Pak. J. Bot., 55(5): 1851-1856, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-5(20)

PACLITAXEL CONTENT IN VARIOUS PARTS OF *TAXUS WALLICHIANA* FROM MOIST TEMPERATE FORESTS OF SWAT AND HAZARA, PAKISTAN

NASREEN GHAFFAR[1*], LINDA S. LEE [2], BUSHRA KHAN[3*], HIZBULLAH KHAN[3], JAVAID IQBAL[3,4*], ISHAQ AHMAD MIAN[5], SHUMAILA IHTISHAM[6], RUKHSANA[7], IFTIKHAR AHMAD[8] AND SHAH KHALID[9]

[1]*Directorate of Academics and Research, Islamia College Peshawar, 25120, Khyber Pakhtunkhwa, Pakistan,*
[2]*Department of Agronomy, Purdue University, West Lafayette, IN 47907, USA*
[3]*Department of Environmental Sciences, University of Peshawar, 25120, Khyber Pakhtunkhwa, Pakistan,*
[4]*University of Lakki Marwat, 28420, Khyber Pakhtunkhwa, Pakistan,*
[5]*Department of Soil and Environmental Sciences, The University of Agriculture Peshawar, 25130, \ Khyber Pakhtunkhwa, Pakistan,*
[6]*Department of Statistics, Islamia College Peshawar, 25120, Khyber Pakhtunkhwa, Pakistan,*
[7]*Department of Pharmacy, University of Malakand, 18800, Khyber Pakhtunkhwa, KP, Pakistan,*
[8]*Director, Directorate of Non-Timber Forest Produce, Forest Department, Khyber Pakhtunkhwa, Pakistan,*
[9]*Department of Botany, Islamia College Peshawar, 25120, Khyber Pakhtunkhwa, Pakistan*
[*]*Corresponding author's email: bushraasu@uop.edu.pk; nasreenghaffar@icp.edu.pk; drjavaidenv@gmail.com*

Abstract

Paclitaxel, isolated from *Taxus wallichiana*, has clinically been used as a standard anticancer agent. Although Paclitaxel has been quantified in species from forests in different countries of the world and its concentration show variation with environmental factors, no studies have been conducted on it in Pakistan. Therefore, the aim of this study was to investigate the presence of Paclitaxel and quantify its concentration in various parts of *T. wallichiana*, sampled from Hazara and Swat Forest Divisions, Khyber Pakhtunkhwa (KP), Pakistan. Paclitaxel levels in 119 samples (leaves, stem, bark, and root) of *T. wallichiana* were measured and compared within and between the two forest divisions. Extraction of biomass was accomplished using Ultrasound-assisted ethanol extraction and liquid chromatography with ultraviolet detection analysis after an ENVI-Carb cleanup step. Paclitaxel content (wt %) measured in plant biomass followed; roots (0.023 ± 0.018) > bark (0.014 ± 0.013) > leaf (0.011 ± 0.006) > stem (0.006 ± 0.006) samples. A significant difference ($p<0.05$) was observed in Paclitaxel content between the two Forest Divisions. The mean Paclitaxel content for different plant parts was significantly greater for trees from Hazara Forests compared to Swat Forests. *T. wallichiana* can be grown in its natural zones "Hazara Forests" for a high yield of Paclitaxel.

Key words: Paclitaxel, *Taxus wallichiana*, Moist temperate, Forest, Khyber Pakhtunkhwa.

Introduction

Taxol, a complex diterpene amide, was discovered as a clinically effective anti-leukemic and anti-tumor agent and is currently used as anticancer medicine in hospitals and clinics (Zaiyou *et al*., 2017; Lasala *et al*., 2006; Oberlies & Kroll 2004; Wani *et al*., 1971). Clinical results showed that Paclitaxel has exceptional antitumor activity against neck, lung, breast, and head cancers. It is one of the top chemotherapeutic agents obtained from plant sources (Baker *et al*., 2007; Newman *et al*., 2003; Arbuck & Blaylock, 1995). Since identified and isolated, Paclitaxel has become the subject of numerous studies including those classifying it as a novel anti-microtubule agent. It prevents mitosis from occurring by stimulating microtubule formation and stabilizing them against being dismantled, thus making cell division impossible (Miglietta *et al*., 2002; Altmann 2001; He *et al*., 2001; Schiff & Horwitz, 1980; Schiff *et al*., 1979).

