin

The examination of the total tannins was carried out using tannic acid as standard. In the present study the ethanolic extracts of *P. aquilinum* was examined for its total tannin contents using folin-Denis method. The content of total tannins in *P. aquilinum* was equal to 35.9 mg of TAE/ g of extract. Maximum tannin content was found in *A. raddianum* (145.53 mg TAE/g) and minimum was found in *P. quadriaurita* (88.77 mg TAE/g). In acetone extract the tannin content

Fig.4 Comparision of Total Flavonoid content in Ethanol, Acetone and Aqueous extract of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*

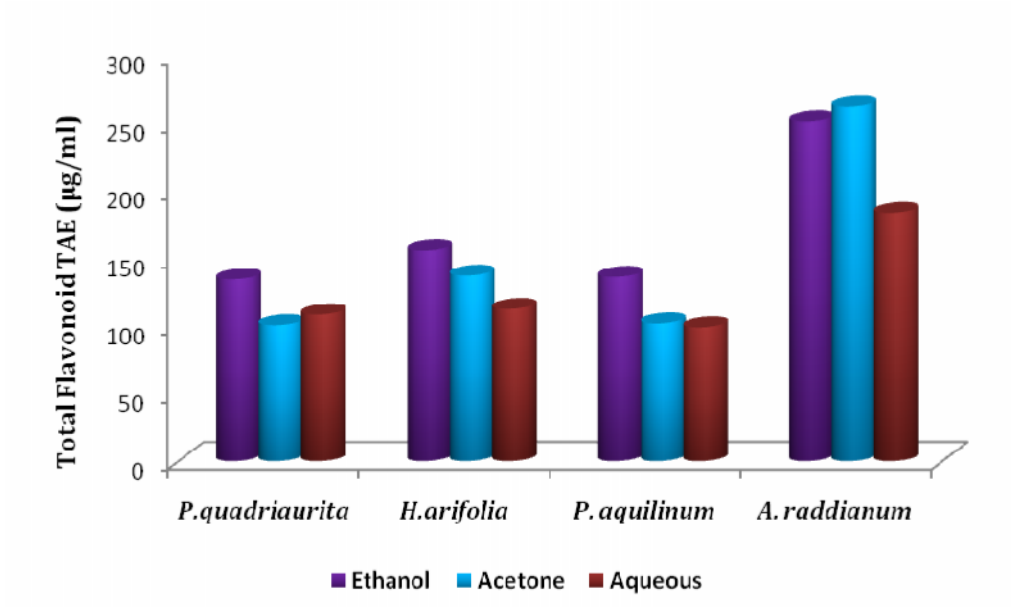


Fig.5 Comparision of Total Phenol content in Ethanol, Acetone and Aqueous extract of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*

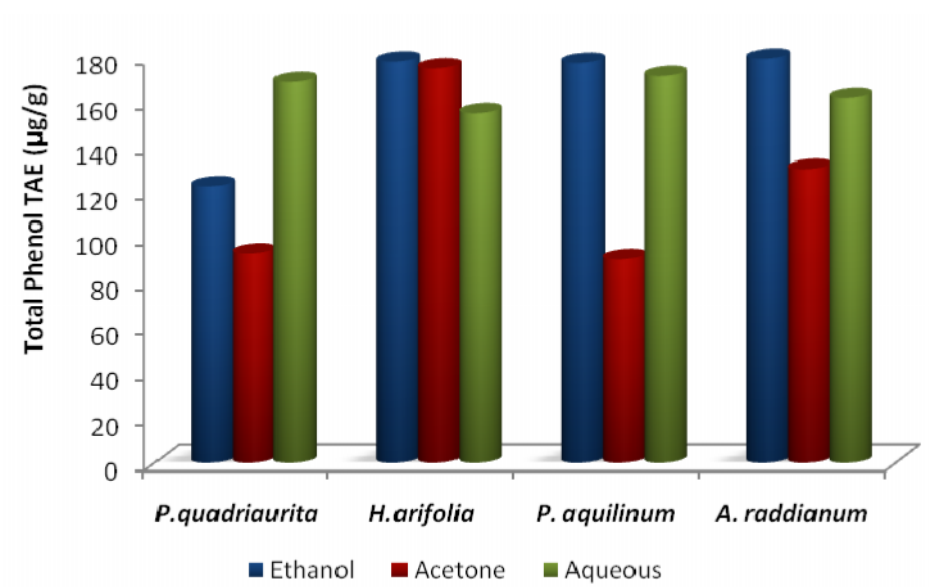
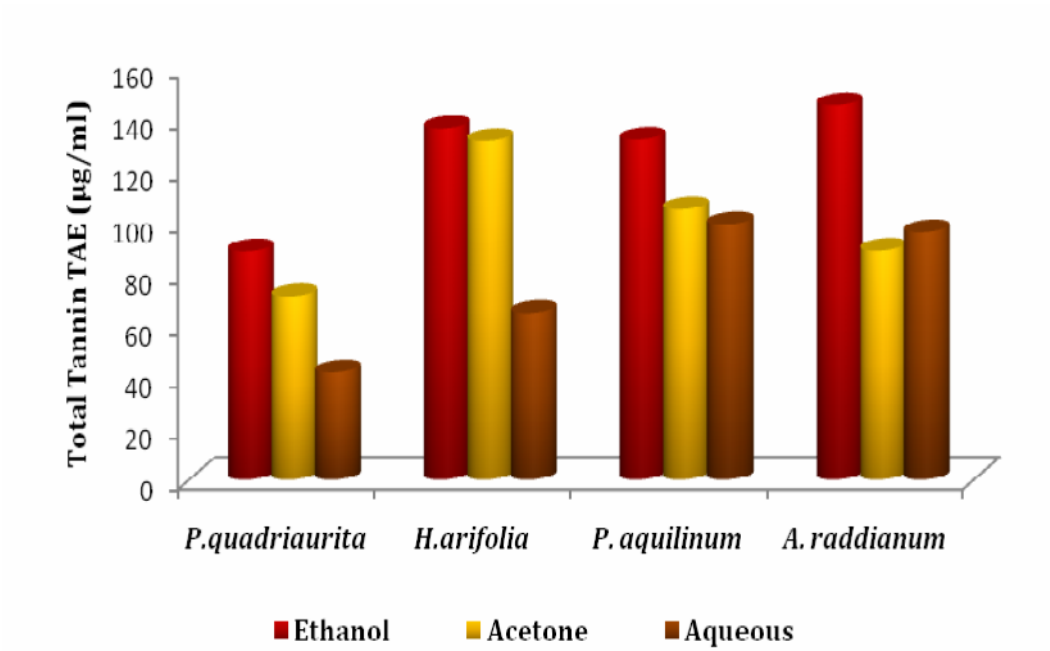


Fig. 6 Comparision of Tannin content in Ethanol, Acetone and Aqueous extract of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*



was present maximum in *H. arifolia* and minimum in *P. quadriaurita* as 131.63 mg TAE/g and 71.33 mg TAE/g respectively. In aqueous extract the maximum amount of tannin was present in *P. aquilinum* (99.17 mg TAE/g) and minimum in *P. quadriaurita* (41.63 mg TAE/g). Though the

fern which had maximum tannin content varied the minimum amount of tannin present in all the three extracts remain to be *P. quadriaurita*.

