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Pharmacognostic Profiling, Phytochemical Investigation, In Vitro Antioxidant And In Vivo Antinociceptive Activities Of Unexploited Species Of Pteris

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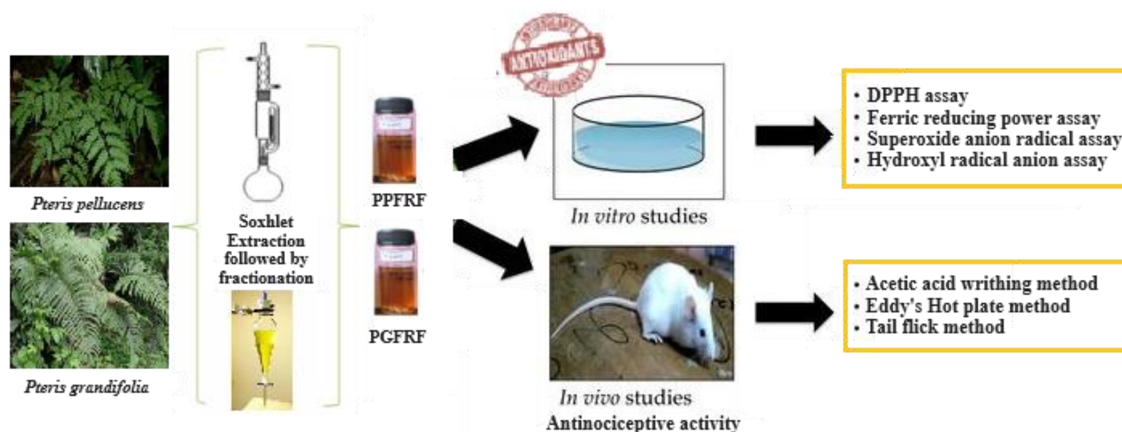
ABSTRACT

Pteridophytes commonly known as ferns play a significant part in traditional medicine, while being ignored nowadays. The primary goal of this study project was to screen various unexploited pteridophyte plants *Pteris pellucens* and *Pteris grandifolia* for their in vitro antioxidant and in vivo analgesic activity. The pharmacognostic standardization of the selected species of *pteris* was done as per standard protocols. The whole plant powders were extracted with ethanol, flavonoid rich fractions of *Pteris pellucens (PPFRF)* and *Pteris grandifolia (PGFRF)* were separated and arsenic content was estimated from their ethanolic extracts as *pteris* species were known to be hyper accumulators of arsenic. *In-vitro* antioxidant activity of ethanol extract and flavonoid rich fraction of the plant species under study were investigated using Superoxide radical scavenging, DPPH radical scavenging, Hydroxyl radical scavenging and Ferric reducing antioxidant power activity. The *in vivo* antinociceptive efficacy of the two plant extracts were analyzed using hot plate, tail flick and acetic acid-induced writhing test. In the powder microscopic studies of the studied plant extracts, V-shaped vascular bundles, a unique characteristic of pteridophytes, was found. Ethanolic extract of both the plantswere found to contain alkaloids, steroids, carbohydrates, glycosides, phenolic compounds, flavonoids and triterpenoids. Flavonoid rich fraction of the the species exhibited better antioxidant activity when compared to their corresponding ethanolic extracts. Comparing the plant extracts' flavonoid rich fractions to their ethanol extracts, it was noteworthy that the former showed significant antioxidant effects. The findings showed that the plant extracts exhibited significant reductions in thermal stimuli and writhing in a dose-dependent manner, indicating their effectiveness in reducing pain perception. These extracts were shown to possess antioxidant phytocompounds with a variety of pharmacologic actions, particularly via reducing oxidative stress, which accounted for their antinociceptive and *in vitro* antioxidant efficacies. To accelerate the design of accessible, affordable, secure, and effective medications, more research on the antinociceptive and antioxidant mechanism(s) as well as the isolation and characterization of relevant compounds is encouraged. Based on the results, we can conclude that the current study implies that this fraction could be very important in the treatment of free radical-related health issues and illnesses associated with age.

Keywords: *Pteris pellucens, Pteris grandifolia,* Flavanoid rich fraction, antioxidant activity, antinociceptive activity.



