Annals of Phytomedicine 6(1): 101-106, 2017
DOI: 10.21276/ap.2017.6.1.15 ; Print ISSN : 2278-9839 and Online ISSN : 2393-9885
Ann. Phytomed., 6(1):101-106 (2017)

Original article

Effect of demographic location on *Phlebodium decumanum* (Willd.) J. Sm. for its phytoconstituents and establishment of antioxidant and novel anthelmintic activity of its aqueous and methanolic leaf extracts

Kuntal Das, R. Rekha, M.A. Ibrahim, S. Yahya Ahmed and Raman Dang
Krupanidhi College of Pharmacy, #12/1, Chikkabellandur, Carmelaram Post, Varthur Hobli, Bengaluru-560035, Karnataka, India

Received May 2, 2017: Revised June 4, 2017: Accepted June 10, 2017: Published online June 30, 2017

Abstract

A fern, *Phlebodium decumanum* (Willd.) J.Sm. (PD) belongs to the family Polypodiaceae. It is a creeping, densely hairy or scaly rhizome bearing fronds at intervals along its length. It is native to tropical and subtropical regions of the America. The present study was investigated to establish the presence of phytoconstituents in leaf samples collected from three different geographical zones (Bengaluru, Nasik, and Munnar) and their impact on antioxidant and anthelmintic activity. Aqueous and methanol leaf extracts were used as solvent for extraction (conventional Soxhlet method) and results were compared to established novel application of fern species. Results revealed presence of alakloids, polyphenols, terpenoids, flavonoids, *etc*., upon various chemical tests. Thereafter, antioxidant study was established with DPPH and FRAP assay method. The IC_{50} value for methanol extract showed higher (210.24 µg/ml) for Munnar sample than the other two samples. An anthelmintic activity was determined by compared with Albendazole standard (25 mg/ml). Results revealed significant anthelmintic activity (p<0.01) for methanol extract of Munnar sample in terms of the time taken for paralysis and death study.

Key words: *Phlebodium decumazum* (Willd.) J.Sm., antioxidant, anthelmintic study, extracts, phytochemical study

1. Introduction

Gastrointestinal nematodosis is a well known serious threat in which helminthes infections are most common infections in human, affecting a large proportion of the world's population. In recent era, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophillia and pneumonia (Bundy, 1994). In general, gastrointestinal nematodosis is controlled by the use of allopathic drugs of benzimidazole, imidothiazole and avermentin groups. Thereafter, the infections also infected animals that are treated with chemical drugs, which showed non-specific clinical signs like diarrhea and are found positive on fecal examination without estimation of intensity of infection. This has led to indiscriminate and regular use of these drugs resulted drug resistance (Barton, 1980). The rapid emergence of resistance to these drugs associated with high cost, food residue and environmental pollution have compelled researchers to focus toward discover of new drug molecules from natural origin as an alternative source of chemical drug. Plants produce over wide range of natural secondary metabolites but the content and nature depends on environmental conditions and geographic location (Behdani *et al*., 2012). In recent years, increasing attention has been paid to the search for natural antioxidants from plants that protects the human body from the attack of free radicals so natural antioxidants have become one of the major focused area of scientific investigation (Chaudhuri *et al*., 2012) . In the present study, *P. decumanum* (PD) belongs to the family Polypodiaceae, was selected which is used generally as ornamental plant in India. The plant is abundantly available in damp region of many places in India, tropical rainforests and subtropical forests of the Caribbean and northern South America (Das and Einstein, 2007). The fern having plant constituents like flavonoids, alkaloids and lipids. Further, various fatty acids like eicosapentaenoic acid, elaidic acid, juglanin, kaempferols, linoleic acids, linolenic acids, melilotoside, oleic acid, adenosine, arachidonic acid, arabinopyranosides, calagualine, ecdysone, ecdysterone, *etc*. (Schultes and Raffauf, 1990; Lakshmi and Rajarlakshmi, 2010) are also present. The presence of these secondary metabolites indicates the plant may have various important medicinal activities like antioxidant, wound healing, antimicrobial, anthelmintic, *etc*. Thereafter, many literatures revealed its potential antimicrobial activity, immunomodulation activity (Tuominen *et al*., 1991; Gridling *et al*., 2009; Gonzalez-Jurado *et al*., 2011), anti-inflammatory and antioxidant activities (Díaz-Castro *et al*., 2012). Many of these metabolites are effective in plant defense against insects and diseases. Hence, identification of these metabolites is effective against pests and pathogens. Therefore, exploitation of plant having anthelmintic activity is important in this direction.