Simillar results were reported by Rukmini *et al.,* (2015) that *H.arifolia* had 0.26 mg/g of tannin, while Gracelin *et al*., (2013) reported 3 mg, 5.3, 4.60 and 6.25 mg in *P. confusa, P. vittata, P. argyreae, P. biaurita* respectively and absent in *P. multiaurita*.

4.5 Antioxidant

4.5.1 Enzymatic Antioxidant

4.5.1.1 Polyphenol Oxidase

Polyphenol oxidases (PPOs; EC 1.10.3.2 or EC 1.14.18.1) catalyzing the oxygen-dependent oxidation of phenols to quinones are ubiquitous among angiosperms and assumed to be involved in plant defense against pests and pathogens. This phenomenon was proved by Li**,** L**.** and J.C. Steffens (2002).

In this present study among the four ferns taken for the study maximum and minimum amount of polyphenol oxidase was present in *P. aquilinum* and *H. arifolia*. The values of all the ferns taken for this study according to their highest to lowest values are given below: 19.70 U/g, 17.57 U/g, 13.57 U/g and 3.33 U/g in *P. aquilinum, P. quadriaurita, A. raddianum* and *H. arifolia* respectively.

4.5.1.2 Peroxidase

Present investigation showed a significant peroxidase activity was in all the ferns, and activity was recorded to be slightly similar in *P. quadriaurita, P.aquilinum* and *H. arifolia* as 106.8 U/g, 105.33 U/g and 104.13 U/g respectively. *A. raddianum* (60.83 U/g) showed a much less activity when compared with the other ferns taken for the study.

Studies done in *P. vittata* by Feng and wei, (2012), showed the presence of peroxidase activity in selenium concentration (mg/l), the higher concentration of se5 (44 U/g) and lower

Table. 7. Levels of various antioxidant enzymes in *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*

Plants	Polyphenol oxidase (U*/g)	Peroxidase (U@/g)	Ascorbate oxidase (U#/g)
P. quadriaurita	17.57 ± 0.51	106.8 ± 1.15	2.6 ± 0.20
H. arifolia	3.33 ± 0.35	104.13 ± 0.23	2.48 ± 0.40
P. aquilinum	19.70 ± 0.36	105.33 ± 0.58	2.30 ± 0.26
A. raddianum	13.57 ± 0.51	60.83 ± 15.91	1.94 ± 0.42
SEd	0.3985	0.6815	0.2347
CD (p<0.05)	0.6291	0.9742	0.3956

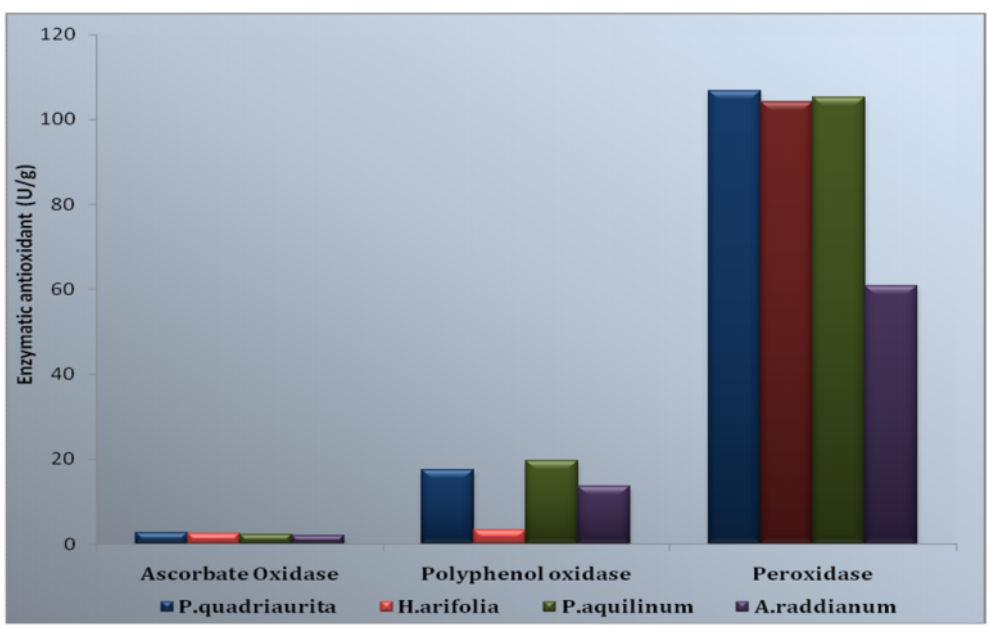
Values are mean ± SD of three samples in each group

* 1 unit - Activity of catechol oxidase which transforms 1 U/mol of dihydro-phenol to quinine/min

@1 unit - change in absorbance / minute at 430 nm

1 unit – U/g

Fig.7 Comparision of enzymatic antioxidants in *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*



concentration of se10 (24 U/g). This study shows that our results were four times higher than the concentration present in *P. vittata*.

4.5.1.3 Ascorbate Oxidase

Ascorbic acid oxidase is widespread in plant tissues. The role of thisenzyme is to regulate the levels of oxidised and reduced glutathione andNADPH. Phenol oxidases are copper proteins catalyse the aerobic oxidationof certain phenolic compounds to quinones. The poly phenol oxidasecomprises catechol oxidase and laccase. The activities these enzymes areimportant with regard to defence mechanism against diseases (Benzie, 1999).

Ascorbate oxidase activity of *P. quadriaurita* and *A. raddianum* was found to be maximum and minimum respectively. The activity of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* were 2.6 U/g, 2.48 U/g, 2.30 U/g and 1.94 U/g respectively.

4.5.2 Non-Enzymatic Antioxidant

4.5.2.1 Ascorbic Acid

Vitamin C, which includes ascorbic acid and its oxidation product-dehydroascorbic acid, has many biological activities in human body have found that vitamin C can reduce levels of C-reactive protein (CRP), a marker of inflammation and possibly a predictor of heart disease. More than 85% of vitamin C in human diets is supplied by fruits and vegetables (Thevasundari and Rajendran, 2011).

In the present investigation non-enzymatic antioxidant Ascorbic acid was present between 30.3 – 1.31.2 µg / ml when compared within all the three extracts. In ethanol extract the highest value in *A. raddianum* (83.1 µg/ml) and the lowest value in *H. arifolia* were noted. In Acetone extract the maximum value and minimum value were observed in *A. raddianum* (85.8 µg/ml) and *P. quadriaurita* (30.3 µg/ml) respectively. While analyzing the aqueous extract the maximum value was registered in *P. quadriaurita* (131.2 µg/ml) and minimum value was registered in *H. arifolia* (32.0 µg/ml)

4.5.2.3 Reduced glutathione

Glutathione, a major non-protein thiol in living organisms, plays a central role in coordinating the body's antioxidant defense processes. Excessive peroxidation causes increased

Table: 8. Estimation of Non-enzymatic antioxidants in *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*