INTRODUCTION

Ferns and their associates subjected to various stress parameters were expected to serve as active antioxidants in the prevention of ageing and long-lasting illnesses. Free radical damage leads to vast spectrum of human ailments and metabolic disorders. Many research studies have also proved a link between the consumption of antioxidant-rich foods and human diseases prevention[1]. Beyond starch, sugar, amino acids and proteins, ferns have alkaloids, glycosides, terpenoids, flavonoids, phenols, sesquiterpenes and sterols etc. as important components which were used in various industries[2]. *Pteris* was a genus of around 300 fern species. *Pteridaceae* was one family under the genus pteris which was the largest medicinal family with 4 medicinal genera and 16 taxa. Pteridophytes, although hyperaccumulators of Arsenic were anticipated to contain higher concentration of beneficial substances than the remainder of plants as well[3]. Though the pteridophytes were used in Ayurveda, Homoeopathy and Unani system of medicine along with folklore medicine; these plants have been overlooked and; rather neglected. In contrast, variety of phytochemicals such as flavonoids, alkaloids, polyphenols, steroids, glycosides etc., were reported in this group of plants which justify their potential use in the preparation of various medicaments against different ailments[4]. Furthermore, they possess distinct phytonutrients which have not been identified in plants of higher altitudes[5]. These plants were tolerant to infections caused by microbes that might have contributed to its evolving success and longevity of over two hundred and fifty million years [6].

Many conventional beneficial fern species have recently been studied and found to contain biophysical responses like free radical associated[7], anticancer[8], anti-retroviral[9], pain killing [10], antimicrobial [11] and antiviral[12] activities. The inventory survey conducted on pteridophytes revealed the importance of pteridophyte species in ethno medicinal practices by various herbalists in the management of a wide range of ailments and illensses, hence may be considered as important source to manufacture novel drugs to fight against different diseases in pharmaceutical industries[13].

The *pteris* species selected for the current study were *Pteris pellucens* and *Pteris grandifolia* as shown in figure 1. These particular species of *Pteris* were selected for the study out of all the others since it is widely dispersed throughout the study area and have not received much attention in the field of ethnobotany. *Pteris pellucens* also known as *Pteris philippinensis* was a terrestrial fern and *Pteris grandifolia* commonly known as Elephant Leaf Brake Fern have considerable economic and ecological value[14]. However, there has been no investigation into the ethnomedicinal applications of these *pteris* species.

Pteris Pellucens *Pteris grandifolia*

Figure 1: *Pteris* species selected for the study

The widest range of bioactivity, including anti-inflammation, is exhibited by plant-derived polyphenolic and related antioxidant phytochemicals, which work mainly by reducing and mitigating oxidative stress in biological systems and so promoting health[15]. As a result, the focus of research has changed to examine natural products, particularly medicinal plants, as one of the most promising sources of anti-inflammatory and pain medicines[16]. As a result, preliminary phytoconstituents identification, evaluation of *in vitro* antioxidant potential and *in vivo* analgesic activity was carried out in the selected pteridophyte plant species to explore their medicinal value.

MATERIALS AND METHODS

Reagents and chemicals

The study employed analytical-grade chemicals from Sigma Aldrich Pvt. Ltd. in India, including DPPH (α-diphenyl-β-picrylhydrazyl), TPTZ (tripyridyl triazine), NBT (Nitroblue tetrazolium), NADH (Nicotinamide adenine dinucleotide hydrogen), PMS (phenazine methosulfate), and TBA (thiobarbituric acid). Additional reagents, such as methyl orange, saffranine, chloral hydrate, and phloroglucinol, were acquired from Himedia Pvt. Ltd. in India.

Collection and Authentication of Plant material

The two plants, *Pteris pellucens* and *Pteris grandifolia*, were recognized and gathered from the wet, hilly regions of the Sathyavedu forest region in Andhra Pradesh. Dr. K. Madhava Chetty, Assistant professor at Sri Venkateswara University in Tirupati, verified the authenticity of the plants. Plants *Pteris Pellucens* (SVCP/2020/23) *and Pteris grandifolia* (SVCP/2020/24) voucher specimens were submitted at Sree Vidyanikethan College of Pharmacy, Tirupati, in the Department of Pharmacognosy. Following a thorough washing, the two plants were dried in indirect sunlight for 15 to 20 days before being ground into a coarse powder (Sieve No. 44) using a grinder. The dried herbs in powder form were stored in airtight bottles.

