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Phytochemical Screening and Quantitative Screening for Total Phenolic and Flavonoid Content in *Taxus wallichiana* Collected from Various Districts of Nepal

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Abstract: For the detailed study on any plant species, primarily knowing its major phytochemical components has become vital prior to starting the research. The availability of phytochemical compound, phenolic and flavonoid content vary plant to plant and species to species. Therefore, the present study investigates the phytochemical content, total phenolic and flavonoid content in *Taxus wallichiana* collected from different districts of Nepal. The needles and bark pieces of *T. wallichiana* collected from 11 sites of Nepal were air dried and extracted using different solvents for phytochemical screening. Further, total phenolic and flavonoid content were determined from the extracts. The major phytochemical compounds found were alkaloids, flavonoids, phenol, glycosides, terpenoids, tannins, coumarin, and quinone. Lignin, saponin, and resin showed low presence whereas starch was completely absent. Acetone, 50% ethanol and methanol were proven best solvents for all the three screening whereas hexane and ether proved to be less effective. Both phenolic and flavonoid content was found to be high in bark samples compared to the needle ones. Among the districts, not much difference in the quantity was accounted for. There wasn't much difference in both phenolic and flavonoid contents between male and female plants. However, female plants showed high flavonoid content than male. Also, female needle samples showed higher phenolic and flavonoid content than male needle samples. *T. wallichiana*, mostly renowned as an anti-

cancerous plant is rich with various phytochemical constituents and contains prominent amount of phenolic and flavonoid compounds in its bark as well as needles.

Keywords: Taxus, Phytochemical Screening, Phenolic Content, Flavonoid Content.

1. INTRODUCTION

Taxus wallichiana, also commonly known as Himalayan Yew, is an evergreen tree species belonging to Taxaceae family. The tree is dioecious in nature, having the male and female parts on separate trees. Found in the temperate Himalayas at an elevation range of 1800 to 3300m, the plant is said to be native to Nepal and known as Lodhsalla. The plant is renowned to contain Taxol, which is considered as the anti-cancerous agent being studied and used to cure breast and ovarian cancer[1]. This has not only increased its economic value but researches have been flooding in to inquire more about this prominent medicinal plant.

Screening of phytochemical compounds basically reveals the bioactive compounds present in the plant [2,3]. Plant usually produce these phytochemical compounds to thrive themselves within nature, to help them with their competitors or predators but studies have shown that these compounds have medicinal value to us humans. For example, Taxol which is a diterpenoid alkaloid found in foliage and barks of *Taxus* species is a prominent drug[4]. But since Taxol is anti-cancerous in nature, all the focus has driven to Taxol extraction. Besides Taxol, *T. wallichiana* has other phytochemical components too and this has been investigated by our study.

Flavonoids and Phenols, naturally occurring plant metabolites are well known to have health benefits as they help in cell signaling; they are antimicrobial [5,6], anti-inflammatory [7], antihyperglycemic [8], antihyperlipidemic [8], and anti-hypertensive [9]; widely used for treatment of Breast Cancer [10], tuberculosis [11]; for radical scavenging [12] and protecting brain functioning . Determining the total flavonoid and phenolic content of a plant species is usually a priority for every research student or scientist as this supports the evidence of plant having medicinal value and gives the researcher backdrop to initiate the research. *T. wallichiana* being a medicinal plant with evidences of its anti-cancerous property is prominent to have both flavonoid and phenolic content.

According to Ethnobotanical Society of Nepal (1997), there are 22 representative districts enlisted for *T. wallichiana* out of which we have selected 11 as our sampling sites. The aim of our study is to determine phytochemical compounds present in Taxus, and quantitative screening of total flavonoid and phenolic content. Since *T. wallichiana* is dioecious in nature, we have done the screening separately for male and female as well which gives the research a broader aspect. *T. wallichiana* has been widely studied worldwide but in the case of Nepal, the research done is limited and close to negligible. According to our review, this study is first of its kind to be done in Nepal with so much sample and parameter variability.

2. MATERIALS AND METHODS

Sampling Sites and Collection of Plant Material: Needles and bark pieces of *T. wallichiana* were collected from 10 districts of Nepal as shown in **Figure 1** which are Gorkha, Taplejung, Rasuwa, Sindhupalchok, Mustang, Manang, Solukhumbu, Parbat, Kaski, and Lamjung. For Kaski district, there were 2 sample sites; Sikles, and ACAP area. So, in total there were 11 sampling sites. They were cleaned

to remove dust particles, air dried in the greenhouse, ground to make powders, labeled appropriately and then stored for further use.