Paclitaxel was first isolated from the bark of Taxus brevifolia (Pacific yew) (Wani *et al*., 1971). Paclitaxel and other taxanes occur commonly in different parts of numerous yew species (Parmar *et al*., 1999). These species include T. canadensis, T. brevifolia, T. baccata, T. cuspidata, and *T. wallichiana* (Witherup *et al*., 1990). *T. wallichiana* is an evergreen, non-coniferous, slow-growing species that can survive thousands of years. Taxus causes severe poisoning which can result in life-threatening cardiac toxicity (Labossiere & Thompson, 2018). All parts of the plant (leaves, stem, and bark) are toxic except the fruit (red berries). The toxicity is due to the presence of cardio-toxic alkaloids (taxine A and taxine B) and not Paclitaxel present in Taxus (Cope, 2015). Taxus species is present in various areas of the world including in the mountains of Asia (Spjut, 2010). In Pakistan, *T. wallichiana* is distributed in the moist temperate forests of Hazara, Swat, Murree, Kashmir, Shangla, Kurram, and Chitral at an elevation of 1500-3500 m (Spjut, 2010; Mulliken and Crofton, 2008). The distribution of *T. wallichiana* is patchy and now present in only a few places due to habitat destruction, forest fires, lack of awareness, agricultural activities, slow growth rate, construction activities, use for decoration, improper harvesting, accidental mortality, animal grazing, illicit cutting, and regeneration failure (Iqbal *et al*., 2020; Paul *et al*., 2013; Mulliken & Crofton, 2008). Taxus wood is used mostly in graves, as fuel wood, and for a wide range of medicinal purposes, which has resulted in the reduction of the species critically (Haq, 2012). With the passage of time, its population has been reduced by almost 87 percent and it is now considered an endangered species (Haq, 2012; Bhuju & Gauchan, 2018). In Pakistan less

work has been done on the conservation of the *T. wallichiana*. Conservation is done both in-situ (Iqbal *et al*., 2022) and ex-situ means.

Demand for the Taxus species has increased throughout the world with a concomitant increase in research on best methods to extract and quantify Paclitaxel in various parts/species of Taxus (Sadeghi-Aliabadi *et al*., 2009; Ballero *et al*., 2003; Mukherjee *et al*., 2002; Wheeler *et al*., 1992; Witherup *et al*., 1990). Previous studies on quantification of Paclitaxel in various Taxus species showed significant variations in the quantity of Paclitaxel within and among species and populations (Rawat, 2011; Cameron & Smith, 2008; Zhang *et al*., 2008; Vidensek *et al*., 1990; Neto & Decosmo 1992).

Pakistan imports Paclitaxel from other countries of the world for treatment of cancer (Faden *et al*., 2009) but it is very expensive with estimated costs of Rs. 5300 ($45.85) for one injection (6 mg/mL). To the best of our knowledge, there has not been any work done to evaluate Paclitaxel levels in Taxus specie in Pakistan. Given the importance and cost of Paclitaxel and the known occurrence of *T. wallichiana* in local forests of the Hazara and Swat Forests, it was judicious to investigate Paclitaxel in local Taxus species. Therefore, the aim of this study was to investigate the presence of Paclitaxel and quantify its concentration in various parts of *T. wallichiana* growing in the Hazara and Swat Forest Divisions of KP, Pakistan. Paclitaxel levels in leaves, stems, bark, and roots of *T. wallichiana* from each forest were measured and compared within and between the two forest divisions.

Materials and Methods

Study area

General experimental procedure: Leaf, stem, bark, and root tissues were sampled from *T. wallichiana* from twelve locations (Ayubia, Beran Gali, Bagnotar, Kaghan, Khanspur, Chetta Cham, Natia Gali, Changla Gali, Donga Gali, Tharati, Balakot, and Jared) in the Hazara Forests and seven locations (Roringar, Miandam, Banjot, Beha, Malam Jaba, Lalku, and Kalam) in the Swat Forests (Fig. 1). A total of 119 leaf, stem, bark, and root samples were collected, which included 64 samples from the Hazara Forests and 55 samples from the Swat Forests. The samples were packed in navel orange mesh bags, labeled, and transported to the laboratory. Samples were then cut into pieces and dried in dark at room temperature. Air-dried samples were grinded using a plant grinder (Thomas Wiley Laboratory mill, Model 4) and then sieved to obtain the 0.2 mm ≤ size ≤ 1.5 mm fraction. Samples were stored in labeled zip-locked bags and shipped to Purdue University, USA for analysis. Voucher specimens no. Bot. 20156 (PUP) was deposited in the herbarium of Department of Botany, University of Peshawar, KP, Pakistan.

The moisture content of each sample was determined gravimetrically (80°C) after air-drying and then kept in a desiccator until cooled. The moisture content (oven-dried basis) of the various leaf, stem, bark, and root samples of *T. wallichiana* ranged from 5.78 to 17.44 % with a mean value of 8.62% so the air-dried samples were used for extraction of Paclitaxel.