Pharmacognostic Study

To prevent adulteration and substitution of medicinal herbs, standardization characteristics were essential[17]. In this investigation, the following criteria were to be assessed: powder study, physicochemical and phytochemical analysis, macroscopic and microscopic examination, and organoleptic features.

Physicochemical analysis

The WHO guidelines for quality control procedures for medicinal plant materials[18,19] and the Indian Pharmacopoeia recommended the physicochemical criteria, such as loss on drying, total ash, water insoluble, and soluble as well as acid insoluble ash for the selected species.

Extraction of the plant material

The plant powders (100 g) of two species were soaked in pet ether (60-80°C) for 72 hours in order to defat them. After the defatted plant powders were extracted with ethanol using Soxhlet extraction equipment, three to four cycles of six hours each were completed until a clear solvent was obtained in the extraction flask. The extracts were filtered and dried in a rotavapour at 40°C to produce two pure ethanol extracts. The extracted materials were then stored in glass vials in a freezer at 4°C until they need to be utilized again.

Determination of Arsenic Content

Since the *pteris* species was one of the hyper accumulators of arsenic, the ethanol extract of two plants were analyzed using Perkin Elmer Optima 5300 DV ICP-OES (Inductively coupled plasma optical emission spectrometry) at a wavelength of 188.979 nm to estimate the amount of arsenic in the plant extract [20].

Phytochemical screening of the plant extracts

Various qualitative tests were conducted for the ethanol extracts of two plants for the identification of chemical constituents using standard methods [21,22].

Separation of Flavonoid Rich Fraction (FRF)

Each of the two herbal extracts were distributed progressively among identical amounts (50 mL each) of water and n-Hexane for flavonoid separation, then the remainder of the water phase was extracted with a similar amount of ethyl acetate to extract all the flavonoid containing compounds into the organic phase, and the ethyl acetate phase was concerted by rotavapour, and the dry fraction was weighed and subjected to flavonoid identification[23]. The scheme of separation of FRF was given in Figure 2.

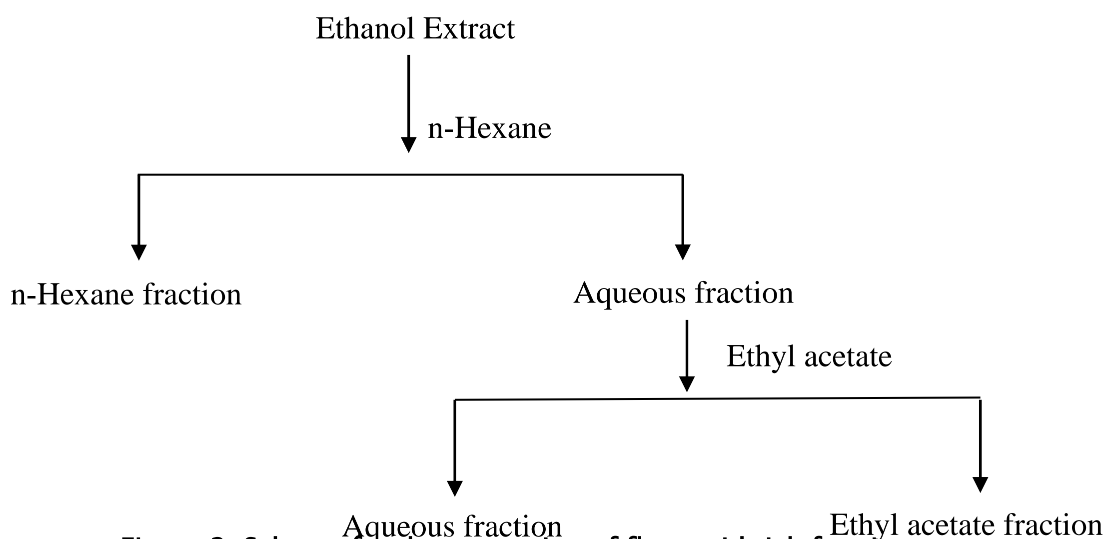


Figure 2: Scheme for the separation of flavonoid rich fraction

***In vitro* antioxidant assays**

Flavonoid rich fractions have been dispersed in purified water to a level equivalent to 1 mg/ml, followed by diluting to prepare concentrations of 100 mg/ml using models such as DPPH radical scavenging, Superoxide radical scavenging, Hydroxyl radical scavenging, and reducing power activity.