2. Materials and Methods

2.1 Collection of plant materials

Leaves of *Phlebodium decumanum* (Willd.) J.Sm. were collected from three different geographical zones of India, *viz*., Bengaluru,

Author for correspondence: Dr. Kuntal Das
Professor, Krupanidhi College of Pharmacy, #12/1, Chikkabellandur, Carmelaram Post, Varthur Hobli, Bengaluru-560035, Karnataka, India
E-mail: drkkdsd@gmail.com
Tel.: +91-9632542846

Nasik and Munnar and samples were kept in our college for future reference as herbarium (No: KCP/PD/2017-BLR, KCP/PD/2017-MUN and KCP/PD/2017-NAS, respectively). The geographical situations are as follows: Bengaluru located in the state of Karnataka with *latitude*: 12°58'38 N and *longitude*: 77°35'14 E, Nasik in the state of Maharashtra with *latitude* of 19°59'50.83"N and a *longitude* of 73°47'23.29"E and Munnar in the state of Kerala with *latitude* of 10°5'20.16"N and a *longitude* of 77°3'34.29"E (Figure 1).

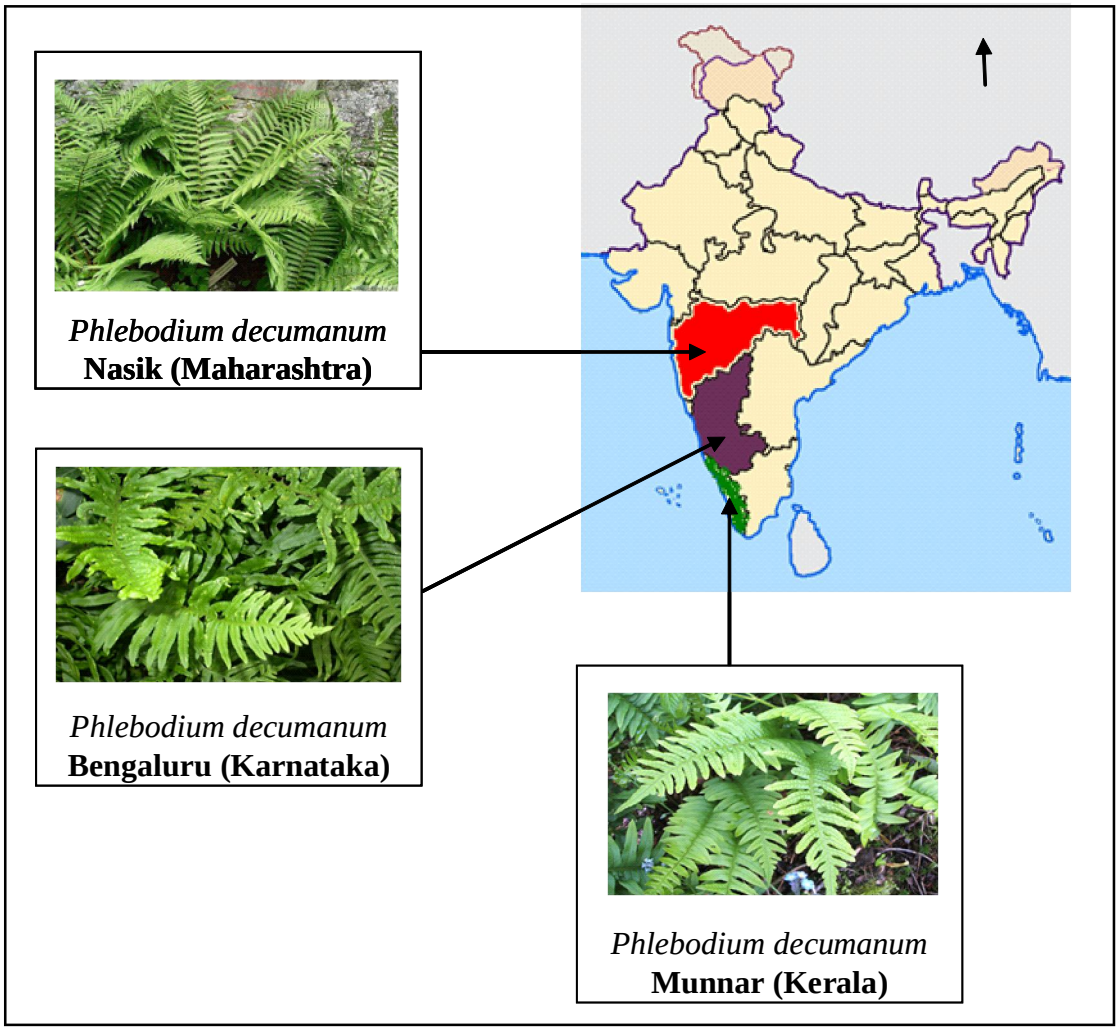


Figure 1: *P. decumanum* leaves collection from different places of India

2.2 Extraction of plant materials

Collected leaves were separately sun dried for 5-6 days after cleaned with the running tap water. Leaves were hand crushed and the coarse powder of leaves (250 g) was extracted with light petroleum and defatted the leaves. After sun dried, the methanol solvent was used and extracted by hot reflux method for 9-10 h, followed by aqueous extracted for 9-10 h to get the methanol (MEPD) and aqueous (AEPD) extracts of the leaves, respectively. The extracts were concentrated by rotary evaporator at 40-45^{o}C for 30-45 min. and stored with proper labeled in small glass bottles in refrigerator at 4-5^{o}C. The percentage yield of extracts was calculated separately and the results were tabulated (Figure 2) as per the following formula:

$$\% \text{ Yield} = \frac{W_1}{W_2} \times 100$$

where, W_1 is the weight of the extract after solvent evaporation, W_2 is the weight of the plant powder.

2.3 Phytochemical study

Both the extracts were subjected to phytochemical studies to identify the presence of secondary metabolites like alkaloids, glycosides, proteins, lipids, tannins, resins, steroids, phenols, flavonoids, *etc.* Various chemical tests were performed for respective constituents and the method carried out as per Harborne (1998) and Sazada *et al.* (2009). The results were tabulated in Table 1.

2.4 Antioxidant study

2.4.1 DPPH method

The antioxidant activity of all the extracts was assessed by their ability to scavenging 2,2-diphenyl-1-picrylhydrazyl stable radicals

(DPPH) by using the method described previously (Shimada *et al*., 1992). Briefly, 1 ml of methanolic extract and 5 ml of freshly prepared 0.1 mM DPPH methanolic solution were mixed and kept in the dark for 60 min. The absorbance of the reaction mixture was measured by spectrophotometer at 517 nm. The blank was prepared for reading correction (without extract) and used as control. The percentage of free radical scavenging activity was calculated as follows:

