Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 7, 2021, Pages. 1493-1501
Received 05 May 2021; Accepted 01 June 2021.

Antimicrobial Activity of Ethyl Acetate, Chloroform and Deionized Water Extract of Leaves of Pteris Cretica

Robina[1], Shubana Hayat[1], Junaid Ahmad[1*],Shahzeb Javed[2], Sahibzada Muhammad Adnan[3],Jawad Zahir[1], Musarat Shagufa[1],Shadman[4], Mehmood khan[5],Waliullah[1], Abdul Baseer[5], Muhammad Shahab[2]

[1]Department of Microbiology, Faculty of Biological Science, Hazara University, Mansehra, Pakistan
[2]Department of Microbiology, Faculty of Biological Science, Abbottabad University of Science and Technology, Abbottabad, Pakistan
[3]Department of Biotechnology, Faculty of Biological Science, COMSATS University, Abbottabad, Pakistan
[4]Department of Environmental Science, Hazara Univeristy, Mansehra, Pakistan
[5]Department of Microbiology, Hazara University, Mansehra, Pakistan
* Corresponding author, Email address:ofridai116@gmail.com

Abstract

Man is constantly in contact with microorganisms. The human pathogenic, as well as microflora, is developing resistance due to different reasons. Microbial resistance is an uprising threat to human life. Microbial resistance is the primary reason for the increased infections and death rates in developing and underdeveloped countries. With the fast-growing resistance, the old and modern antibiotics are getting ineffective. This urged the need to search for new antimicrobial agents. The study aims to find the antimicrobial activity of *Pteris cretica* fern. The water extract, Plant heavy- biomaterial, chloroform extract and ethyl acetate extract antimicrobial activity was tested against five bacterial strains *E.coli, Shigella, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus* and one fungal strain, *Candida albicans,* using the agar well diffusion method. The *Pteris cretica* showed significant antimicrobial activity against all studied microorganisms. The results indicate that *Pteris cretica* could be a new and efficient source for the drug, which could be used as a therapeutic agent.

Keywords: Antimicrobial activity, microorganisms, *Pteris cretica,* chloroform extract, ethyl acetate,

Introduction

Antibiotic resistance in bacteria emphasizes the importance of discovering novel, effective, and promising medicinal compounds derived from natural sources (Bax, Mullan, & Verhoef, 2000). Multidrug resistance provides significant hurdles to medicine, and infections caused by multidrug-resistant bacteria, particularly in intensive care units, are a major concern (Suffredini, Paciencia, Varella, & Younes, 2006). Antibiotic resistance is not a problem exclusive to the Pakistan subcontinent; it is a worldwide issue. Antibiotic resistance is a common occurrence in

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 7, 2021, Pages. 1493-1501
Received 05 May 2021; Accepted 01 June 2021.

bacteria as a result of natural adaptation to agents. Apart from being inexpensive to manufacture, using plant compounds to combat human illnesses offers several advantages. Natural plant products have been widely employed in this context since they are safer, more dependable, less poisonous, and less expensive than accessible synthetic medications (Newman & Cragg, 2004). From the very beginning of humanity, plants have been utilized as natural medicines for different ailments. Many pharmaceutical companies use the whole plant or its various parts as the main ingredient to produce antimicrobial medication. According to the WHO, 80 percent of the population in underdeveloped nations relies on herbs for medical treatment. Although the use of medicinal plants as a source of healing from disease dates all the way back to the early civilizations of China, India, and the north east, it is recognized as an art as ancient as humanity (Malesh & Satish, 2008). Effective plant extracts can combat human pathogenic bacteria without toxic side effects and environmental hazards (Raghavendra, Satish, & Raveesha, 2006). Additionally, 25% of the medications included in the new pharmacopoeia are derivatives of plants or semi-synthetic substances (Akhtar et al., 2019). Thus, plants can be extensively explored to discover novel antibacterial compounds that are both effective and therapeutically useful(Karaman et al., 2003; Raghavendra et al., 2006; Ray, Sarma, & Singh, 2004). Pteris cretica L., a perennial evergreen herb that belongs to the genus Pteris, is a member of the genus Pteris (Pteridaceae). It is estimated that there are over 250 species in the genus Pteris, which is geographically dispersed in tropical and subtropical regions throughout the world. Many of these species have been grown for aesthetic, culinary, and medicinal applications (Chao, Rouhan, Amoroso, & Chiou, 2014; Cordell, 2000). Additionally, it is used topically to wounds as an antibacterial in fronds paste (Testo, Watkins, Pittermann, & Momin, 2015). Modern research on the Pteris species has been substantial, revealing that these plants contain a variety of bioactive components, including flavonoids (Harinantenaina, Matsunami, & Otsuka, 2008; Kala et al., 2011; Wang et al., 2010), sesquiterpenoids (Harinantenaina, Matsunami, & Otsuka, 2009; Imperato & Nazzaro, 1996; Luo et al., 2016), and diterpenoids(Ge et al., 2008; Kim, Seo, Oh, & Sung, 2017; Shi et al., 2017). The present work aims at the separation and identification of bioactive components from P. cretica.