DPPH radical scavenging activity

This test was measured as described by Blois[24]. The test's ability to liberate the DPPH radical was expressed as a percentage inhibition and computed using the equation:

$$\text{DPPH scavenging effect (\% inhibition)} = \{(A0 - A1)|A0\} \times 100$$

Where A_0 = absorbance of the control and A_1 = absorbance of the sample.

Ferric reducing antioxidant power (FRAP) activity

The capacity of ferric ions to be reduced has been investigated using the methods given by Benzie & Strain[25] and Zarghami[26] .

Superoxide radical scavenging (SRS) activity

It was estimated using the Garrat DC technique[27]. The percent inhibition was estimated by using the following equation

$$\text{Superoxide radical scavenging activity (\% inhibition)} = \{(A0 - A1)|A0\} \times 100$$

Where, A0 = absorbance of the control and A1 = absorbance in presence of the extract and reference. All the *in vitro* antioxidant tests were done in triplicates and the findings were taken as the mean.

Hydroxyl radical scavenging (HRS) activity

It was conducted using the revised procedure given by Halliwell[28]. The herb's capacity to neutralise hydroxyl radicals was assessed as percent decrease in deoxyribose degradation utilising the following equation:

$$\text{Hydroxyl radical scavenging activity (\% inhibition)} = \{(A0 - A1)|A0\} \times 100$$

Where, A0 = absorbance of the control and A1 = absorbance considering the extract and reference both exist. All *in vitro* scavenger experiments were carried out in a trio, with the findings taken as the mean.

STATISTICAL ANALYSIS

The outcomes were given in the form of mean ± standard error mean (SEM). Results found to be statistically significant using one-way ANOVA followed by a Dunnet's test. The threshold for the significance level was $p < 0.05$.

Acetic acid induced writhing method

***In vivo* Antinociceptive assays**

This test was carried out as previously described by Collier et al[29]. The purpose of the test was to determine whether *PPFRF* and *PGFRF* could influence the nociceptive response brought on by a painful chemical stimulation. The decrease in the average quantity of writhings in the test groups when compared to the control group suggested antinociceptive action. A formula outlined by Dambisya and Lee [30] was used to calculate the percentage of antinociception.

$$\% \text{ of antinociception} = \frac{(\text{control group mean}) - (\text{test group mean})}{(\text{control group mean})} \times 100\%$$

Eddy's Hot plate method

The hot-plate test, as outlined by Eddy and Leimbach[31], was conducted to assess the antinociceptive effects of *PPFRF* and *PGFRF* against heat-induced pain responses. Latency periods were measured prior to (BF) and at 30, 60, 120, and 180 minutes post i.p. administration of the test substances. Statistical analysis compared the prolongation of latency times in the test groups to those in the control group.

Tail flick method

The experiment followed the method described by Sewell et al [32]. Response time, measured with a cutoff of 15 seconds, was recorded when the tail was swiftly removed from hot water.

RESULTS

Macroscopy

The whole plants of two species were evaluated for morphological parameters. The observations for color, odour, shape, taste, fracture and size were noted were tabulated in Table 1.

Parameter	*P.pellucens*	*P.grandifolia*
Colour	Brownish green	Yellowish green
Odour	Strong	Characteristic
Shape	Morphological circumscription of this clade is difficult; potential diagnostic features include veins free and lamina one-pinnate to two-pinnatifid.	Tropical fern produces large, pinnate, leathery leaves from an underground rhizome. The big ones, with fronds about six feet long (2m). Morphologically, the two clades share single-pinnate lamina and the terminal pinna larger than the adjacent lateral pinnae.
Fracture	Brittle when dried	Brittle when dried
Size	Leaves were 40-60 cm long and 15-30 cm wide	Leaves were 20-50 cm long and 45-70 cm wide