$$\text{DPPH scavenging activity } (\%) = (1\text{-}A_{sample} / A_{blank}) \text{ X } 100$$

where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the test extracts. The extracts were made up various concentrations (50, 100, 200, 300 and 400 µg/ml) by addition of 0.1 mM DPPH solution in the presence of Tris-HCl buffer (50 mM, pH 7.4). In the same manner, standard ascorbic acid solution was also prepared and decreased absorbance was measured at 517 nm. The IC_{50} value of the sample, *i.e.*, the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using the calibration curve by linear regression.

2.4.2 Reducing ability (FRAP) assay

The reducing ability of the ferric ion by all the extracts was determined by modified FRAP assay (Ferric reducing antioxidant power) method (Benzie and Strain, 1996). The stock solution of standard FRAP reagent was prepared by mixed 300 mM sodium acetate buffer (pH 3.6), 10.0 mM tripyridyl triazine solution with 20.0 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume. Samples at different concentrations (50, 100, 200, 300 and 400 µg/ml) was then added to 3 ml of FRAP reagent and the reaction mixture was incubated at 37°C for 30 min. Finally, the absorbance measured at 593 nm, using $FeSO_4$ as working solution for calibration. The antioxidant capacity of sample was calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per gram of sample.

2.5 Anthelmintic property

2.5.1 Selection of experimental organisms

The assay was performed on adult earthworm, *Pheretima posthuma*, belong to class Oligochaeta. Because of their anatomical and physiological resemblance with the intestinal round worm parasites of human beings, they are used for preliminary evaluation of anthelmintic activity in the present study. They were collected from moist soil of medicinal garden of Krupanidhi College of Pharmacy, Bengaluru and washed with normal saline to remove all foreign matters from body and further used for anthelmintic study. The earthworms of 2-6 cm in lengths and 0.1-0.2 cm in width were used for all the experimental protocol. Albendazole (25 mg/ml) was used as standard solution (prepared by dissolved in DMF), purchased from local market of Bengaluru and each of the test solutions of extracts (50, 100, 150, 200 mg/ml) were evaluated for anthelmintic activity. The time taken for paralysis and death of individual worms were observed during the study. When there was no movement of any part of the body, then time noted for the paralysis condition followed by the death time was noted when no movement of any part of the body even after shaken vigorously and also followed by fading away of their body colors of worms. Experiment was carried out as per the guideline of the Institutional Biosafety and Ethical Committee (Chandrashekhar *et al*., 2008).