Materials and methods

The antimicrobial activity of pteridophytes *Pteris cretica* against pathogenic bacteria and fungi was conducted in Microbiology Research Lab, Hazara University, Mansehra, Pakistan.

Study area

The study area is district Mansehra, located in the Hazara division of Khyber Pakhtunkhwa, Pakistan Mansehra district is rich in flora, a large diversity is spread throughout the district. The majority of the herbs are used as Medicinal plants.

Plant collection

The plant Pteris cretica was collected in March from Shinkayari, district Mansehra. The plant was confirmed by the Botany Professor Mam Alia Gul of Hazara University.

Plant preservation

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 7, 2021, Pages. 1493-1501
Received 05 May 2021; Accepted 01 June 2021.

The vegetative plant leaves were washed thoroughly with Tap water followed by distilled water to remove the containments. The leaves were then shade dried for 15 to 20 days. The dried leaves are powdered using an electric motor. The powder was stored in sterilized sealed bags for further use.

Plants extraction

The powder of the plant was processed for extraction. Three solvents of different polarities were used: polar solvent deionized water, semi-polar solvent ethyl acetate, and non-polar solvent chloroform.

Water extraction procedure

The 5g of dried powder of Pteris cretica was soaked in 100ml of deionized water and then heated at 100^{o}C until boiling. After boiling for a few minutes, the temperature is shifted to 80oC and maintained for 30-40 minutes for complete plant extraction. The colour of deionized water changes from colourless to greenish colour, confirming extraction. The flask was removed and held for cooling. After cooling, the extract was filtered using

Whatman filter paper. The filtrate is then centrifuged at 4000rpm for 40 minutes. The supernatant was removed using a pipettor and stored in a sterilized container at 4^{o}C for further use. The plant bio-heavy mass is transferred to Eppendorf and kept at 4^{o}C for antimicrobial activity.

Ethyl Acetate and Chloroform extraction procedure

Powder drug weighing 5g was added to 100ml of ethyl acetate and chloroform solvent each and soaked for ten days at room temperature 25^{o} C with gentle shaking twice a day. The extract was filtered using Whatman filter paper. The filtrate was refilter followed by drying at 60^{o}C using a hot plate. The dried extract was scratched and stored in a container for antimicrobial activity.

Microorganisms used

The pure microorganism culture for the study was obtained from different diagnostic labs of Pakistan. The selected microorganism includes Gram-negative bacteria *E. coli, Shigella, Salmonella typhi, Pseudomonas aeruginosa*, gram-positive, Staphylococcus aureus, and fungal strain, including *Candida albicans.*

Inoculum preparation

The pure bacterial cultures were subculture using nutrient broth. The media was prepared by manufacturer's instructions (Oxoid, UK). Bacterial cultures were inoculated on nutrient broth and incubated for 24hr at 37^{o}C.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 7, 2021, Pages. 1493-1501
Received 05 May 2021; Accepted 01 June 2021.