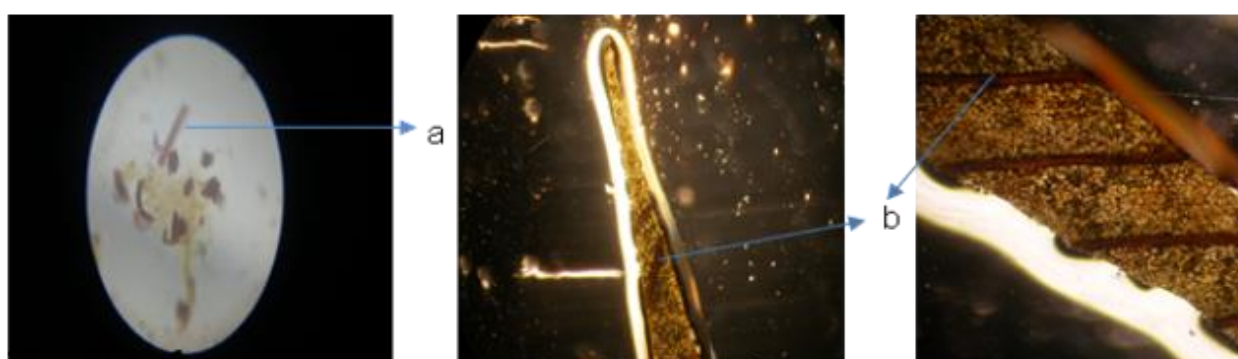
Table 1: Organoleptic charaters of the *Pteris* species

Microscopy

Upon microscopic examination, both leaf and powder samples from two distinct *Pteris* species revealed the presence of V-shaped vascular bundles within the leaf and various structures such as calcium oxalate crystals, xylem vessels, epidermis, and covering trichomes in the powder samples. This was observed in transverse (TS) and longitudinal (LS) sections, as depicted in Figures 3 and 4.

a. Xylem Vessels b. Calcium oxalate crystals c. Vascular bundles

Figure 3: Powder micrsocopy, leaf T.S and L.S of *P.pellucens*



a. Xylem Vessels b. Vascular bundles

Figure 4: Powder micrsocopy, leaf T.S and L.S of *P.grandifolia*

Physicochemical Analysis

The powder's physical and chemical characteristics, outlined in Table 2, conformed to the standards specified in the Indian Ayurvedic Pharmacopoeia. Parameters including pH, moisture content, soluble sugar content, crude fiber content, chlorophyll content, and proline content were evaluated using established analytical methods. Additionally, the percentage of extractive value soluble in both alcohol and water was determined and presented in Table 2.

Parameter	*P. pellucens*	*P. grandifolia*
pH	6.82	6.95
Moisture Content	0.0015	0.0012
Total Crude fiber content	0.0482	0.0245
Ash Value	0.059	0.062
Acid insoluble ash Value	0.105	0.092
Water soluble ash value	0.062	0.054
Water soluble extractive	0.024	0.03
Alcohol soluble extractive	0.05	0.04
Total soluble sugar Content	1.3µg/ml	0.2µg/ml
Starch Content	4 µg/ml	0.5 µg/ml
Crude fiber Content	0.0626	0.0454
Total Chlorophyll	15.75mg/1g	12.25 mg/1g

Chlorophyll a	10.42mg/1g	9.22 mg/1g
Chlorophyll b	5.25mg/1g	4.55 mg/1g
Proline content	0.35 µg/ml	0.22 µg/ml

Table 2: Physico-chemical parameters of the *pteris* species

Extraction and Fractionation

The yield of the ethanolic extract (EE) obtained per 100 g of dry plant material. Subsequently, the ethanolic extract underwent partitioning with different solvents to isolate the flavonoid-rich fraction, with the outcomes detailed in Table 3.

Solvent	Ethanol extract (EE) of Pteris species	Total extract (g/100 g of dry plant material)
Ethanol (99% v/v)	PPEE	45.8 ± 0.35
	PGEE	39.2 ± 0.22

PPEE and PGEE - Ethanol extracts of *P.pellucens, P.grandifolia* respectively.

Flavonoid rich Fraction (FRF)	% Yield
PPFRF	20.2 ± 0.54
PGFRF	15.6 ± 0.88

Table 3: Ethanol extract yields and flavonoid rich fractions of *pteris* species

PPFRF and PGFRF - Flavonoid rich fractions of *P.pellucens and p.grandifolia* respectively.

Arsenic Content

Given the hyperaccumulating nature of the *pteris* species with respect to arsenic, their extracts underwent ICP-OES (Inductively coupled plasma - optical emission spectrometry) analysis to ascertain the presence of arsenic. It was determined that the arsenic content in the prepared ethanolic extracts was below the level of detection. These findings are detailed in Table 4.