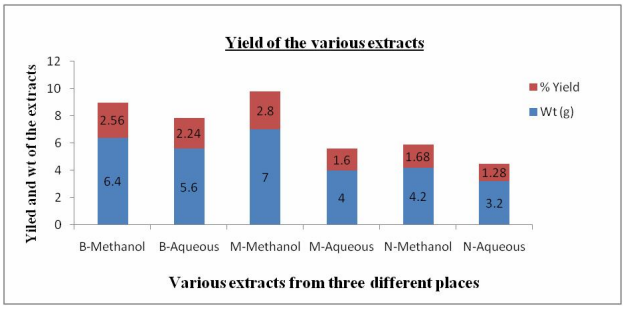
2.6 Statistical analysis

Data are expressed as mean ± SEM from three replications. For antioxidant assays and anthelmintic activity, one-way ANOVA test followed by Dennett's test (p<0.05) was used to analyze the differences among IC_{50} of various extracts for different antioxidant assays. The IC_{50} values were determined using the Graph Pad Prism 5 software. Correlation coefficient (r) was carried out among the extract and the activities. p values less than 0.05 were considered to be statistically significant.

3. Results

3.1 Yield of the extract

The higher percentage of yield was found with methanol extract of sample collected from Munnar, Kerala (2.8%), followed by Bengaluru, Karnataka (2.56%) and Nasik, Mumbai (1.68%). Aqueous extracts of the same was resulted higher in leaf sample collected from Bengaluru (2.24%), followed by Munnar (1.6%) and Nasik (1.28%). Among the extracts, methanol extracts showed more yield than aqueous extracts (Figure 2).



B = Bengaluru; M = Munnar; N = Nasik

Figure 2: Weight (w/w) and % yield for various extracts from three different PD leaves samples.

3.2 Phytochemical screening

Various chemical tests were performed to detect the presence of secondary metabolites. The results were tabulated in Table1. Based on the availability of the secondary metabolites, further estimation of vital plant constituents', *viz*., total alkaloids, phenols and total flavonoid were determined for methanol, aqueous and combined aqueous and methanol extracts.

Table 1: Preliminary phytochemical study of various PD extracts

Chemical tests	PD sample (Bangalore)		PD sample (Munnar)		PD sample (Nasik)	
	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous
Carbohydrate	-	-	-	-	-	-
Alkaloids	+	+	+	+	+	+
Glycosides	-	+	-	+	-	-
Tannins	++	+	++	+	++	+
Resins	-	-	-	-	-	-
Phenols	++	+	++	+	++	+
Steroids	+	-	+	-	-	-
Flavonoids	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Protein	-	-	-	-	-	-
Lipids	-	-	-	-	-	-

(++) = Positive; (-) = Negative

3.3 Antioxidant study

3.3.1 DPPH assay

PD aqueous extract exerted an inhibition of 58.01% with extract prepared from Munnar sample, followed by Bengaluru and Nasik sample and that of ascorbic acid was 89.24% at 400 µg/ml. The IC_{50} of the extract was 196.34 µg/ml, while that of ascorbic acid was 266.7 µg/ml. In case of methanol extract, sample collected from Munnar sample showed higher percentage of inhibition (66.28%) as compared to standard 89.24% at 400 µg/ml. The IC_{50} value of the same was 210.24 µg/ml for Munnar, followed by Bengaluru and Nasik samples. The DPPH radical scavenging (%) activity is shown in the Figure 3 and 4, respectively.

3.3.2 FRAP Assay

Reducing power of the ferric ion by the extracts was determined and revealed methanol extract of Munnar sample showed more reducing power than Bengaluru and Nasik samples. The activity was concentration dependent wherein 400 µg/ml concentrations, standard $FeSO_4$, showed higher absorbance, followed by Munnar sample. Thereafter, in case of aqueous extracts, sample collected from Bengaluru zone showed higher activity than Munnar and Nasik samples when compared with standard $FeSO_4$ at 400 µg/ml concentrations. The results depicted in Figures 5 and 6, respectively.