Media preparation

About 28g of agar media was added to 1L of distilled water for media preparation. Both ingredients were mixed and sterilized in an autoclave at 121^{o} temperature and 15PSI pressure for 20 minutes. Media was then loaded on perti dishes and stored at room temperature for solidification.

Agar well diffusion method

The fresh, pure cultures were inoculated on Petri plates using the streak plate method. Antimicrobial activity was tested using the agar well diffusion method. The borer of 6 mm was used to make wells. The perti dishes had three well inoculated with bacterial strains and had four well inoculated with fungal strain. Drug of the choice disc (Positive control)

Were used according to bacteria strain and fungal strain. For fungal strain, the drug of the chosen solution was made by adding 500mg fluconazole tablet to 1ml deionized water, then 50µl was filled in well. All solvent extracts at a concentration of 50ul and 100ul were injected into the wells. For water extract and Plant bio-heavy mass (PBHM) negative control, deionized water was used, and DMSO was used as a negative control for the ethyl acetate and chloroform.

Results

In the current study, the antimicrobial and antifungal activity of various extracts (water extract, PBHM, Chloroform and ethyl acetate) of *Pteris cretica* leaf were studied in two volumes (50µl and 100µl) against five pathogenic bacterial strains, four gram-negative bacteria (*E.coli, Shigella, Salmonella typhi, Pseudomonas aeruginosa*), one gram-positive bacteria (*Staphylococcus aureus*) and one fungal strain (*Candida albicans*) (Table 1). The antibacterial and antifungal activity was determined in terms of the zone of inhibition.

The antibacterial activity of water extract and PBHM at volumes (50µl and 100µl) did not display a zone of inhibition against *Salmonella typhi* and *Pseudomonas aeruginosa*. It showed significant activity against *E.coli, Shigella, Staphylococcus aureus* at both volumes (50µl and 100µl) (Table 1). Both the water extract and PBHM displayed the biggest zone of inhibition at both books against *staphylococcus aureus*. The water extract at 50µl and 100µl exhibited zone of inhibition 2.2mm and 2.6mm against *Staphylococcus aureus,* respectively (Figure 4). Likewise, the PBHM showed 2.4mm and 2.9mm at 50µl and 100µl, respectively, against *Staphylococcus aureus*.

Table 1: Water, PBHM, Chloroform and Ethyl acetate extracts antimicrobial activity.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 7, 2021, Pages. 1493-1501
Received 05 May 2021; Accepted 01 June 2021.

Microorganism	Water extract (µl)		PBHM (µl)		Chloroform extract (µl)		Ethyl acetate extract (µl)		Positive l (µl)
	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	Antibiotics
	Zone of inhibition (mm)		Zone of inhibition (mm)		Zone of inhibition (mm)		Zone of inhibition (mm)		Zone of inhibition (mm)
E. coli	1.8	2.3	2.0	2.2	2.4	2.7	2.1	2.4	Ciprofloxacin ≈ 3.5
Shigella	1.6	1.9	2.1	2.4	1.9	2.0	1.7	2.1	Cefotaxime ≈ 0
Salmonella Typhi	-	-	-	-	1.4	1.7	1.6	1.9	Cefixime ≈ 0
Pseudomonas Aeruginosa	-	-	-	-	2.1	2.4	2.3	2.6	Gentamycin ≈ 2.3
Staphylococcus Aureus	2.2	2.6	2.4	2.9	3.0	3.2	3.4	3.8	Ciprofloxacin ≈ 4
Candida Albicans	2.7	3.0	2.9	3.1	3.3	3.5	3.9	4.1	Fluconazole ≈ 4.5
Negative Control	DI Water				DMSO				

The chloroform and ethyl acetate extracts at both volumes (50µl and 100µl) showed the zone of inhibition against all studied bacterial strains *E.coli, Shigella, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* (Table 1). The chloroform and ethyl acetate showed the biggest zone of inhibition at both volumes against *Staphylococcus aureus*. The area of inhibition at 50µl volume of chloroform and ethyl acetate extract was 3.0mm and 3.4mm, respectively, and at 100µl volume were 3.2mm and 3.8 mm, respectively.