Sample	Element symbol and Wavelength (nm)	Concentration in ppm
PPEE	As 188.980	BDL
PGEE	As 188.981	BDL

Table 4: Arsenic content of *pteris* species

Phytochemical Analysis

The analysis of phytochemical properties revealed that two of the examined plants contained compounds with medicinal potential including alkaloids, steroids, sugars, glycosides, phenols, flavonoids, and terpenoids as indicated in Table 5. Conversely, both species of *pteris* plants lacked tannins, proteins, amino acids, and saponins.

Chemical Constituents	*Pteris pellucens*	*Pteris grandifolia*
Alkaloids	+	+
Steroids	+	+
Carbohydrates	+	+
Glycosides	+	+
Saponins	-	-

Proteins	–	–
Aminoacids	–	–
Tannins	–	–
Phenolic compounds	+	+
Flavanoids	+	+
Triterpenoids	+	+

+ indicates Present – indicates Absent

Table 5: Phytochemical analysis of the *pteris* species

Antioxidant Studies

The percentage inhibition, indicating the capacity to inhibit reactive oxygen species, of the studied fractions at a concentration of 100 mg/ml was presented in Table 6. In the DPPH scavenging assay, the ability of PPFRF to scavenge free radicals was comparable to that of the standard Ascorbic acid. Similarly, in the FRAP assay, the free radical scavenging capacity of PPFRF was on par with Ascorbic acid. Moreover, in the SRS radical scavenging and HAS assays, PPFRF demonstrated a capability to neutralize reactive oxygen species similar to that of Ascorbic acid.

Antioxidant Activity	Extracts				Ascorbic acid
	PPEE	PPFRF	PGEE	PGFRF	
DPPH Radical Scavenging Activity	25.13 ± 0.38*	45.63 ± 0.33*	18.58 ± 0.33*	23.37 ± 0.19*	60.57 ± 0.16
FRAP Activity	35.43 ± 0.4*	44.96 ± 0.32*	11.59 ± 0.27*	17.93 ± 0.12*	54.3 ± 0.13
SOA Activity	22.74 ± 0.26*	43.13 ± 0.41*	18.37 ± 0.33*	20.51 ± 0.23*	54.93 ± 0.17
HRS Activity	26.74 ± 0.30*	40.23 ± 0.31*	15.33 ± 0.24*	19.13 ± 0.16*	58.49 ± 0.19

Table 6: Effect of *pteris* species on Antioxidant activity

Anti nociceptive studies

Acetic Acid-Induced Writhing Method

The extract, at all doses, significantly (P < 0.001) decreased the number of writhes in mice in the acetic acid-induced writhing method when compared to the control as shown in Table 7. When compared to the lower dosage of the extract (25 mg/kg), the higher dose of the extract (50 mg/kg) demonstrated a significant (p < 0.001) difference.

Table 7: Effect of *PPFRF* and *PGFRF* on Acetic Acid-Induced Writhing in Mice

Group	Test sample dose (mg/kg bw)	Total writhing count (Mean±SEM)	% Inhibition
Control (1% Tween-80 in normal saline)	0.2 ml/10g of body wt	26±0.7	-
Standard (Diclofenac Sodium)	50	7.6±0.67*	70.77
P.pellucens (PPFRF)	25	10.2 ± 0.9*	60.2
	50	9.7 ± 0.5*	62.1
P.grandifolia (PGFRF)	25	11.4 ± 1.02*	56.15

	50	10 ± 0.7*	61.53

SEM=Standard Error of mean. *p<0.001; Probability values as compared to control using one way ANOVA followed by Dunnet's test.

Hot plate method

The study compared the effects of different doses of *P. pellucens* and *P. grandifolia* extracts (25 and 50 mg/kg) with a control and standard (Tramadol) on reaction time at various time intervals (0, 15, 30, 60, 120 min). PPFRF at 50 mg/kg showed a considerable analgesic effect, approaching the efficacy of Tramadol, especially at later time points. PGFRF also demonstrated a significant analgesic effect, though slightly less pronounced than PPFRF at the same doses as tabulated in Table 8.