Extraction and Analysis of Paclitaxel: Air-dried plant biomass samples were extracted through a heated ultrasound-assisted extraction (UAE) with ethanol and paclitaxel quantified using high-performance liquid chromatography with UV detection (HPLC-UV) after an ENVI-Carb cleanup step as described in Ghaffar *et al*., (2019). Briefly, ~0.5 g samples were extracted three times sequentially with 4-mL ethanol each time with UAE using an Elmasonic Model P70H bath sonicator for 60 min at 180 W and 60ºC. HPLC-UV analysis was performed on an automated Shimadzu HPLC with a Shimadzu UV-Vis Detector (Model SPD 10A) using a Waters Novapak Phenyl column (4 µM, 60 Å, 15 cm x 3.9 mm) maintained at 30ºC using a gradient mobile phase consisting of 0.01 M ammonium acetate in DI water and 0.01 M ammonium acetate in acetonitrile (B) at 0.8 mL/min. The limits of detection (LOD) and quantitation (LOQ) were determined to be 0.018 mg/L and 0.55 mg/L, respectively.

Statistical Analyses

Statistical analysis was carried out using SPSS (version 25). Means and standard deviation (mean ± SD) were calculated. Analysis of variance (ANOVA), followed by Tukey test, Post Hoc test, and t-Test (Tekade *et al*., 2013) were applied.

Results and Discussion

Paclitaxel concentrations in the various parts of *T. wallichiana*: Paclitaxel was detected in all the samples of different parts of *T. wallichiana* collected from all geographical locations. Mean Paclitaxel content (air dried wt %) measured in different parts of *T. wallichiana* samples from the Hazara and Swat Forests of KP are presented in (Table 1).

Paclitaxel content (wt %) in samples of leaf, stem, bark, and roots ranged 0.003 ─ 0.025, 0.001 ─ 0.023, 0.001 ─ 066 and 0.02 ─ 087, respectively. Overall, Paclitaxel content in different tree parts is given in (Fig. 2).

From (Table 2) it has been concluded that there is a significant difference (p<0.001) in the quantity of Paclitaxel content between different tree parts. Generally, the results showed that root samples had the highest Paclitaxel content present followed by bark, leaf, and stem. The Paclitaxel content extracted from roots was 2 and 4 times higher than leaves, and stem, respectively.

A pairwise comparison using the Post Hoc Test between tissue types (root, leaf, bark, and stem) for Paclitaxel content on all *T. wallichiana* samples collected from both forests indicated that the Paclitaxel content between tissue types is significantly different for leaf-root, stem-bark, stem-root and root-bark comparisons for each Division. No significant difference in Paclitaxel content was observed for the leaf-stem and leaf-bark combinations (Table 3).

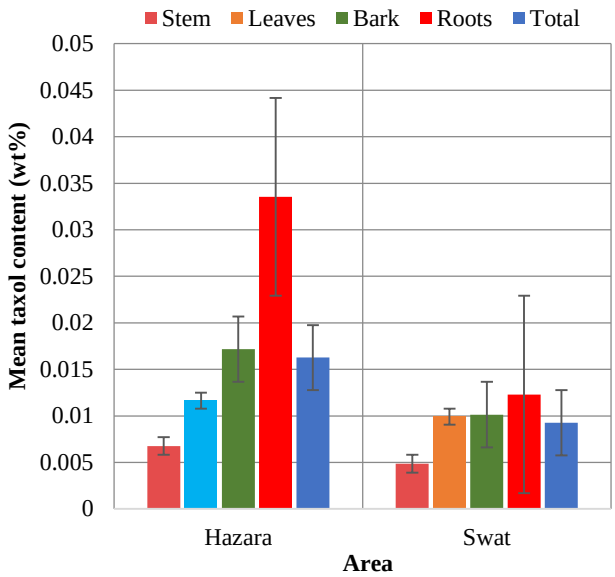

Fig. 1. Location wise overall mean Taxol content (% wt) in *T. wallichiana*.

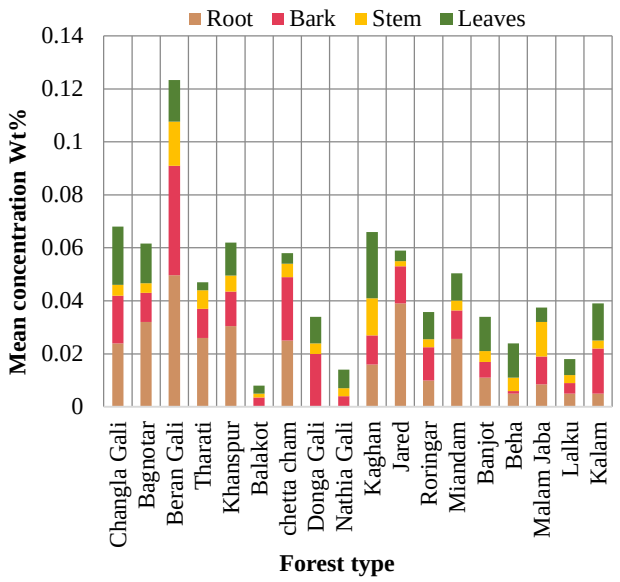

Fig. 2. Mean Taxol content (wt %) in plants sampled from different sampling locations.