Plant Name	Ascorbic Acid (Ug / ml)			Total Polyphenol (mg / 100 g)			Reduced glutathione (Ug / ml)		
	Ethanol	Acetone	Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone	Aqueous
P. quadriaurita	59.1±0.002	30.3±0.002	131.2±0.002	531±0.004	433±0.002	383±0.002	696±0.002	595±0.003	417±0.002
H. arifolia	53.6±0.003	67.4±0.002	32.0±0.001	552±0.001	484±0.003	292±0.001	620±0.001	612±0.002	131±0.001
P. aquilinum	63.9±0.003	32.4±0.002	36.4±0.002	518±0.03	444±0.001	46±0.003	577±0.003	533±0.001	204±0.001
A. raddianum	8.31±0.002	85.8±0.002	60.3±0.002	560±0.002	561±0.002	149±0.002	699±0.014	603±0.005	274±0.004
SEd	0.0015			0.0012			0.0616		
CD (p<0.05)	0.0033			0.0048			0.1702		

Values are mean ± SD of three samples in each group

* - Significant at 5% level (p<0.05)

Fig.8 Comparision of Non-enzymatic antioxidant Ascorbic acid in *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*

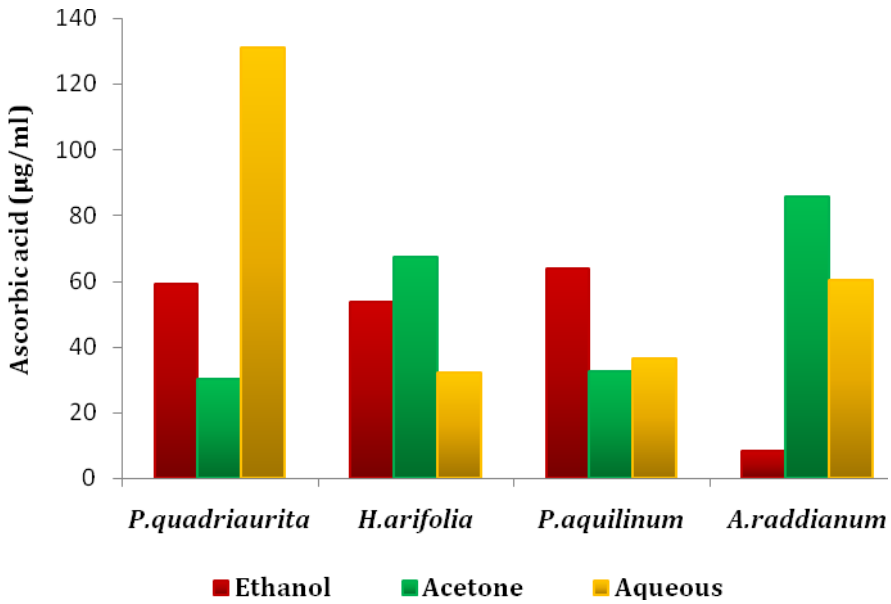


Fig.9 Comparision of Non-enzymatic antioxidant total polyphenol in *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*

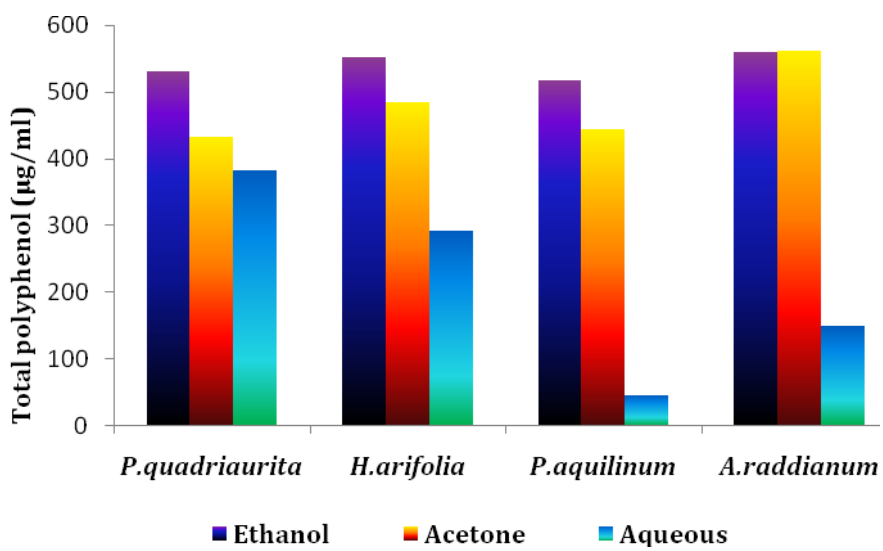
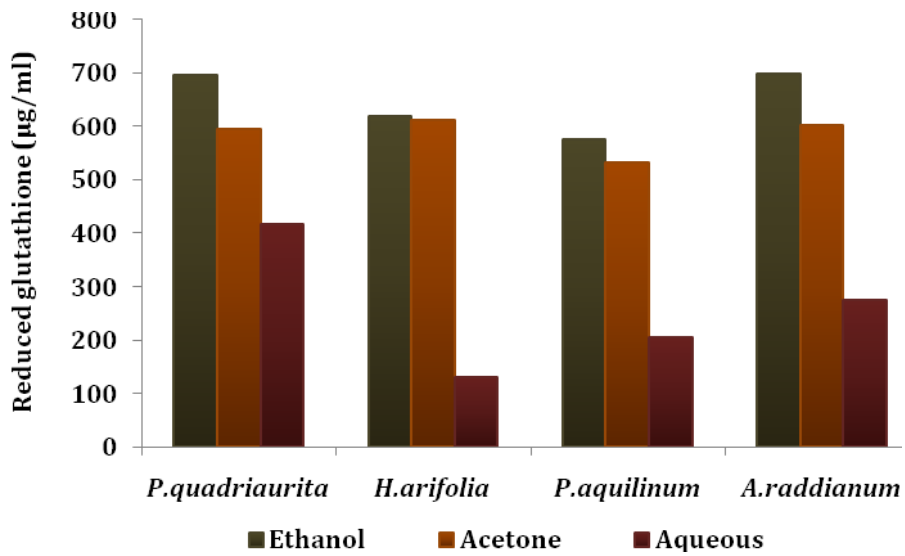


Fig.10 Comparision of Non-enzymatic antioxidant Reduced Glutathione in *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*



glutathione consumption. Reduced thiols have long been reported to be essential for recycling of antioxidants like vitamin E and vitamin C (Weis, 1975).

The present study revelaed the presence of reduced glutathione in all the four ferns in all the three extracts, while *H. arifolia* showed a very low amount in aqueous extract and *A. raddianum* showed the highest value in overall compariosion. In ethanol extract the maximum activity of reduced glutathione was present in *A. raddianum* (699 µg/ml) and the minimum was found in *P. aquilinum* (577 µg/ml). In acetone extract activity of reduced glutathione was found to be maximum in *H. arifolia* and minimum in *P. aquilinum* with the values of 0.612 µg/ml and 533 µg/ml respectively. In aqueous extract highest activity was observed in *P. quadriaurita* (417 µg/ml) and lowest activity in *H. arifolia* (131 µg/ml).

Simillar results were obtained by Kumar, 2009 in 20 µl of *Adiantum capillus veneris* leaves extract 4.90±0.03 (µm/mg of protein) reduced glutathione was observed. Feng and wei, 2012, observed the presence of glutathione reductase activity in selenium concentration (mg/L) of *P.vittata*. the higher concentration of se20 (27.5) and lower concentration of se5 (17.5).

4.5.2.2 Total Polyphenol

The present study is done to investigate the amount of total polyphenol present in the four ferns in different extract. Overall comparision reveals that the maximum amount is present in acetone extract of *A. raddianum* (561 µg/ml) and minimum amount was present in aqueous extract of *P. aquilinum* (46 µg/ml).