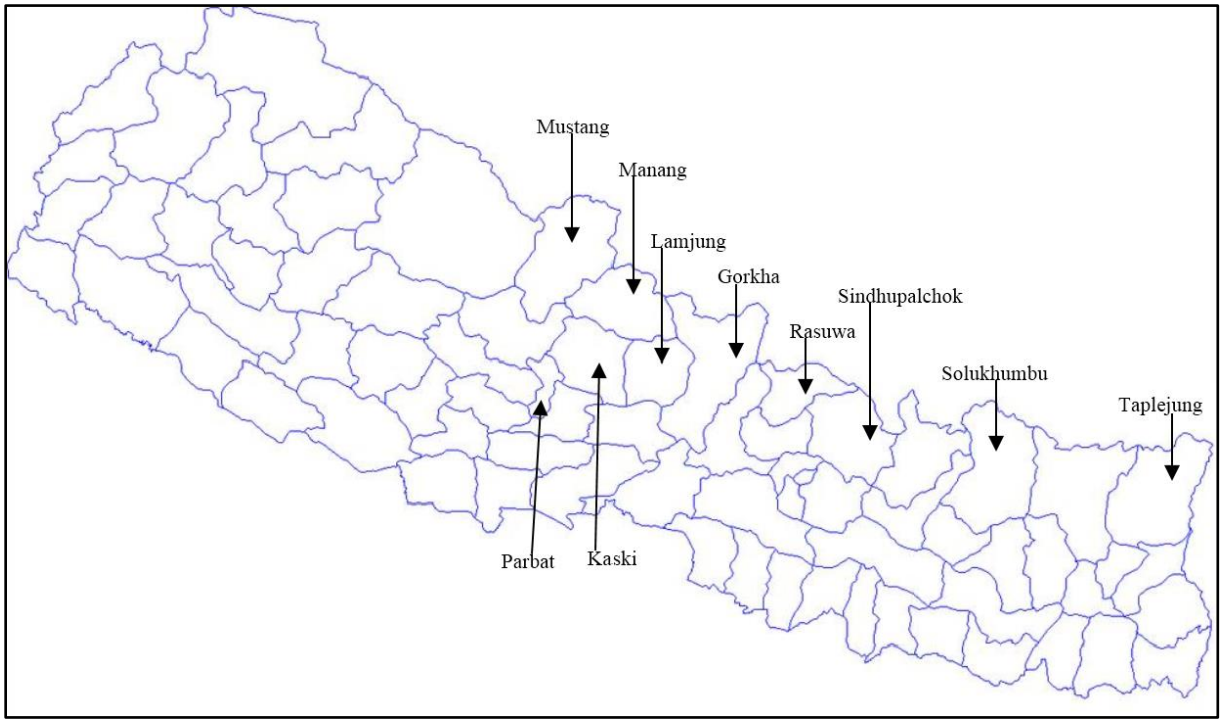

Figure 1: Districts selected as the sample collection site

Extract Preparation: Dried and finely powdered plant materials were extracted with different solvent in 1:10 ratio. The extract was subjected to the rotary shaker for 48 hours after which the extract was filtered through filter paper and hence the filtrate was used for the phytochemical analysis. Each sample was subjected to 10 different solvents to determine which solvent gave the better result. For the phenolic and flavonoid content, the above process was repeated thrice for complete extraction from the explant.

Phytochemical Screening: Phytochemical screening was carried out by summarizing the methods given by Tiwari *et. al.*[13], Rathore *et. al.*[14] and Jayashree[15]. The extracts were evaluated for the qualitative screening of major phytochemical constituents which include: Alkaloids, Flavonoids, Glycosides, Phenol, Lignin, Saponin, Sterols and Triterpenes, Tannins, Proteins and Amino-acids, Carbohydrates (Reducing Sugar), Coumarin, Steroids, Terpenoids, Quinone, Resin, Starch and Diterpenes. Here the parameters were bark sample, needle sample, and 10 solvent systems.

Quantitative Screening for Total Phenolic and Flavonoid Content: Quantitative screening was done for 11 districts which we selected as our sampling site with one bark and one needle sample from each district. The enriched flavonoid and phenol content were clearly visible through the phytochemical screening which boosted our work further. Like in case of phytochemical screening, the study was done also for 10 solvent system for one bark and one needle sample. Considering the outcome, we chose to continue the research with methanolic extract as methanol is considered the standard solvent for extraction and is also easily available, cheaper, neither evaporates too fast nor too slow and importantly safest solvent to handle besides water.