Table 1. Mean Paclitaxel content in air dried wt % measured in different parts of *Taxus wallichiana*.

Tree parts	Area	N	Mean ± SD (wt %)
Leaf	Hazara	17	0.012 ± 0.008
	Swat	14	0.010 ± 0.004
	Total mean	**31**	**0.011 ± 0.006**
Stem	Hazara	17	0.007 ± 0.007
	Swat	14	0.005 ± 0.005
	Total mean	**31**	**0.006 ± 0.006**
Bark	Hazara	17	0.017 ± 0.016
	Swat	14	0.010 ± 0.006
	Total mean	**31**	**0.014 ± 0.013**
Root	Hazara	13	0.033 ± 0.019
	Swat	13	0.012 ± 0.008
	Total mean	**26**	**0.023 ± 0.018**
Total	Hazara	64	0.016 ± 0.016
	Swat	55	0.009 ± 0.006
	Total mean	**119**	**0.013 ± 0.013**

Table 2. Analysis of Variance

	Degrees of freedom	Mean square	F	P
Between tree parts	3	0.001	10.329	0.000
Within tree parts	115	0.000		

Table 3. Pair wise comparison of Paclitaxel content wt % measured in various tree parts from samples of *T. wallichiana*

Tree part pairs for comparison	Significance
Leaf-stem	0.349
Leaf-bark	0.722
Leaf-root	0.001**
Stem-bark	0.038*
Stem-root	0.00001**
Bark-root	0.026*

**0.001 level; *0.05 level

Table 4. Overall mean paclitaxel content in hazara and swat forest divisions.

Area	N	Mean paclitaxel (wt %)	T	Sig
Hazara	17	0.015623	2.472	0.020
Swat	14	0.009244		

Paclitaxel content in *T. wallichiana* at hazara and swat forest divisions: Sixty-four and fifty-five samples were collected from the Hazara and Swat Forest Divisions, respectively. Mean Paclitaxel content in samples (combine root, bark, stem, and leaf) of Hazara and Swat Forest Divisions was 0.016 ± 0.016 wt % and 0.009 ± 0.006 wt %, respectively. Using a t-Test showed that the Paclitaxel content from the two different forest divisions is significantly different (p<0.05) (Table 4).

Table 5. Paclitaxel content (wt %) in different tree parts of *T. wallichiana* in Hazara and Swat.

Source	Sum of square	Df	Mean square	F	Sig
Corrected model	0.008	7	0.001	9.653	0.0001
Intercept	0.021	1	0.021	183.412	0.0001
Tree parts	0.004	3	0.001	12.685	0.0001
Area	0.002	1	0.002	16.488	0.0001
Tree parts * Area	0.002	3	0.001	5.073	0.002
Error	0.013	111	0.000		
Total	0.040	119			
Corrected Total	0.020	118			