In ethanol extract the maximum value was observed in *H. arifolia* (552 µg/ml) and the minimum value was observed in *P. aquilinum* (518 µg/ml). In acetone extract *A. raddianum* had the highest value of 561 µg/ml and the lowest value of 433 µg/ml in *P. quadriaurita*. In aqueous extract the maximum and minimum values were observed by *P. quadriaurita* (383 µg/ml) and *P. aquilinum* (46 µg/ml) respectively.

4.6 Free Radical Scavenging activity

4.6.1 DPPH Scavenging activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. DPPH is a stable nitrogen – centered free

Table. 9. DPPH scavenging activity of in Ethanol Extract

Conc. (Ug/ml)	*P. quadriaurita*	*H. arifolia*	*P. aquilinum*	*A. raddianum*	Ascorbic Acid
5	17.43±0.38	3.71±0.19	12.27±0.26	17.70±0.52	10.67±0.17
10	35.20±0.18	16.11±0.21	19.74±6.16	34.45±0.61	29.11±0.96
15	55.44±0.12	26.06±0.24	34.68±1.25	51.76±0.74	68.45±1.30
20	78.65±0.11	39.21±0.48	58.24±0.74	74.09±0.56	78.46±1.30
25	81.96±0.83	54.09±0.68	65.25±0.42	95.92±0.42	83.24±.0.92
30	83.23±0.25	68.15±0.74	73.10±0.59	98.59±0.55	84.52±0.45
SEd	0.3258	0.3913	2.1246	0.4679	0.7735
CD (p<0.05)	0.7099	0.8526	4.6291	1.0194	1.6854

Table. 10. DPPH scavenging activity in Acetone Extract

Conc. (Ug/ml)	*P. quadriaurita*	*H. arifolia*	*P. aquilinum*	*A. raddianum*	Ascorbic Acid
5	15.02±0.67	11.12±0.76	11.17±0.76	20.63±0.90	10.67±0.17
10	26.93±0.53	39.78±0.75	21.24±0.78	34.15±0.68	29.11±0.96
15	36.13±0.73	50.69±0.65	42.43±0.68	41.97±1.29	68.45±1.30
20	46.61±0.57	56.12±0.62	61.67±1.22	55.43±0.99	78.46±1.30
25	61.31±1.14	57.81±0.82	72.13±0.94	62.68±1.37	83.24±.0.92
30	69.71±0.56	60.81±0.89	77.51±0.83	80.11±0.69	84.52±0.45
SEd	0.5962	0.6464	0.7237	0.8330	0.7735
CD (p<0.05)	1.2989	1.4085	1.5769	1.8150	1.6854

Fig. 11 Comparison of DPPH free radical scavenging ability of Ethanol extract of four ferns in different concentrations

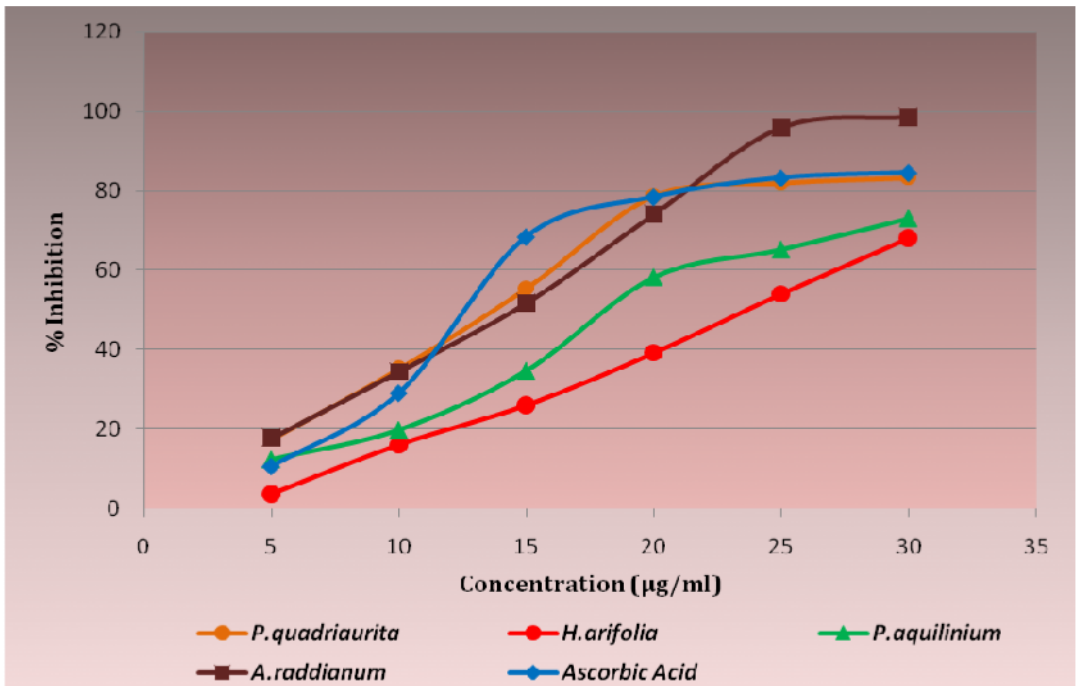
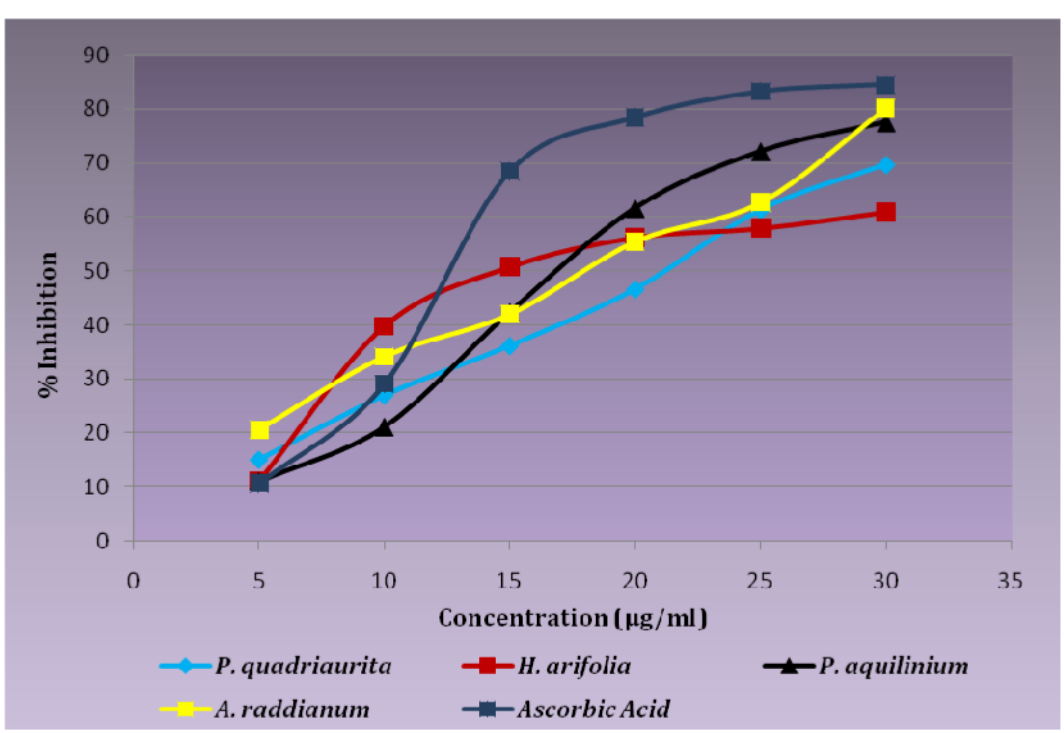


Fig. 12 Comparison of DPPH free radical scavenging ability of Acetone extract of four ferns in different concentrations



radical whose color changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Brand-Williams *et al.*, 1995; Ebrahimzadeh *et al*., 2010; Moyo *et al*., 2012).