Table 8: Effect of *PPFRF* and *PGFRF* on Hot Plate Method

Group	**Test sample dose (mg/kg bw)**	**Reaction time in seconds**				
		0 min	**15 min**	**30 min**	**60 min**	**120 min**
Control (1% Tween-80 in normal saline)	0.2 ml/10g of body wt	6.4 ± 0.54	6.64 ± 0.91	6.82 ± 0.68	7.04 ± 0.42	6.95 ± 0.7
Standard (Tramadol)	30	7.82 ± 0.95	12.84* ± 1.79	14.76** ± 2.38	16.76** ± 2.09	19.3±1.8**
P. pellucens (PPFRF)	25	6.2±0.20	7.67±0.21**	8.85±0.45	13.36±0.18*	17.5±1.5**
	50	8.53 ± 0.77	11.52 ± 1.64	13.28* ± 1.02	14.65 ± 0.82**	18.4± 1.29**
P.grandifolia (PGFRF)	25	5.1±0.21	6.2±0.42	8.67 ± 1.04	9.87 ± 1.53	11.81±0.19**
	50	5.6±0.31	8.1±0.30*	9.4±0.50	10.13 ± 0.64	15.85 ± 2.03**

Values expressed as Mean ±SEM, n=6. One way ANOVA followed by Dunnett's multiple comparison test *p<0.05, **p<0.01 compared with control group.

Tail flick method

The presented data show the effects of different treatments on the reaction time of subjects in a tail flick method, highlighting their analgesic potential. The analgesic efficacy of *PPFRF* was noted at both dosages (25 mg/kg and 50 mg/kg), with the 50 mg/kg dose showing a particularly strong impact and a considerable increase in reaction times. At both doses, *PGFRF* likewise delayed reaction times; however, its effects were somewhat less noticeable than those of *PPFRF*. Overall, *PGFRF* and *PPFRF* both demonstrate notable dose-dependent analgesic effects; however, *P. pellucens* appears to have greater potential, particularly at higher doses, approaching the effectiveness of Tramadol.

Table 9: Effect of *PPFRF* and *PGFRF* on Tail flick Method

Group	**Test sample dose (mg/kg bw)**	**Reaction time in seconds**				
		0 min	**15 min**	**30 min**	**60 min**	**120 min**

Control (1% Tween-80 in normal saline)	0.2 ml/10g of body wt	3.86±0.38	4.43±0.39	4.58±0.69	4.85±0.24	4.94±0.24
Standard (Tramadol)	30	8.23±0.15**	11.40±0.51**	14.52±0.78**	14.90±0.68**	17.20±0.1**
P.pellucens (PPFRF)	25	6.43±0.47*	7.05±0.11**	8.60±0.27**	10.40±0.12**	11.06±0.11**
	50	6.80±0.64**	8.98±0.14**	9.22±0.13**	11.51±0.27**	13.36±0.18*
P.grandifolia (PGFRF)	25	5.03±0.13**	6.02±0.22**	6.98±0.29*	8.55±0.36*	10.98±0.35*
	50	5.96±0.11**	6.75±0.55*	7.82±0.85	8.95±0.14**	11.11±0.25*

Data were presented as Mean ± SEM, with n=6. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test, indicating significance as *$p<0.05$ and **$p<0.01$ compared to the control group.