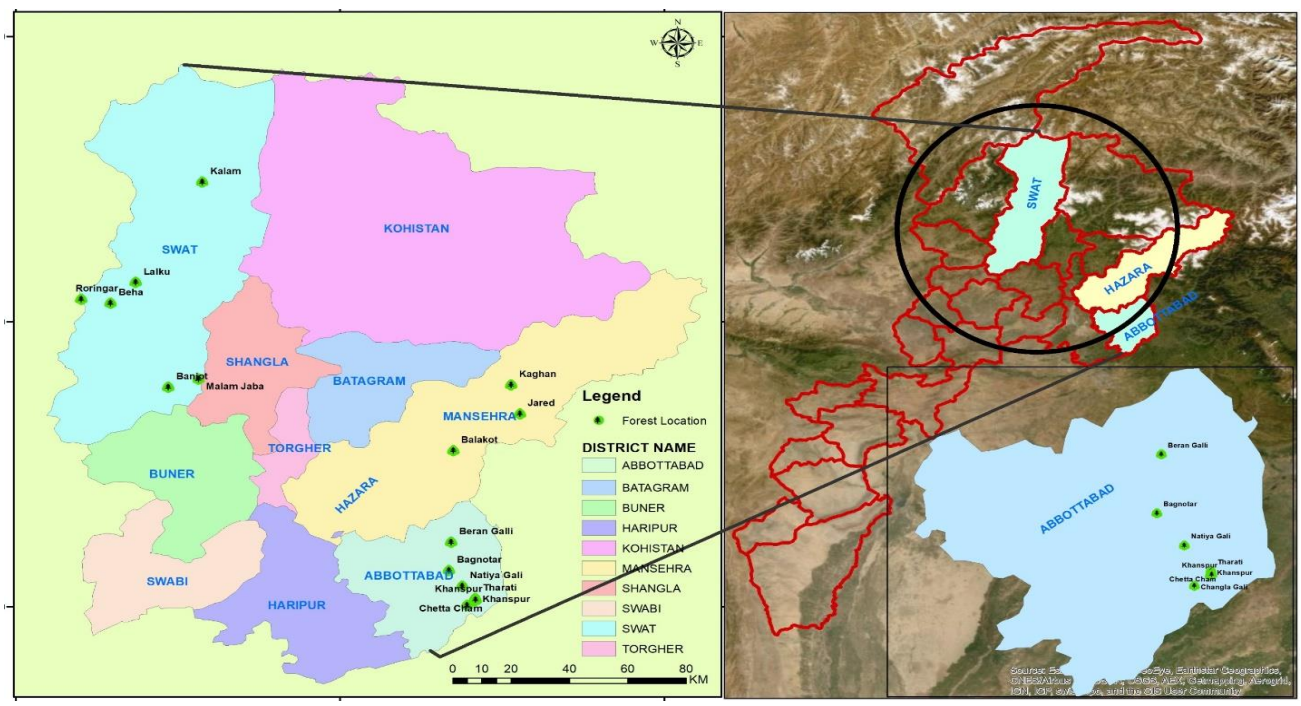

Fig. 3. Map of Hazara and Swat Forest Divisions' sampling areas.

Likewise, significant differences were observed in Paclitaxel content extracted from same tree parts sampled at geographically distinct forests (Table 5).

Graphical representation of Paclitaxel content in samples of leaves, stem, bark, and roots is shown in (Fig. 3).

Our results showed that the highest Paclitaxel content was present in roots of *T. wallichiana*. Mean Paclitaxel content followed: root (0.023 ±0.018 wt %) > bark (0.014±0.013 wt %) > leaf (0.011 ±0.006 wt %) > stem (0.006±0.006 wt %). These findings are contrary to what has been reported in some studies but consistent with other studies. For example, Vidensek *et al*. (1990) reported that Paclitaxel content (%) from several Taxus species is highest in the bark followed by the root: bark (0.015 ± 0.018) > root (0.004 ± 0.004) > leaf (0.0015 ± 0.0012) > stem (0.0006 ± 0.000) [31]. The present results agree with trends reported by Enaksha *et al*., (1994) who described a higher concentration of Paclitaxel in roots compared to the leaves of Taxus x media Rehd. Cultivars Brownii, Densiformis, Fieldii, Hicksii, and T. cuspidate Sieb and Zucc (Enaksha *et al*., 1994). Wang *et al*., (2006) also reported root and bark are richer in Paclitaxel content than leaf and stem.

The mean Paclitaxel content (0.014±0.013 wt %) in bark samples of *T. wallichiana* is comparable to those in T. brevifolia of 0.012 ± 0.005 wt % and 0.015 ± 0.018 wt %, respectively (Videnseк *et al*., 1990; Neto & DeCosmo, 1992). Nadeem, *et al*. (2002) reported higher Paclitaxel content (0.0558 ± 0.008 wt %) in bark [36]. Similarly, Kelsey and Vance (1992) & Németh-Kiss *et al*., (1996) found bark richer in Paclitaxel content than needles. Less Paclitaxel in leaves as compared to bark may be due to the reason that leaf extracts have higher amounts of waxy, non-polar components (Whitherup, *et al*., 1990).

In the present study, the mean average quantity of Paclitaxel (0.011±0.006 wt %) in leaf samples is like that reported by Whitherup *et al*. (1990) of 0.01 wt % for T. X media cv. Hicksii. However, Neto & DiCosmo (1992) (0.035 ± 0.006 wt %) and Elsohly *et al*., (1997) (0.028 to 0.063 g %) observed much higher leaf Paclitaxel contents for T. cuspidata and T. media, respectively. Leaves, being renewable, can be easily used as a constant supply source of Paclitaxel to help meet what is required for cancer treatment (Witherup *et al*., 1990).

The current study shows that stem has the lowest Paclitaxel content which supports the studies of Hook *et al*., (1999) and Mukherjee *et al*., (2002) conducted previously. Hook *et al*., (1996) also reported that leaves produced higher amounts of taxoids than stems in Irish yew and other Taxus species.