The DPPH scavenging activity of ethanol extract of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* was concentration dependent (increasing from 5 µg/ml – 30 µg/ml) and it was able to inhibit the formation of DPPH radicals. The antioxidant activity of ethanol extract increased in a dose dependent manner with IC_{50} value of 13.56, 22.81, 16.97 and 14.7 in *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* respectively.

The DPPH scavenging activity of ethanol extract of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* was concentration dependent (increasing from 5 µg/ml – 30 µg/ml) and it was able to inhibit the formation of DPPH radicals. The antioxidant activity of ethanol extract increased in a dose dependent manner with IC_{50} value of 21.73, 14.63, 17.99 and 18.4 in *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* respectively.

The DPPH scavenging activity of ethanol extract of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* was concentration dependent (increasing from 5 µg/ml – 30 µg/ml) and it was able to inhibit the formation of DPPH radicals. The antioxidant activity of ethanol extract increased in a dose dependent manner with IC_{50} value of 24.32, 18.8, 17.56 and 19.11 in *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* respectively.

Comparing all the IC_{50} values of all the four ferns in three extracts, it seems *P. quadriaurita* had a good value of 13.56 in ethanol extract, while *H. arifolia* showed a good value of 14.63 and in aqueous extract *P. aquilinum* showed a good value of 17.56. Among these ethanolic extract of *P. quadriaurita* is found to possess a better IC_{50} value for DPPH scavenging activity.

Results obtained seemed to differ from that of the findings stated by Kumar *et al*., (2016). According to them in *Pteris cretica* the IC_{50} value for DPPH ranged from 90.33, 61.33 and 32 µg/ml in 95%, 50% ethanol and rutin respectively. DPPH activity in n-Hexane, chloroform, and ethanol extracts of *Pteris cretica* L. and standard ascorbic acid showed 60%, 67%, 65% and 85% free radical scavenging activity (%RSA), respectively.

Table. 11. DPPH Scavenging Activity of Aqueous Extract

Conc. (Ug/ml)	*P. quadriaurita*	*H. arifolia*	*P. aquilinum*	*A. raddianum*	Ascorbic Acid
5	10.01±0.87	10.24±0.36	10.77±0.97	13.76±0.83	10.67±0.17
10	17.33±0.52	37.66±0.81	12.56±0.83	34.15±0.88	29.11±0.96
15	27.30±0.90	45.34±1.28	33.60±1.07	40.64±0.81	68.45±1.30
20	29.58±1.06	53.43±1.38	56.47±1.19	53.49±1.09	78.46±1.30
25	51.21±0.93	57.41±0.94	63.17±0.76	61.60±0.93	83.24±.0.92
30	60.59±0.91	58.15±0.86	65.51±0.67	77.54±1.12	84.52±0.45
SEd	0.7186	0.8130	0.7621	0.7765	0.7735
CD (p<0.05)	1.5657	1.7714	1.6605	1.6919	1.6854

Table 12. IC_{50} Value for DPPH activity of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* in Ethanol, Acetone and Aqueous extracts

Extract	*P. quadriaurita*	*H. arifolia*	*P. aquilinum*	*A. raddianum*	Ascorbic acid
Ethanol	13.56	22.81	16.97	14.7	11.19
Acetone	21.73	14.63	17.99	18.4	
Aqueous	24.32	18.8	17.56	19.11	

Fig. 13 Comparison of DPPH free radical scavenging ability of Aqueous extract of four ferns in different concentrations

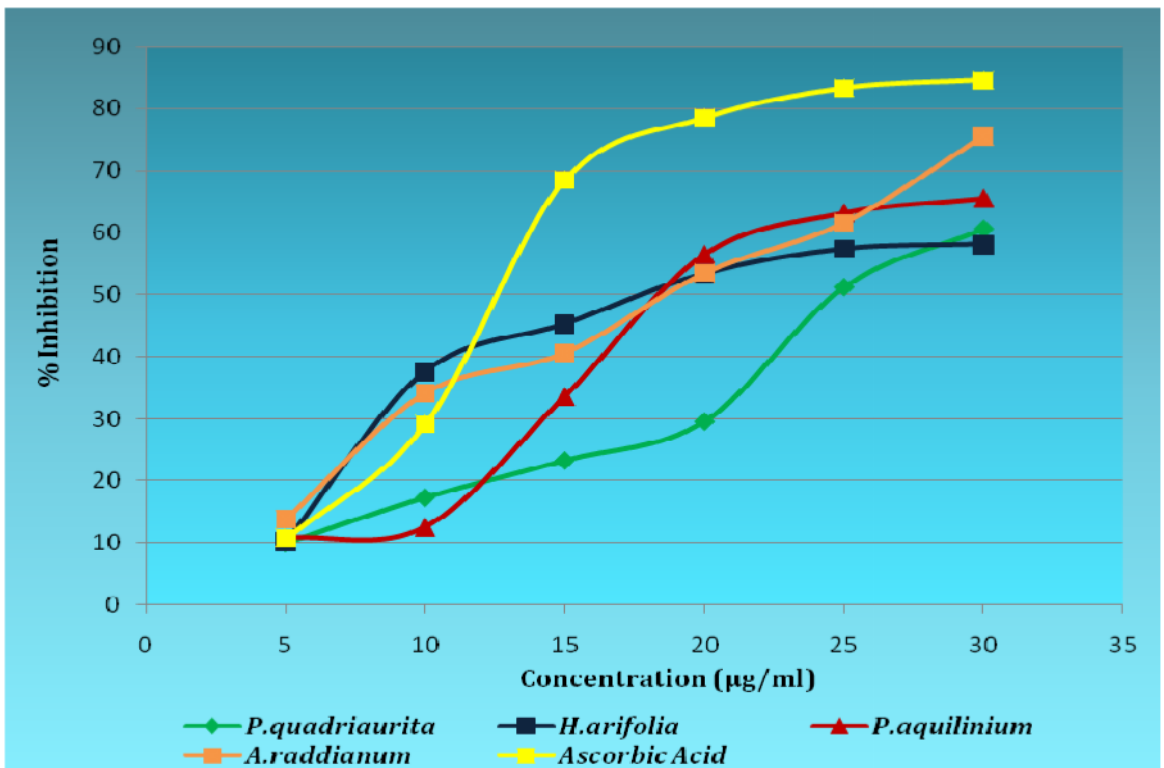
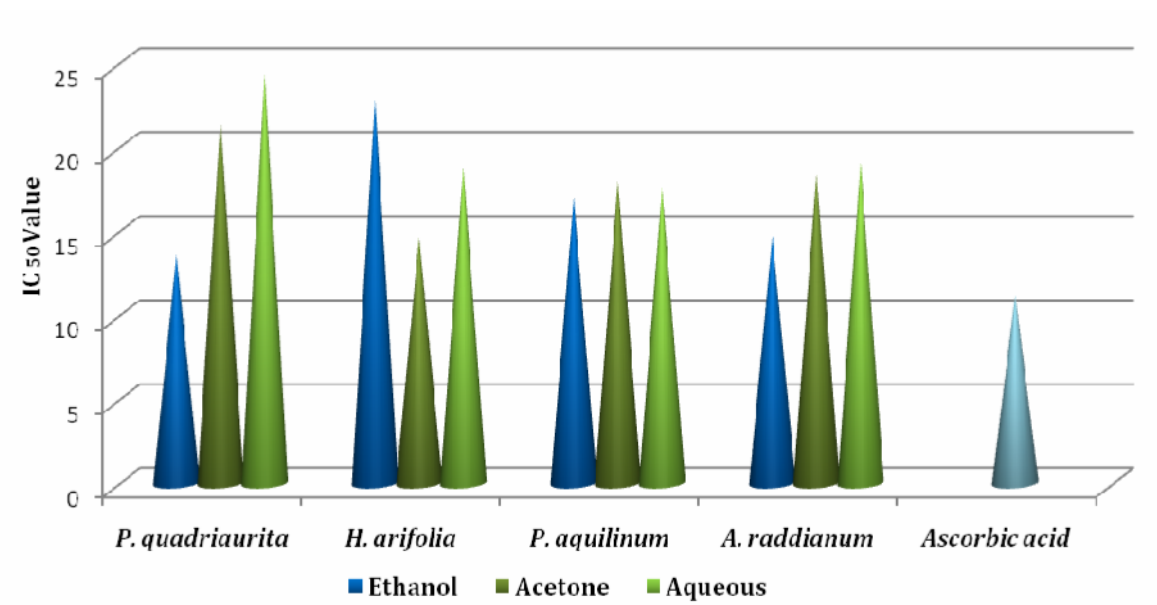