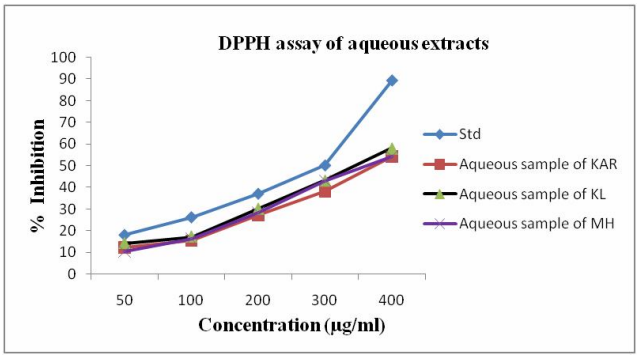


Figure 3: DPPH assay of aqueous extracts from three different geographical zone samples.

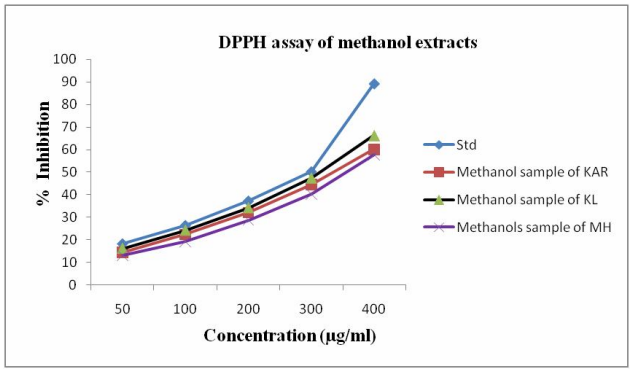


Figure 4: DPPH assay of methanol extracts from three different geographical zone samples.

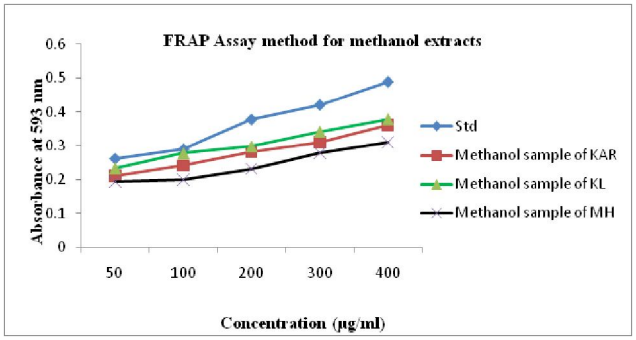


Figure 5: FRAP assay of methanol extracts from three different geographical zone samples.

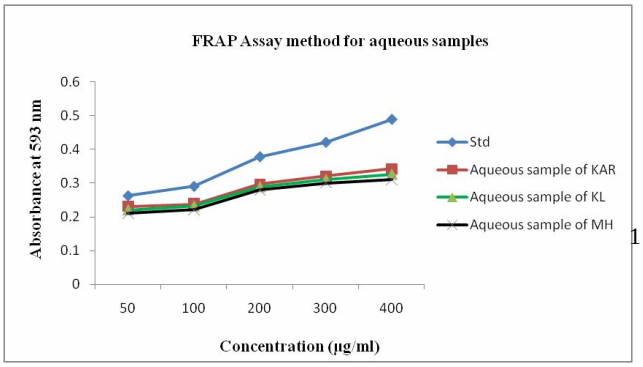


Figure 6: FRAP assay of aqueous extracts from three different geographical zone samples.

Table 2: Anthelmintic activity of various extracts

Groups	Bangalore sample		Munnar sample		Nasik sample	
	TTP	TTD	TTP	TTD	TTP	TTD
Control	--	--	--	--	--	--
Methanol extract (mg/ml)						
50	34.32**± 0.11	40.20**± 0.23	28.21**± 0.01	0.43	42.01**± 0.31	47.15**± 0.02
100	31.04**± 0.10	37.16**± 0.11	25.61*± 0.31	32.27**± 0.11	36.21**± 0.01	42.12**± 0.11
150	27.17**± 0.20	34.11**± 0.02	23.11*± 0.11	29.01**± 0.40	32.01**± 0.11	0.08
200	24.07**± 0.13	31.43**± 0.02	21.21*± 0.31	27.12**± 0.43	26.22**± 0.01	0.43
Aqueous extract (mg/ml)						
50	35.03**± 0.21	38.03**± 0.21	35.42**± 0.05	42.42**± 0.11	44.03**± 0.01	49.15**± 0.12
100	31.74**± 0.14	36.10**± 0.06	32.21**± 0.20	0.04	38.11**± 0.11	0.05
150	29.17**± 0.21	35.44**± 0.01	29.23*± 0.10	0.02	34.01**± 0.01	0.03
200	26.04**± 0.02	33.03**± 0.11	27.44**± 0.33	34.12**± 0.01	30.04**± 0.02	0.14
Albendazole 25 mg/ml	20.11± 0.11	24.02± 0.04	20.11± 0.11	24.02± 0.04	20.11± 0.11	24.02± 0.04