The difference in Paclitaxel quantities in the different tree parts may be due to several factors involved in it such as storage, post-harvest procedures, and extraction protocol (Kelsey & Vance, 1992). During extraction, solvent type, tissue particle size, length of extraction, and solvent temperature seem to influence the final concentrations. Moreover, the source of plant material may also be important. Even within limited populations, there is considerable inter-tree variation, and diverse geographic sources are likely to have even greater differences (Kelsey & Vance, 1992). Variation in Paclitaxel content in two forest divisions may be due to the environmental conditions and genetic variations of the tree species at the two different locations. The number of *T. wallichiana* trees found in Hazara Forests is greater than that of Swat Forests. The reason may be that forests at Swat are protected forests where local people have more rights which results in higher anthropogenic activities and changes in land use while Hazara forests are reserved and comparatively less affected by the local community. Deforestation, habitat destruction, over-exploitation, agricultural practices, un-sustainable extraction, developmental activities, fuel, growth of population, local farming, enlarged animal husbandry practices, and unsustainable extraction are the anthropogenic activities that may affect the existence of *T. wallichiana* as well as production of Paclitaxel. The difference in the quantity of Paclitaxel may also be due to heavy grazing, herbivory of seeds or seedlings, adverse soil conditions, poor soil water relations, competition for light among the trees, fungal diseases, and changes in microclimate (Thomas & Polwart, 2003; Holtan, 2001; Lewandowski *et al*., 1995; Pridnya, 1984; Krol, 1978).

The demand of Paclitaxel for the treatment of cancer is very high. Taxus trees not only grow slowly, but also are insufficient in number to meet the demand for Paclitaxel throughout the world. The best approach to trying to maintain consistent supplies of Paclitaxel is to gather leaves through pruning. Removal of leaves unlike other parts (roots and bark) does not cause destruction of the plant (Elias & Korzhenevsky, 1992).

Conclusion

The Paclitaxel content was observed in all the samples (100%) of the leaf, stem, bark, and root of *T. wallichiana* at the Hazara and Swat Forest Divisions, KP. The significant difference in mean Paclitaxel content ($p<0.05$) was found between different parts (leaf, stem, bark, and root) of *T. wallichiana* and the study areas. The order of Paclitaxel content observed was root > bark > leaf > stem. *T. wallichiana* from Hazara Forests has greater Paclitaxel content than the same species from Swat Forests. Further studies need to be carried out in other parts of Pakistan to identify and explore productive areas for the extraction of Paclitaxel. *T. wallichiana* at both the forest divisions especially Hazara Forests must be grown on large-scale plantations to yield Paclitaxel in great quantity and the present species must be conserved to cease its extinction from its natural zones. Paclitaxel content may be affected by environmental factors like temperature, rainfall, moisture, altitude, and soil properties which must be probed out. Further studies are needed to be carried out to determine the effect of these factors on the quantity of Paclitaxel in these areas.

Acknowledgements

This work was supported by the Higher Education Commission (HEC) of Pakistan under the International Research Support Initiative Program (IRSIP).

References

Altmann, K.H. 2001. Microtubule-stabilizing agents: a growing class of important anticancer drugs. *Curr. Opin. Chem. Biol*., 5: 424-431. doi: 10.1016/S1367-5931(00)00225-8.

Arbuck, S.G. and B.A. Blaylock. 1995. Taxol: clinical results and current issues in development. *Taxol: Sci. & Appl*., 379.

Baker, D.D., M. Chu, U. Oza and V. Rajgarhia. 2007. The value of natural products to future pharmaceutical discovery. *Nat. Prod. Reports*, 24(6): 1225-1244.

Ballero, M., M.C. E.L.M. Loi, T.A. Van Rozendaal, C. Van Beek, F. Van de Haar and G. Poli. Appendino. 2003. Analysis of pharmaceutically relevant taxoids in wild yew trees from Sardinia. *Fitoterapia*, 74(1-2): 34-39.

Bhuju, S. and D.P. Gauchan. 2018. *Taxus wallichiana* (Zucc.), an Endangered Anti-Cancerous Plant: A Review. *Int. J. Res*., 21(5): 10-21.

Cameron, S.I. and R.F. Smith. 2008. Seasonal changes in the concentration of major Taxanes in the biomass of wild Canada Yew (*Taxus canadensis*. Marsh.). *Pharm. Biol*., 46(1-2): 35-40.

Cope, R.B. 2005. The dangers of yew ingestion. *Vet. Med*., 9: 646-650.

Elias, T.S. and V.V. Korzhenevsky. 1992. The presence of paclitaxel and related compounds in Taxus baccata native to the Ukraine (Crimea), Georgia, and southern Russia. *Syst. Evol. Bot*., 13(3): 463-470.