Fig. 14 Concentration of Different extracts required to reduce the initial DPPH radical by 50%



The scavenging potential of the plant extract can be estimated by the degree of discoloration (in DPPH the absorbance decreases due to presence of antioxidant activity. Due to decrease of absorbance the purple colour was turns to yellow. It was reported by (Priyanka and Deepak, 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. It has been showed that the scavenging effects on the DPPH radical increases sharply with the increasing concentration of the samples and standards to a certain extent (Motalleb *et al.*, 2005) and hence are said to be strongly dependent on the extract concentration.

Phytochemical analysis in both stem and leaf of *Adiantum* and *Pteris* IC_{50} for DPPH was reported by them as 26.42, 27.90, 26.42, 26.42 and 27.14 in *Adiantum* leaf, *Adiantum* stem, *Pteris* leaves, *Pteris* stem and ascorbic acid respectively. DPPH scavenging activity of *Adiantum capillus veneris* absorbed the IC_{50} of 51.33 µg/ml in petroleum ether extract and 39.01 µg/ml in methanol extract (Kumar *et al*.,2013).

Similar work were done in *H. arifolia* by Udayabhanu *et al*., (2014) where the DPPH radical scavenging capacity of *Hemionitis arifolia* was found to be 65.7. it was found by Lai and lim *et al.,* (2011) that DPPH scavenging activity of ferns with high Total phenolic content in *Adiantum raddianum* contains 0.27 mg/ml and low phenolic content in *Pteris venulosa* 1.99 mg/ml. DPPH assay of different *Pteris* sample collected from different are showed IC_{50} values ranging from 260-395 µg/ml (Singh *et al.,* 2015). *Pteridium aquilinum* contain good amount of antioxidant activity 84% inhibition in mg/ml. which is comparable with that of ascorbic acid (Kardong *et al*., 2013).

The DPPH radical scavenging capacity of *H. arifolia* was observed by Udayabhanu *et al.,* (2014) to be 65.7± 4.18. Ethanolic extract of *Adiantum capillus-veneris* showed good antioxidant activity as compared to ascorbic acid, it exhibits low IC_{50} value, 0.3986 mg/gm for DPPH assay. DPPH radical scavenging capacity of PME extract of *P.vittata* (28.83 µg/ml) was reported by Kaur *et al*., (2014).

Table 13. Hydrogen peroxide Scavenging activity in Ethanol Extract

Conc. (Ug/ml)	*P. quadriaurita*	*H. arifolia*	*P. aquilinum*	*A. raddianum*	Ascorbic Acid
5	16.40±4.72	11.44±1.19	13.79±0.95	13.65±0.73	10.67±0.17
10	24.47±1.21	25.41±0.97	27.47±0.72	22.36±0.73	29.11±0.96
15	30.54±1.20	36.42±0.79	33.52±0.83	31.45±0.94	68.45±1.30
20	39.57±1.01	45.68±1.22	41.78±0.86	46.37±0.99	78.46±1.30
25	50.45±0.82	57.04±1.37	58.58±1.25	58.58±0.78	83.24±0.92
30	67.22±0.79	62.47±0.90	76.53±0.74	62.46±1.17	84.52±0.45
SEd	1.7487	0.8911	0.7410	0.7394	0.7735
CD (p<0.05)	3.8101	1.9416	1.6145	1.6109	1.6854

Table 14. Hydrogen peroxide Scavenging activity in Acetone Extract

Conc. (Ug/ml)	*P. quadriaurita*	*H. arifolia*	*P. aquilinum*	*A. raddianum*	Ascorbic Acid
5	16.57±0.99	12.55±0.85	12.57±0.76	12.64±0.82	10.67±0.17
10	20.60±1.03	21.70±1.11	22.62±1.03	27.67±0.90	29.11±0.96
15	36.57±1.23	33.39±0.93	38.49±1.13	36.68±1.13	68.45±1.30
20	41.55±0.95	41.55±1.23	42.50±1.38	42.51±0.99	78.46±1.30
25	54.71±1.04	44.57±0.50	50.36±1.02	52.67±1.20	83.24±0.92
30	61.51±1.15	61.53±1.26	60.50±1.08	61.49±0.97	84.52±0.45
SEd	0.8719	0.8276	0.8835	0.8095	0.7735
CD (p<0.05)	1.8997	1.8032	1.9250	1.7637	1.6854

Fig. 15 Comparison H_2O_2 activity of Ethanol extract of four ferns in different concentrations