The antifungal activity was displayed by water extract, PBHM, chloroform extract and ethyl acetate extract *of Pteris cretica* at volumes (50µl and 100µl). The biggest zone of inhibition, 3.9mm and 4.1mm against *Candida albicans*, was shown by the ethyl acetate extract at any volumes (50µl and 100µl), respectively. The water displayed the smallest zone of inhibition of 2.7mm and 3.0mm against Candida albicans at 50µl and 100µl volumes.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 7, 2021, Pages. 1493-1501
Received 05 May 2021; Accepted 01 June 2021.

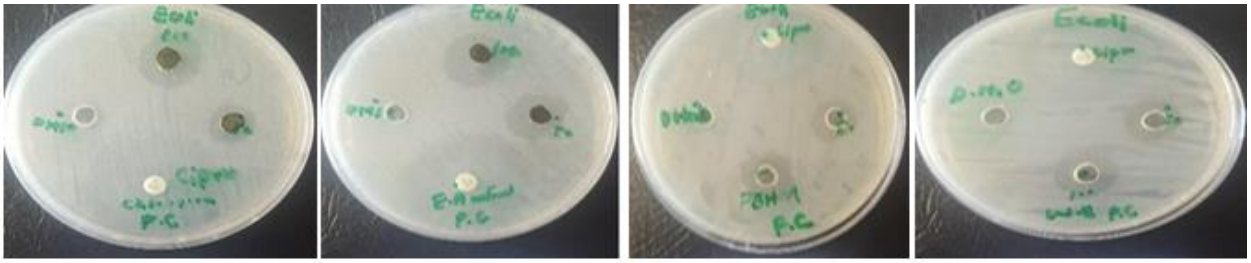

Figure 1: (a) chloroform (b) ethyl acetate (c) PBHM (d) water extract of *Pteris cretica* leaves the zone of inhibition against *E.coli*

Figure 2: (a) chloroform (b) ethyl acetate (c) PBHM (d) water extract of *Pteris cretica* leaves the zone of inhibition against *Shigella.*

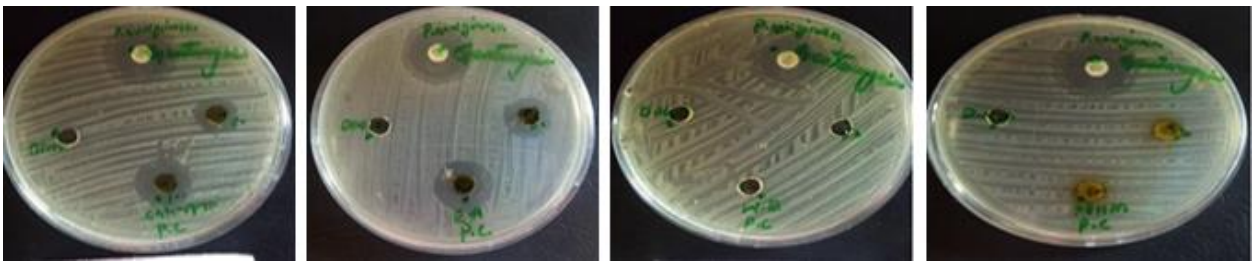

Figure 3: (a) chloroform (b) ethyl acetate (c) PBHM (d) water extract of Pteris cretica leaves the zone of inhibition against Salmonella typhi.