TTP=Time taken for paralysis (Min); TTD=Time taken for death (Min); One-way ANOVA studies followed by Dennett's test as compared to control

Statistical representation of the effective paralysis and dead time by PD methanol and aqueous extract, positive anthelmintic control (Albendazole, 25 mg/ml) processed by paired *t*-test analysis (Dennett's test.

Mean ± SEM (n =3); $^{*}p < 0.05$, $^{**}p<0.01$ represented statistical significant.

3.4 Anthelmintic study

The results (Table 2) showed methanol extract of Munnar sample produced higher anthelmintic activity in terms of paralysis and death of the organisms in lower time than Bengaluru and Nasik samples but the activity was little higher than the standard Albendazole (25 mg/ml). The activity was concentration dependent where at 200 mg/ml, showed higher inhibition of parasite movement followed by death. Methanol extract of Munnar sample (21.21 min and 27.12 min, respectively) as well as aqueous sample of Bengaluru (26.04 min and 33.03 min, respectively) showed more significant results for paralysis and death activities as compared to control and standard.

4. Discussion

4.1 Yield of the extract

The results showed the higher percentage of yield, was found with methanol extract of sample collected from Munnar, Kerala, followed by Bengaluru, Karnataka and Nasik, Mumbai. Whereas, an aqueous extracts of the same was resulted higher for Bengaluru sample, followed by Munnar and Nasik. This variation of the result was due to the climatic condition of the states and the soil nature of the states. Many literatures revealed that plant biomass depends on the soil nature, soil fertility as well as climatic condition and nature of solvent used for the extraction (Anwar *et al*., 2013; Braga *et al*., 2016, Das *et al*., 2016). Thereafter, yield of the extract directly proportional with the plant biomass (Das and Tribedi, 2015).

4.2 Phytochemical study

Chemical test of various extracts was performed and results showed the presence of alkaloids, tannins, phenols, flavonoids, steroids, terpenoids, *etc*., which are most essentials for any pharmacological activities. Earlier literatures also showed the same constituents present in this fern plant (Gomez and Wallace, 1986; Schultes and Raffauf, 1990).

4.3 Antioxidant study

DPPH is a stable free radical which is freshly prepared solution. In methanol, it produces dark purple colour and while reduced by antioxidants its colour turns yellow. The mechanism is based on the acceptance of an electron or hydride radical and converts to a stable diamagnetic molecule by reacting with suitable reducing agents. DPPH radicals formed into the corresponding hydrazine by electron acceptor and donor mechanism (Stankovic, 2011; Alam *et al*., 2012). Several publications reported that the free radical scavenging activity and reducing power of botanical extracts are dependent on their total phenolics and total flavonoids (Veeru *et al*., 2009; Karpe and Lawande, 2014) which is same correlated with our study. Preliminary phytoconstituents like phenols, flavonoids, *etc.*, are present in PD extracts which showed the pharmacological activities. Methanol extract of PD samples showed the more potent than aqueous extracts. This reduction of DPPH by the extract was may be due to either by the transfer of a hydrogen atom or the transfer of an electron. Phenolic and flavonoid compounds are also effective hydrogen donors, which indicates good source of antioxidants (Michalak, 2006).

FRAP assay had a similar trend to DPPH assay method. In this assay, the presence of antioxidants in the extracts reduced Fe^{+3}-extract complex to the ferrous form which is indicated by the intensity of the blue color formation. In FRAP assay, the extracts showed antioxidant capacity which are electron donors. The reducing capacity of the extracts may serve as an indicator of potential antioxidant activities through the breaking the free radical chain by hydrogen atom donation (Zhou and Yu, 2004). In our study, methanol extract showed more breaking of free radicals by donated hydrogen atom which was similar study as reported earlier (Barchan *et al*., 2014) where the methanolic extract of *Mentha* tree showed high reducing power.