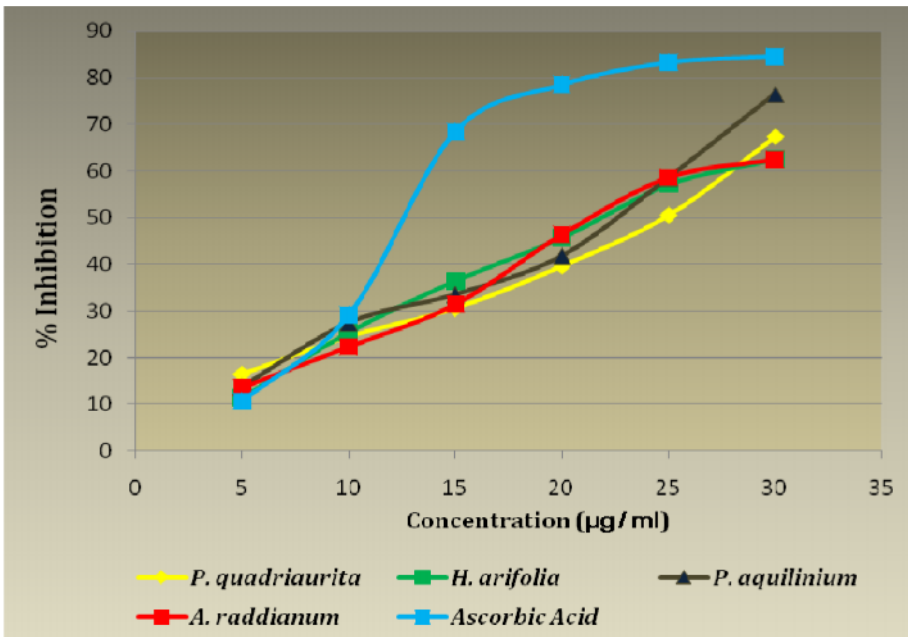


Fig. 16 Comparison H_2O_2 activity of Acetone extract of four ferns in different concentrations

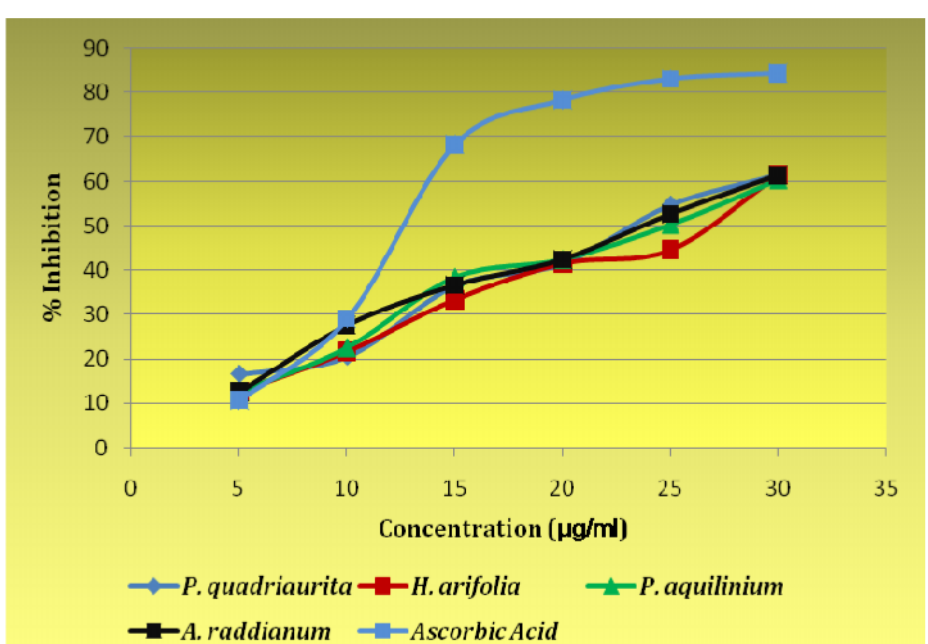


Table 15. Hydrogen peroxide Scavenging activity in Aqueous Extract

Conc. (Ug/ml)	*P. quadriaurita*	*H. arifolia*	*P. aquilinum*	*A. raddianum*	Ascorbic Acid
5	15.74±0.87	11.51±1.07	13.35±0.92	11.55±1.19	10.67±0.17
10	24.58±1.31	20.27±0.90	22.44±0.80	21.54±0.90	29.11±0.96
15	33.33±1.31	36.56±0.66	31.41±1.04	32.60±0.85	68.45±1.30
20	42.10±1.49	43.50±0.70	41.47±0.96	41.39±1.17	78.46±1.30
25	50.48±1.30	50.41±1.15	53.65±1.29	50.42±0.93	83.24±0.92
30	58.39±1.40	60.50±0.83	58.64±1.09	60.65±1.02	84.52±0.45
SEd	1.0083	0.7379	0.8380	0.8319	0.7735
CD (p<0.05)	2.1969	1.6078	1.8260	1.8125	1.6854

Table 16. IC_{50} Value for H_2O_2 activity of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* in Ethanol, Acetone and Aqueous extracts

Extract	*P. quadriaurita*	*H. arifolia*	*P. aquilinum*	*A. raddianum*	Ascorbic acid
Ethanol	24.79	21.98	23.46	21.21	11.19
Acetone	22.42	27.72	24.79	23.82	
Aqueous	24.59	24.2	22.76	24.79	

Fig. 17 Comparison H_2O_2 activity of Aqueous extract of four ferns in different concentrations

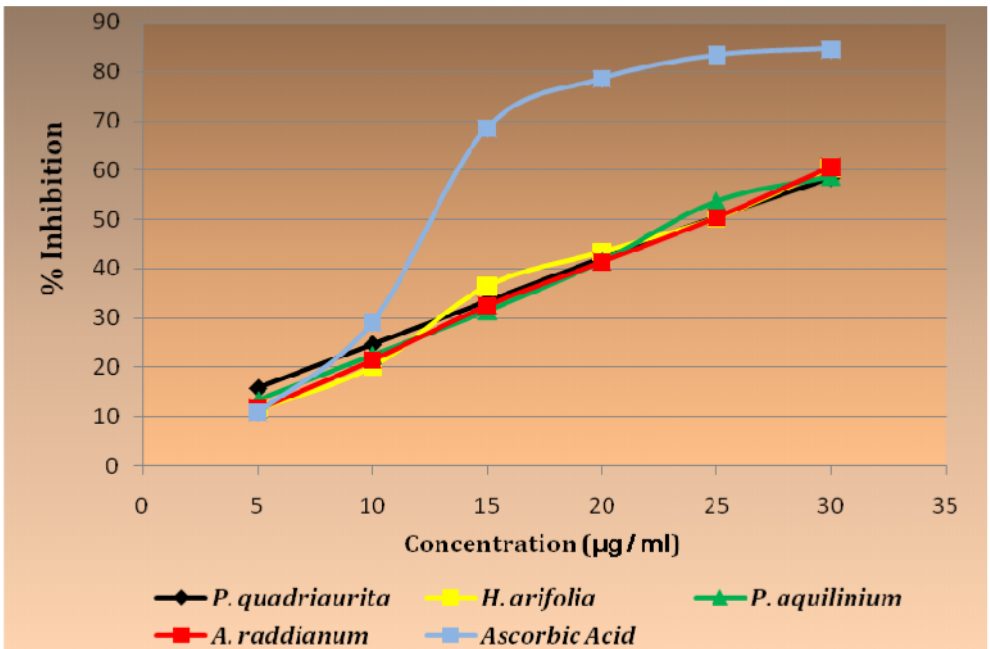
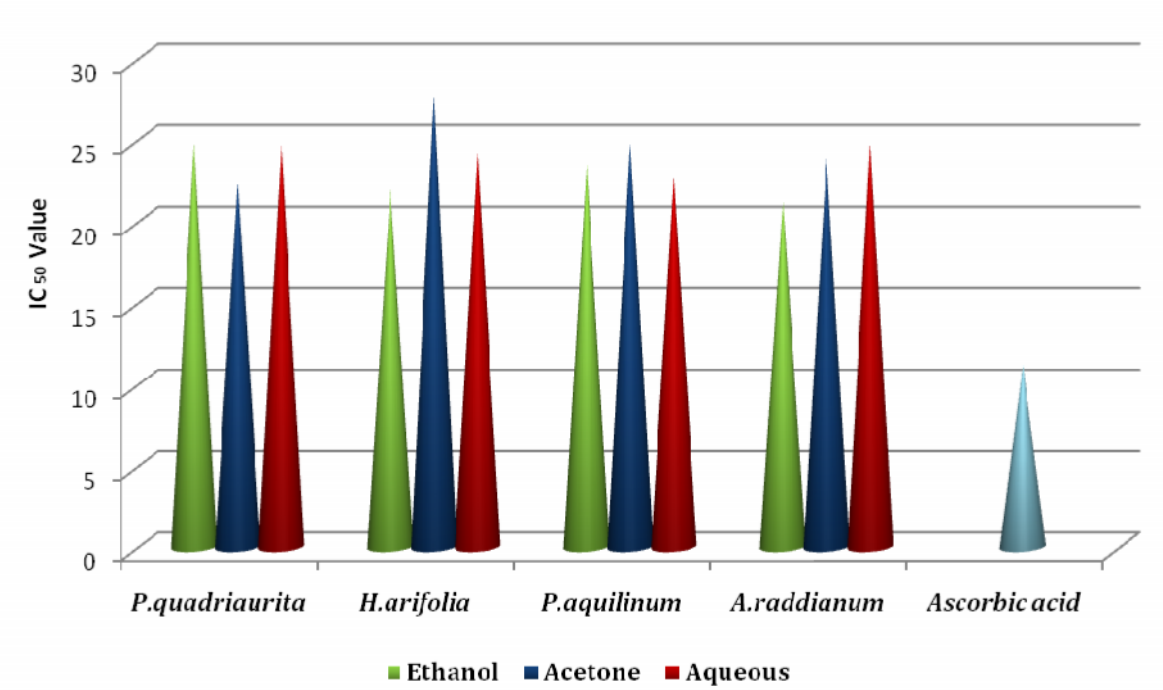