DISCUSSION

Physicochemical studies would help in identification and authentication of the herb. Furthermore, it might work as a tool for detecting adulterants, assisting in the preservation of natural medicinal quality, repeatability, and efficacy. Phytochemical study of plant samples revealed the presence of components known to have biological and medicinal activities[33]. After the ethanol extract was extracted for each plant, the ethyl acetate fraction was found to be flavonoid-rich, which was consistent with earlier research by Saeed et al[34]. The two of the understudied preparations had arsenic levels that were found to be below the detection limit. Despite being hyperaccumulators of arsenic, *pteris* species were safe to use because of their medicinal worth, according to the OES analysis's findings. Multiple illnesses in humans were significantly influenced by free radicals and other reactive species. Radical scavenging activity was crucial because it protects biological systems from their damaging impacts. Secondary metabolites with scavenging action, such as flavonoids, triterpenoids, and phenolic compounds, were sources of antioxidants. The recent study's findings revealed that the plants under investigation had components with significant therapeutic value. As the herbs studied possess phytoconstituents such as flavonoids and phenols, the plant species under research may have antioxidant activity, and this activity aids in disease prevention by scavenging free radicals. Plant phenolic components such as flavonoids, phenolic acids, tocopherols, and others are the main source of naturally occurring antioxidants[35].
The *in vitro* antioxidant activity was performed for flavonoid rich fractions and ethanolic extracts of the two *pteris* species and the data depicted that flavonoid rich fraction of *p.pellucens* showed maximum inhibitory effect as compared to the standard Ascorbic acid in DPPH radical Scavenging activity, Ferric reducing antioxidant power activity, Superoxide radical activity and Hydroxyl radical scavenging activity compared to the other species of *pteris* under the present study. The scavenging abilities of studied plant extracts against the four antioxidant assays were as follows:
DPPH Assay – Ascorbic acid >PPFRF>PPEE>PGFRF>PGEE
FRAP Assay – Ascorbic acid > PPFRF>PPEE>PGFRF>PGEE
SOA Assay – Ascorbic Acid>PPFRF>PPEE>PGFRF>PGEE
HRS Assay – Ascorbic Acid>PPFRF>PPEE>PGFRF>PGEE
This clearly suggests that *Pteris pellucens and Pteris grandifolia* extracts have strong antioxidant action. The flavonoid-rich fraction of *Pteris pellucens* demonstrated powerful *in vitro* antioxidant

activity in DPPH, FRAP, SOA, and HRS assays, surpassing typical antioxidants like ascorbic acid, thus providing a basis for its medicinal use in traditional treatments.

The central effects of the extract in generating antinociception were evaluated using the tail flick and hot plate tests. The tests can be further differentiated by means of their inclination to react to pain stimuli via distinct neural pathways. Although the hot plate approach includes higher brain activity and is thought to be a supraspinally structured response, tail flick conveys spinal reflex to nociceptive stimuli[36]. Both plant extracts demonstrated a dose-dependent increase in reaction time, with higher doses resulting in greater analgesic effects centrally. This was indicative of their potential utility as analgesics. The results suggest that both *P. pellucens* and *P. grandifolia* possess significant analgesic properties, with *P. pellucens* at higher doses showing particularly strong effects. These findings support further investigation into these plants as potential sources of new analgesic compounds. The comparison with Tramadol confirms their potential, though Tramadol remains more effective overall.

In peripheral analgesic model, both *P. pellucens* and *P. grandifolia* showed significant analgesic properties, with *P. pellucens* showing slightly higher efficacy, particularly at the 50 mg/kg dose, though both are less effective than Diclofenac Sodium. The frequency of writhing action in *PPFRF* was significantly reduced. It is likely that the antinociceptive action is due to the inhibition or antagonism of the mediators, primarily prostaglandin.

Overall, *P. pellucens* and *P. grandifolia* both exhibit significant analgesic properties across different pain models. *P. pellucens* shows a slightly higher potential, particularly at higher doses, and its analgesic effect in thermal and chemical pain models suggests a broad spectrum of pain-relief capabilities. While neither plant extract reached the efficacy of the standard drugs (Tramadol and Diclofenac Sodium), their significant effects indicate potential for development as analgesic agents. Further studies are warranted to explore their mechanisms of action and potential therapeutic applications.

According to the current research, this fraction may be crucial in the management of illnesses induced by free radicals and those related to ageing and chronic pain.

CONCLUSION

Research into the phytochemical and pharmacological properties of pteridophytes, a class of plants with therapeutic potential, may help find potentially active substances that may be developed into effective drugs. Pharmacognosy, phytochemistry and pharmacology on these *pteris* species were poorly researched. It is crucial to support substantial investigation in science on these undiscovered plant species may be beneficial to the ongoing exploration for innovative and low cost remedies in order to generate novel medications from these understudied plant groupings. According to this study, ethnobotanical and medicinal research is important that may help produce revolutionary, innovative medicines. Thus, if this ethnomedicinal study is paired with biological assessment or physiological studies, beneficial medications that may be used to treat chronic pain may be found. Further study on these pteridophyte species may lead to the discovery of a great number of different compounds with potential therapeutic significance.

AUTHOR'S CONTRIBUTION

The research protocol was designed and developed by Sujatha Dodoala. Experimental work and manuscript preparation was done by Padmini Karnatham.

CONFLICT OF INTEREST

It is declared here that none of the authors have any conflicts of interest.

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