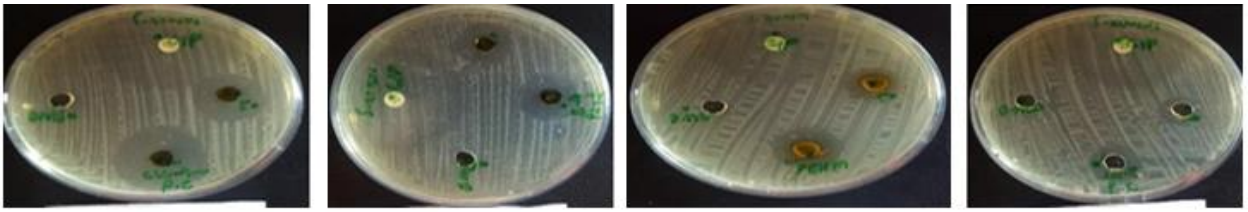

Figure 4: (a) chloroform (b) ethyl acetate (c) PBHM (d) water extract of Pteris cretica leaves the zone of inhibition against Staphylococcus aureus.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 7, 2021, Pages. 1493-1501
Received 05 May 2021; Accepted 01 June 2021.

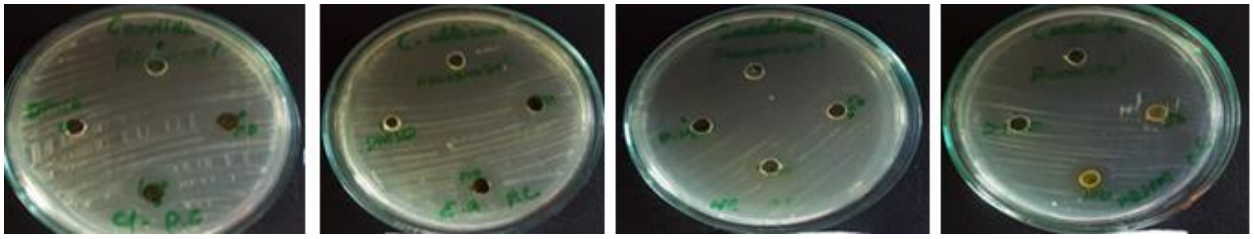

Figure 5: (a) chloroform (b) ethyl acetate (c) PBHM (d) water extract of Pteris cretica leaves the zone of inhibition against Candida albicans.

Discussion

Bacterial resistance to antibiotics has become a global problem impacted partly by antibiotics in various environments. New antimicrobial agents are therefore urgently needed to overcome this problem. Pteris is a common fern of the study region in the present study, which showed antibacterial and antifungal activity. In the previous studies, antimicrobial activity of n-hexane, chloroform and ethanol extract of *Pteris cretica* has been reported against bacterial and fungal strains. The n-hexane inhibited the *Bacillus subtilis, Staphylococcus aureus, Clostridium sporogenous* and *Klebsiella pneumonia*. Ethanol extract was effective against *B. subtilis, S. aureus, P. aeruginosa, C. sporogens* and *K. pneumonia*. All three extracts were ineffective against *Fusarium saloni* but effective against *Candida albicans* and *Aspergillus oryzae* (Saleem, Law, Sahib, Pervaiz, & Zhang, 2018). In another study, the methanol extract of eight ferns *Polypodium interjected, Polysticum woronowii, Polystichum aculeatum, Dryopteris affinis, Athyrium filix-femina, Asplenium scolopendrium, Asplenium adiantum* and *Pteris cretica* were tested against *S. aureus* and *E. coli*. The eight ferns' methanol extracts of leaves and rhizome showed significant activity against *S. aureus* and *E. coli* (Che, Kale, Li, Bahadori, & Liu, 2015). Many other species of genus Pteris, *Pteris inaequalis* (Gavins, Dalli, Flower, Granger, & Perretti, 2007), *Pteris vitatta (Abbas et al., 2017), Pteris quadriaurita* (Thomas, 2011), *Pteris biaurita L* (de Britto, de Moura, Aouada, Mattoso, & Assis, 2012) were reported with significant antimicrobial activity. Water, 70% ethanol, acetone and ether extract of 114 Pteridophytes was written for antimicrobial activity. Twenty species showed zone of inhibition against Penicillin resistant *S. aureus*, 16 against *Mycobacterium pheli*, 24 against *S. typhi*, 16 against *V. cholera*, three against *P. aeruginosa*. These results of previous studies are verification of current developments. The present study also confirms the ethnomedical use of *Pteris cretica* leaf paste for wound healing (Ilyas et al., 2017).