4.4 Anthelmintic study

Cestodes are one of the major groups of helminthes that infect human intestine as well as the blood and tissues (Romero *et al*., 2014; Mwale and Masika 2015). Due to similarities with tape worm, earthworms are used preliminary in this present study. The effect of anthelmintic activity directly depends on the processes of hatching, development and motility of parasites without disturbing any internal physiological functions of the host (Shaibani *et al*., 2008). The activity was concentration dependent. The plant possesses significant anthelmintic activity due to presence of more polar bioactive phytochemicals such as tannins, phenolics, alkaloids, glycosides, *etc*., in the polar solvents such as methanol and aqueous (Stankovic, 2011). Our study also trend the similar results as reported earlier. Commonly alcoholic extracts are reported for their potent biological activities and inhibits the worms by the efficient release of phenols. On other way, the decrease in activity of aqueous extract is ascribed to the enzyme polyphenol oxidase, which degraded phenols in water extracts. Although modern medicines may be available, due to socio-economical, cultural and historical reasons, herbal medicines have maintained their importance. Hence, as per our understanding, anthelmintic activity of PD leaf extract is reported for the first time in this paper as a natural biosource beneficial fern.

5. Conclusion

The present study underpins that the methanol extract of PD leaves are the effective sources of antioxidant and anthelmintic fern in the conventional drug framework. The studies are obliged to identify the presence of various secondary metabolites, potent antioxidant and an anthelmintic impact. The discoveries provided much lauded impact of *P. decumanum* as an anthelmintic fern rather than ornamental plant.

Acknowledgements

Authors are thankful to the Management of Krupanidhi College of Pharmacy, Bengaluru for providing facilities in accomplishment of this research work.

Conflict of interest

We declare that we have no conflict of interest.

References

Alam, M.B.; Hossain, M.S.; Asadujjaman, M.; Islam, M. M.; Mazumder, M.E.H. and Haque, M.E. (2010). Peroxynitrite scavenging and toxicity potential of different fractions of the aerial parts of *Bacopa monniera* linn. IJPSR, **1**(10):78-83.

Anwar, F.; Kalsoom, U.; Sultana, B.; Mushtaq, M.; Mehmood, T. and Arshad, H.A. (2013). Effect of drying method and extraction solvent on the total phenolics and antioxidant activity of cauliflower (*Brassica oleracea* L.) extracts. International Food Research Journal, **20**(2): 653-659.

Barchan, A.; Bakkali, M.; Arakrak, A.; Pagan, R. and Laglaoui, A. (2014). The effects of solvents polaritiy on the phenolic contents and antioxidant activity of three *Mentha* species extracts. Int. J. Curr. Microbiol. App. Sci., **3**(11):399-412.

Barton, N.J. (1980). Emergence of *Haemonchus contortus* resistant to thiabendazole. Austr. Vet. J., **56**:46-47.

Behdani, M.; Pooyan, M. and Abbasi, S. (2012). Evaluation of antifungal activity of some medicinal plants essential oils against *Botrytis cinerea*, causal agent of postharvest apple rot, *in vitro*. International Journal of Agriculture and Crop Sciences, **4**(14):1012-1016.

Benzie, I.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': The FRAP assay. Analytical Biochemistry, **239**:70-76.

Braga, G.C.; Melo, P.S.; Bergamaschi, K.B.; Tiveron, A.P.; Massarioli, A.P. and De Alencar, S.M. (2016). Extraction yield, antioxidant activity and phenolics from grape, mango and peanut agroindustrial by-products. Ciencia Rural, Santa Maria, **46**(8):1498-1504.

Bundy, D.A. (1994). Immunoepidemiology of intestinal helminthic infection: The global burden of intestinal nematode disease. Trans. Royal Soc. Trop. Med. Hyg., **8**:259-61.

Chandrashekhar, C.H.; Latha, K.P.; Vagdevi, H.M. and Vaidya, V.P. (2008). Anthelmintic activity of the crude extracts of *Ficus racemosa*. Int. J. Green Pharm., **2**;100-103.

Chaudhuri, D.; Baban Ghate, N.; Sarkar, R. and Mandal, N. (2012). Phytochemical analysis and evaluation of antioxidant and free radical scavenging activity of *Withania somnifera* root. Asian J. Pharm. Clin. Res., **5**(4):193-199.