ElSohly, H.N., E.M. Croom Jr, W.J. Kopycki, A.S. Joshi and J.D. McChesney. 1997. Diurnal and seasonal effects on the taxane content of the clippings of certain Taxus cultivars. *Phytochem. Anal*., 8(3): 124-129.

Enaksha., R., M. Wickremesinhe and R.N. Arteca. 1994. Roots of hydroponically grown Taxus plants as a source of paclitaxel and related taxanes. *Plant Sci*., 101(2): 125-135.

Faden, R., R., K. Chalkidou, J. Appleby, H.R. Waters and J.P. Leider. 2009. Expensive cancer drugs: a comparison between the United States and the United Kingdom. *The Milbank Q*., 87(4): 789-819.

Ghaffar, N., L.S. Lee, Y.J. Choi, C.D. Perre and B. Khan. 2019. Efficient heated ultrasound assisted extraction and clean-up method for quantifying paclitaxel concentrations in *Taxus wallichiana*. *J. Environ. Anal. Chem.*, 101(4): 549-560.

Haq, F. 2012. The critically endangered flora and fauna of district Battagram Pakistan. *Adv. Life Sci*., 2(4): 118-123.

He, L., G.A. Orr and S.B. Horwitz. 2001. Novel molecules that interact with microtubules and have functional activity similar to Taxol. *Drug Discov.*, 6: 1153-1164.

Holtan, D. 2001. Barlinda Taxus baccata L. i Møreog Romsdal– på veg ut. *Blyttia*, 59: 197-205.

Hook, I., C. Poupat, A. Ahond, D. Guénard, F. Guéritte, M.T. Adeline and P. Potier. 1999. Seasonal variation of neutral and basic taxoid contents in shoots of European Yew (*Taxus baccata*). *Phytochem*., 52(6): 1041-1045.

Hook, S., F. Griffin, C. Mackintosh and G. Buchan. 1996. Activation of an interleukin-4 mRNA-producing population of peripheral blood mononuclear cells after infection with Mycobacterium bov is or vaccination with killed, but not live, BCG. *Immunology*, 88(2): 269-274.

Iqbal, J., B. Khan, S. Khan, N. Ghaffar, I A. Mian, I. Ahmad, N. Yousaf, I. Aamir, I. Ahmad and S. Manzoor. 2022. Effects of hormones (IBA & IAA) on the propagation of himalayan yew in Pakistan: a conservation approach. *Pak. J. Bot*., 54(4): 1237-1243.

Iqbal, J., R. Meilan and B. Khan. 2020. Assessment of risk, extinction, and threats to Himalayan yew in Pakistan. *Saudi J. Biol. Sci*., 27(2): 762-767.

Kelsey, R.G. and N.C. Vance. 1992. Paclitaxel and cephalomannine concentrations in the foliage and bark of shade-grown and sun-exposed Taxus brevifolia trees. *J. Nat. Prod*., 55(7): 912-917.

Krol, S. 1978. An outline of ecology. The yew Taxus baccata L. Foreign Scientific Publication, Department of the National Centre for Scientific and Technical, and Economics Information (for the Department of Agriculture and the National Science Foundation, Washington, DC), Warsaw, Poland, 65-86.

Labossiere, A.W. and D.F. Thompson. 2018. A review of the clinical toxicology of yew poisoning. *Ann. Pharmacother*. 1060028017754225.

Lasala, J.M., G.W. Stone, K.D. Dawkins, P.W. Serruys, A. Colombo, E. Grube and S. Ellis. 2006. An Overview of the TAXUS Express, Paclitaxel-Eluting Stent Clinical Trial Program. *J. Intervent. Cardiol*., 19(5): 422-431.

Lewandowski, A., J. Burczyk and L. Mejnartowicz. 1995. Genetic structure of English yew (*Taxus baccata* L.) in the Wierzchlas Reserve: Implications for genetic conservation. *For. Ecol. and Manag*., 73(1-3): 221-227.

Miglietta, A., C. Bocca and L. Gabriel. 2002. Comparative studies on biological activity of certain microtubule interacting taxanes. *Chem. Biol. Interact*., 139: 283-299.

Mukherjee, S., B. Ghosh, T.B. Jha and S. Jha. 2002. Variation in content of paclitaxel and related taxanes in Eastern Himalayan populations of *Taxus wallichiana*. *Planta Med*., 68(08): 757-759.

Mulliken, T. and P. Crofton. 2008. Review of the Status, Harvest, Trade and Management of Seven Asian CITES-listed Medicinal and Aromatic Plant Species, Germany BfN – Skripten, 227: 114-132.

Nadeem, M., H.C. Rikhari, A. Kumar, L.M.S. Palni and S.K. Nandi. 2002. Paclitaxel content in the bark of Himalayan Yew in relation to tree age and sex. *Phytochem*., 60(6): 627-631.