The antimicrobial activity of the genus *Pteris cretica* is due to the presence of bioactive molecules confirmed by previous studies. Among different secondary metabolites, the alkaloids, flavonoids, saponin, terpenoids and tannins are confirmed by phytochemical analysis by various studies (Zengin et al., 2019).Three flavonoids were reported in *Pteris cretica* were luteolin-7-o-rustinoside and luteolin 7- o-glucoside and flavonoid that is luteolin 7-O-robino-bioside (Cai, Fratianni, Gautier, & Imperato-McGinley, 1994).The Phenolic content presence was also shown in *Pteris cretica* (Naseri, Valizadeh, & Zakeri-Milani, 2015).

Conclusion

The extracts of *Pteris cretica* leaf showed a significant antibacterial and antifungal activity against *E.coli. Shigella, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureu*s and *Candida albicans*. Among four extracts, the chloroform and ethyl acetate showed a wide range of activities compared to water extract and PBHM. These extracts should be used for extraction purposes. The present study results demonstrated that Pteris cretica could be new and an efficient source for a drug used as a therapeutic agent.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

Abbas, M., Saeed, F., Anjum, F. M., Afzaal, M., Tufail, T., Bashir, M. S., . . . Suleria, H. A. R. (2017). Natural polyphenols: An overview. *International Journal of Food Properties, 20*(8), 1689-1699.

Bax, R., Mullan, N., & Verhoef, J. (2000). The millennium bugs—the need for and development of new antibacterials. *International journal of antimicrobial agents, 16*(1), 51-59.

Cai, L., Fratianni, C. M., Gautier, T., & Imperato-McGinley, J. (1994). Dihydrotestosterone regulation of semen in male pseudohermaphrodites with 5 alpha-reductase-2 deficiency. *The Journal of Clinical Endocrinology & Metabolism, 79*(2), 409-414.

Chao, Y.-S., Rouhan, G., Amoroso, V. B., & Chiou, W.-L. (2014). Molecular phylogeny and biogeography of the fern genus Pteris (Pteridaceae). *Annals of Botany, 114*(1), 109-124.

Che, Z., Kale, D., Li, W., Bahadori, M. T., & Liu, Y. (2015). *Deep computational phenotyping.* Paper presented at the Proceedings of the 21th ACM SIGKDD International Conference on Knowledge Discovery and Data Mining.

Cordell, G. A. (2000). Biodiversity and drug discovery—a symbiotic relationship. *Phytochemistry, 55*(6), 463-480.

de Britto, D., de Moura, M. R., Aouada, F. A., Mattoso, L. H., & Assis, O. B. (2012). N, N, N-trimethyl chitosan nanoparticles as a vitamin carrier system. *Food Hydrocolloids, 27*(2), 487-493.

Gavins, F. N., Dalli, J., Flower, R. J., Granger, D. N., & Perretti, M. (2007). Activation of the annexin 1 counter-regulatory circuit affords protection in the mouse brain microcirculation. *The FASEB Journal, 21*(8), 1751-1758.

Ge, X., Ye, G., Li, P., Tang, W.-J., Gao, J.-L., & Zhao, W.-M. (2008). Cytotoxic diterpenoids and sesquiterpenoids from Pteris multifida. *Journal of natural products, 71*(2), 227-231.

Harinantenaina, L., Matsunami, K., & Otsuka, H. (2008). Chemical and biologically active constituents of Pteris multifida. *Journal of natural medicines, 62*(4), 452-455.

Harinantenaina, L., Matsunami, K., & Otsuka, H. (2009). Chemical constituents of Pteris cretica Linn.(Pteridaceae). *Biochemical Systematics and Ecology, 37*(2), 133-137.

Ilyas, N., Gull, R., Mazhar, R., Saeed, M., Kanwal, S., Shabir, S., & Bibi, F. (2017). Influence of salicylic acid and jasmonic acid on wheat under drought stress. *Communications in soil science and plant analysis, 48*(22), 2715-2723.