Das, K. and Einstein, J.W. (2007). Samambaia - The future focus for Indian researchers in the treatment of psoriasis. Thai. J. Pharm. Sci., **31**: 45-51.

Das, K. and Tribedi, S. (2015). Effect of Zn, Fe and Cu content on phytochemical investigations and antimicrobial potential of *Alternanthera brasiliana* (L.) O. Kuntze leaf extracts procured from two different states of India. Turk. J. Pharm. Sci., **12**:345-356.

Das K,; Deb, S.; Karanth, T.; Upreti, S. and Dang, R. (2016). Effect of cultural condition on element contents in raw material vis-a-vis impact of solvent nature on estimation of phytochemicals and screening of anthelmintic activity of *Melia dubia* Cav. Leaf. Ann. Phytomed., **5**(2):58-68.

Díaz-Castro, J.; Guisado, R.; Kajarabille, N.; García, C.; Guisado, I.M.; De Teresa, C. Ochoa, J.J. (2012). *Phlebodium decumanum* is a natural supplement that ameliorates the oxidative stress and inflammatory signalling induced by strenuous exercise in adult humans. European J. of Applied Physiology, **112**(8):3119-3128.

Gomez, L.D. and Wallace, J.W. (1986). Flavonoids of Phlebodium. Biochem. Syst. Ecol., **14**(4):407-408.

Gonzalez-Jurado, J.A.; Pradas, F.; Molina, E.S. and De Teresa, C. (2011). Effect of *Phlebodium decumanum* on the immune response induced by training in sedentary university students. Journal of Sports Science and Medicine, **10**(2):315-21.

Gridling, M.; Stark, N.; Madlener, S.; Lackber, A.; Popescu, R.; Benedek, B.; Diaz, R.; Tut, F.M.; Nha Vo, T.P.; Huber, D.; Gollinger, M.; Saiko, P.; Azmen, A.; Mosgoeller, W.; De Martin, R.; Eytner, R.; Wagner, K.H.; Grusch, M.; Fritzer-Szekeres, M.; Szekeres, T.; Kopp, B.; Frisch, R. and Krupitza, G. (2009). *In vitro* anticancer activity of two ethnopharmacological healing plants from Guatemala, *Pluchea odorata* and *Phlebodium decumanum*. International Journal of Oncology, **34**:1117-1128.

Harborne, J.B. (1998). (eds.), "Phytochemical Methods: A guide to modern techniques of plant analysis", 3rd edition. Chapman and Hall, London ISBN: 0-412-57270-2, pp:302.

Michalak, A. (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress, Polish J. Environ. Stud., **15**:523-530.

Mwale, M. and Masika, P.J. (2015). *In vivo* anthelmintic efficacy of *Aloe ferox, Agave sisalana*, and *Gunnera perpensa* in village chickens naturally infected with *heterakis gallinarum*. Trop. Anim. Health Prod., **47**(1):131-138.

Romero, M.C.; Navarro, M.C.; Martin-Sanchez, J. and Valero, A. (2014). Peppermint (*Mentha piperita*) and albendazole against anisakiasis in an animal model. Tropical Med. Int. Health, **19**(12):1430-1436.

Sazada, S.; Verma, A.; Rather, A.A.; Jabeen, F. and Meghvansi, M.K. (2009). Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Adv. Biol. Res., **3**:188-195.

Schultes, R.E. and Raffauf, R. (1990). The healing forest: Medicinal and toxic plants of the northwest Amazonia, R.F. Dioscorides Press, Portland Oregon.

Shaibani, I.R.M.A.; Phulan, M.S.; Arijoand, A. and Qureshi, T.A. (2008). Ovicidal and larvicidal properties of *Adhatoda vasica* (L.) extracts against gastrointestinal nematodes of sheep *in vitro*. Pakistan Vet. J., **28**(2):79-83.

Shimada, K.; Fujikawa, K.; Yahara, K. and Nakamura, T. (1992). Antioxidative properties of xanthan on the anti-oxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., **40**; 945-948.

Stankovic, M.S. (2011). Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. Kragujevac Journal of Science, **33**:63-72.

Tuominen, M.; Bohlin, L. and Rolfsen, W. (1991). Anti-inflammatory and immunomodulatory activity of the fern *Polypodium decumanum*. Planta Medica, **58**;306-310.

Zhou, K. and Yu, L. (2004). Effects of extraction solvent on wheat bran antioxidant activity estimation. Lebennsmittel-Wissenscharft und-Technologie., **37**:717-721.