Fig. 18 Concentration of Different extracts required to reduce the initial DPPH radical by 50%



4.6.2 H_2O_2 Scavenging activity

The effect of ethanolic extract on hydroxyl radical is concentration dependent (increasing from 5 Ug/ml – 30 µg/ml) with a good percentage inhibition of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* with IC_{50} value of 24.79, 21.98, 23.46 and 21.21 respectively. It was found that the value was good in *A. raddianum* that other ferns.

The effect of ethanolic extract on hydroxyl radical is concentration dependent (increasing from 5 µg/ml – 30 µg/ml) with a good percentage inhibition of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* with IC_{50} value of 22.42, 27.72, 24.79 and 23.82 respectively. Comparing the activity of the four ferns it was observed that P. quadriaurita showed good activity.

The effect of ethanolic extract on hydroxyl radical is concentration dependent (increasing from 5 µg/ml – 30 µg/ml) with a good percentage inhibition of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* with IC_{50} value of 24.59, 24.2, 22.76 and 24.79 respectively. Compared with all the four ferns *P. aquilinum* was found to possess activity good than the rest of the ferns.

The overall comparision proves that *A. raddianum* ethanolic extract proved to posses a good IC_{50} value than the four ferns in three extracts.Tartar *et al*., (2009) reported that H_2O_2 scavening activity was 84.7 UM, 86.7 UM in *P. vittata* and *P. ensiformis* respectively. Jaishee and Chakraborty, 2014 reported that *P. biaurita*

IC_{50} values of vitamin C, MeOH, HW and EtOH extract to be 71.749, 146.608, 152.351 and 398.759 µg/ml respectively. Das *et al.,* (2012) from their study reported that the ethanolic extract at 250 µg/ml showed 40.21% inhibition whereas the aqueous extract at 250 µg/ml showed 38.07% inhibition of hydrogen peroxide in *Diplazium esculentum*. Earlier research proves that total flavonoids from *Adiantum capillus-veneris* showed high scavenging activity on hydroxyl radicals (Pourmorad *et al*., 2006; Lin and Ding, 2008).

5. SUMMARY AND CONCLUSION

In this present study phytochemical analysis was done in *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum*. The moisture content of the leaf sample collected for this study had very less moisture, which indicates that the season when the sample collected was dry. The solubility test proves that aqueous extract had a very good solubility. Fluorescence property was analyzed and shows presence of secondary metabolites.

Preliminary investigation of phytochemicals showed the presence of carbohydrates, reducing sugar, proteins, flavonoids, glycosides, phenols, tannins in all the ferns in all the three extracts. It showed the absence of alkaloids in all the extracts. Only in aqueous extract anthroquinones, terpenoidscatechin, quinines, sterols were present.

Analysis of biochemical parameters revealed that both carbohydrates and proteins are found to be abundant in all the ferns in all the extract. Ethanolic extract of *A. raddianum* showed the highest amount of total flavonid, and *P. aquilinum* showed the lowest amount of flavonoid. Phenolic compounds are widely distributed in plants and have gained much attention due to their antioxidant activities and free radical scavenging abilities. Results obtained in the present study revealed that while comparing the level of these phenolic compounds in various extracts it was found that total phenolic content was considerably higher in ethanolic extract of *A. raddianum* than in other extracts and lower in aqueous extract of *P. aquilinum*. These findings could be due to different degree of polarity of the solvents used for the extraction of polyphenolic compounds, and to the nature of compounds present in these plant extracts.

Antioxidant study of *P. quadriaurita, H. arifolia, P. aquilinum* and *A. raddianum* proved that these ferns have good enzymatic antioxidant activity. Polyphenol oxidase activity was found to be high in *P. aquilinum*. Peroxidase activity was found to be at its maximum when compared with the four ferns in *P. quadriaurita*. Ascorbate oxidase activity was found to be more in *H. arifolia* than the other ferns. Non-enzymatic antioxidant of ascorbic acid was good in aqueous extract of *P. quadriaurita*, while total polyphenol was found to be maximum in ethanolic extract of *A. raddianum*, and minimum in aqueous extract of *P. aquilinum*. GSH was found to be high in ethanolic extract of *A. raddianum* and

low in aqueous extract of *H. arifolia*. Maximum amount is present in ethanolic extract and lowest in aqueous extracts irrespective of the plant material.

IC_{50} values of all the four ferns in three extracts were compared, to study the plant and also the extract which has the best value. Ethanolic extract of *P. quadriaurita* had maximum, while *H. arifolia* proved to be good in acetone extract *P.aquilinum* showed the maximum value in aqueous extract. Overall comparative study reveals that ethanolic extract of *P. quadriaurita* is found to possess a better IC_{50} value for DPPH scavenging activity.

Compared with all the four ferns for H_2O_2 scavening activity revealed that *P. aquilinum* possess activity good than the rest of the ferns. The overall comparision proves that *A.raddianum* ethanolic extract proved to posses a good IC_{50} value than the four ferns in three extracts.

In conclusion, the key research areas to be considered in future in relation to the present study include:

- ✓ Studying the toxicity of the selected plants.
- ✓ Analyzing the ABTS scavenging activity.
- ✓ Analyzing the Metal chelating activity.

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