Imperato, F., & Nazzaro, R. (1996). Luteolin 7-O-sophoroside from Pteris cretica.

Phytochemistry, *41*(1), 337-338.

Kala, S., Johnson, M., Janakiraman, N., Arockiaraj, A. A., Raj, S. I., & Bosco, D. (2011). Pharmacognostic and phytochemical studies on some selected ethnomedicinal plants of Tamilnadu, South India. *Int. J. Med. Arom. Plants, 1*(2), 89-94.

Karaman, I., Şahin, F., Güllüce, M., Öğütçü, H., Şengül, M., & Adıgüzel, A. (2003). Antimicrobial activity of aqueous and methanol extracts of Juniperus oxycedrus L. *Journal of ethnopharmacology*, *85*(2-3), 231-235.

Kim, J. W., Seo, J. Y., Oh, W. K., & Sung, S. H. (2017). Anti-neuroinflammatory ent-kaurane diterpenoids from Pteris multifida roots. *Molecules*, *22*(1), 27.

Luo, X., Li, C., Luo, P., Lin, X., Ma, H., Seeram, N. P., . . . Gu, Q. (2016). Pterosin sesquiterpenoids from Pteris cretica as hypolipidemic agents via activating liver X receptors. *Journal of natural products*, *79*(12), 3014-3021.

Malesh, B., & Satish, S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogen. *World J Agric Sci, 4*(5), 839-843.

Naseri, N., Valizadeh, H., & Zakeri-Milani, P. (2015). Solid lipid nanoparticles and nanostructured lipid carriers: structure, preparation and application. *Advanced pharmaceutical bulletin*, *5*(3), 305.

Newman, D. J., & Cragg, G. M. (2004). Advanced preclinical and clinical trials of natural products and related compounds from marine sources. *Current medicinal chemistry*, *11*(13), 1693-1713.

Raghavendra, M., Satish, S., & Raveesha, K. (2006). Phytochemical analysis and antibacterial activity of Oxalis corniculata; a known medicinal plant. *My science*, *1*(1), 72-78.

Ray, A. B., Sarma, B. K., & Singh, U. P. (2004). Medicinal properties of plants.

Saleem, M., Law, A. D., Sahib, M. R., Pervaiz, Z. H., & Zhang, Q. (2018). Impact of root system architecture on rhizosphere and root microbiome. *Rhizosphere*, *6*, 47-51.

Shi, Y.-S., Zhang, Y., Hu, W.-Z., Zhang, X.-F., Fu, X., & Lv, X. (2017). Dihydrochalcones and diterpenoids from Pteris ensiformis and their bioactivities. *Molecules*, *22*(9), 1413.

Suffredini, I. B., Paciencia, M. L. B., Varella, A. D., & Younes, R. N. (2006). Antibacterial activity of Brazilian Amazon plant extracts. *Brazilian journal of Infectious diseases*, *10*, 400-402.

Testo, W. L., Watkins, J. E., Pittermann, J., & Momin, R. (2015). Pteris× caridadiae (Pteridaceae), a new hybrid fern from Costa Rica. *Brittonia*, *67*(2), 138-143.

Thomas, G. (2011). A typology for the case study in social science following a review of definition, discourse, and structure. *Qualitative inquiry*, *17*(6), 511-521.

Wang, H.-B., Wong, M.-H., Lan, C.-Y., Qin, Y.-R., Shu, W.-S., Qiu, R.-L., & Ye, Z.-H. (2010). Effect of arsenic on flavonoid contents in Pteris species. *Biochemical Systematics and Ecology*, *38*(4), 529-537.

Zengin, G., Atasagun, B., Aumeeruddy, M. Z., Saleem, H., Mollica, A., Bahadori, M. B., & Mahomoodally, M. F. (2019). Phenolic profiling and in vitro biological properties of two Lamiaceae species (Salvia modesta and Thymus argaeus): A comprehensive evaluation. *Industrial Crops and Products, 128*, 308-314.