Németh-Kiss, V., E. Forgács, T. Cserháti and G. Schmidt. 1996. Taxol content of various Taxus species in Hungary. *J. Pharm. Biomed. Anal*., 14(8-10): 997-1001.

Neto, A.G.F. and F. DiCosmo. 1992. Distribution and amounts of paclitaxel in different shoot parts of *Taxus cuspidata*. *Planta Med*., 58(5): 464.

Newman, D.J., G.M. Cragg and K.M. Snader. 2003. Natural products as sources of new drugs over the period 1981−2002. *J. Nat. Prod*., 66(7): 1022-1037.

Oberlies, N.H. and D.J. Kroll. 2004. Camptothecin and taxol: historic achievements in natural products research. *J. Nat. Prod*., 67(2): 129-135.

Parmar, V.S., A. Jha, K.S. Bisht, P. Taneja, S.K. Singh, A. Kumar, Poonam, R. Jain and C.E. Olsen. 1999. Constituents of the yew trees. *Phytochem*., 50: 1267-1304.

Paul, A., S., Bharali, M.L. Khan and O.P. Tripathi. 2013. Anthropogenic disturbances led to risk of extinction of *Taxus wallichiana* Zuccarini, an endangered medicinal tree in Arunachal Himalaya. *Nat. Areas J*., 33(4): 447-454.

Pridnya, M.V. 1984. Phytocenotic status and structure of the Khosta common-yew population in the *Caucasus biosphere* reserve. *Soviet J. Ecol*., 15: 1-6.

Rawat, J.S. 2011. Cancer of illegal trade threatens the anti-cancer Yews. *Sci. & Cul*., 77: 46-51.

Sadeghi-Aliabadi, H., G. Asghari, S.A. Mostafavi and A. Esmaeili. 2009. Solvent optimization on Taxol extraction from *Taxus baccata* L., using HPLC and LC-MS. *Daru*, 17(3): 192-198.

Schiff, P.B. and S.B. Horwitz. 1980. Taxol stabilizes microtubules in mouse fibroblast cells. *PNAS*, 77(3): 1561-1565.

Schiff, P.B., J. Fant and S.B. Horwitz. 1979. Promotion of microtubule assembly *In vitro* by taxol. *Nature*, 277(5698): 665.

Spjut, R.W. 2010. Overview of genus taxus (Taxaceae): The species, their classification, and female reproductive morphology. The World Botanical Associates, Inc Bakersfield, California, 29.

Tekade, R.K., A. D'Emanuele, A. Elhissi, A. Agrawal, A. Jain, B.T. Arafat and N.K. Jain. 2013. Extraction and RP-HPLC determination of taxol in rat plasma, cell culture and quality control samples. *J. Biomed. Res*., 27(5): 394.

Thomas, P.A. and A. Polwart. 2003. Biological flora of the British Isles: *Taxus baccata* L. *J. Ecol*., 91(3): 489-524.

Vidensek, N., P. Lim, A. Campbell and C. Carlson. 1990. Paclitaxel content in bark, wood, root, leaves, twig, and seedling from several *Taxus* species. *J. Nat. Prod*., 53(6): 1609-1610.

Wang, C.W., S.L. Peng, M.G. Li, W.J. Li and F.L. Lin. 2006. Review of factors affecting the taxoids content of *Taxus* spp. *Acta Ecol. Sin*., 5: 1583-1590.

Wani, M.C., H.L. Taylor, M.E. Wall, P. Coggon and A.T. McPhail. 1971. Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. *J. Amer. Chem. Soc*., 93(9): 2325-2327.

Wheeler, N.C., K. Jech, S. Masters, S.W. Brobst, A.B. Alvarado, A.J. Hoover and K.M. Snader. 1992. Effects of genetic, epigenetic, and environmental factors on paclitaxel content in *Taxus brevifolia* and related species. *J. Nat. Prod*., 55(4): 432-440.

Witherup, K.M., S.A. Look, M.W. Stasko, T.J. Ghiorzi, G.M. Muschik and G.M. Cragg. 1990. Taxus spp. needles contain amounts of paclitaxel comparable to the bark of *Taxus brevifolia*: Analysis and isolation. *J. Nat. Prod*., 53(5): 1249-1255.

Zaiyou, J., M. Li and H. Xiqiao. 2017. An endophytic fungus efficiently producing paclitaxel isolated from *Taxus wallichiana* var. *mairei*. *Medicine*, 96(27):

Zhang, Jie., X.P. Xu, J. Liu, C.X. Tian and S. Zhou. 2008. Study on the paclitaxel distribution in different parts of difference sources Taxus growing in China. *Chinese J. Pharm. Anal*., 28(4): 16-19.

(Received for publication 21 March 2022)