Since *T. wallichiana* is dioecious in nature, we did the study separately on both male and female plants as well. From 4 sites; Lamjung, Mustang, Manang, and ACAP, we were able to collect male and female plants. Hence, we have results for 10 solvents (Bark/ Needle), 11 sites (Bark/Needle) and Male/Female (Bark/Needle).

Determination of Total Phenolic Content: The amount of phenol in the aqueous extract was determined by Folin-Ciocalteu reagent method with some modifications. 0.5 ml Folin-Ciocalteu reagent was added to 0.5 ml of extract to which 2.25 ml of distilled water was added followed by 2 ml of 7% Na_2CO_3. The resulting mixture was incubated at 40℃ for 20 mins. The absorbance of the sample was measured at 760 nm. Gallic acid was used as standard (1mg/1ml). All the tests were performed in triplicates. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/gm dry weight of extracted sample).

Determination of Total Flavonoid Content: Aluminum chloride colorimetric method was used with some modifications to determine total flavonoid content. 0.3 ml of extract was mixed with 2.7 ml of 30% methanol, 0.15 ml of 0.5M $NaNO_2$ and 0.15 ml of 0.3M $AlCl_3.6H_20$. The mixture was incubated at room temperature for 5 mins. 1 ml of 1M NaOH was added finally and mixed well. The absorbance of the sample was measured at 506 nm. Quercetin was used as standard (1mg/1ml). All the tests were performed in triplicates. The results were determined from the standard curve and were expressed as quercetin equivalent (mg/gm dry weight of extracted sample).

3. RESULTS AND DISCUSSIONS

Phytochemical Screening: The extracts obtained after 48 hours were filtered using Whatman filter paper. All the extracts gave herbal aroma and were all colorful just the difference was their color intensity. Non-polar solvents like hexane, ether solvents gave light color whereas polar solvents like acetone, methanol, acetic acid, water gave dark-colored extracts. Bark samples' extracts were light to dark red in color whereas needle samples' extracts were light to dark green in color.

The screening conducted on the *T. wallichiana's* bark and needle samples revealed the presence of various phytochemicals tested. **Table 1** shows the phytochemical screening for bark samples whereas **Table 2** shows results for needle samples. The results were near to similar for both cases as seen in the **Tables 1 and 2.** Starch was completely absent in both bark and needle samples whereas lignin was found to be absent in bark sample but was present in detectable amount in needle sample. Alkaloids, flavonoids, glycosides, phenol, tannins, coumarin, terpenoids, and quinone were the major phytochemicals detected. Our result concurs with that of Vohora and Kumar[16] and Kazmi *et. al.*[17] who also showed alkaloids, flavonoids, phenol, terpenoids, tannins to be the major phytochemical compounds in Taxus species.

From Table 1 and 2, we can deduce that out of the 10 solvents used, acetone, 50% ethanol and methanol gave the best results with visible, clear detection whereas almost all non-polar solvents used which were hexane, diethyl ether, petroleum ether gave negative results for most of the cases proving they are ineffective solvents for the extraction. Significant phytochemicals like alkaloids, flavonoids, glycosides, phenols were easily detected in the polar and semi-polar solvents indicating the presence of polar components in *T. wallichiana*. Positive results for diterpenes and alkaloids explain the accuracy of the procedure as *T. wallichiana* is rich in Taxol and other taxanes which fall under these categories.

Table 1: Phytochemical Screening for Bark Sample of *T. wallichiana*

S. N.	Test	Hexane	Diethyl Ether	Petroleum Ether	Chloroform	Ethyl Acetate	Acetone	50% Ethanol	Methanol	Acetic Acid	Water
1.	Alkaloids	+	++	+	+++	+++	++	+++	++	++	+++
2.	Flavonoids	-	-	-	+	+++	+++	+++	+++	+++	+++
3.	Glycosides	-	-	-	+	+++	++	+++	++	+++	+++
4.	Phenol	-	-	-	-	++	+++	+++	+++	+	+++
5.	Lignin	-	-	-	-	-	-	-	-	-	-
6.	Saponins	+	+	+	+	++	-	-	-	-	-
7.	Sterols & Triterpenes	-	-	-	-	+	+++	+++	-	+	++
8.	Tannins	-	-	-	-	+	+++	+++	+++	-	+++
9.	Proteins/Amino Acids	-	-	-	+	+	+	+	+	+	+
10.	Carbohydrates	-	-	-	-	+++	+++	+++	+++	-	+++
11.	Coumarin	-	-	-	-	++	++	+	+	+++	+
12.	Steroids	-	-	-	-	+	+++	+++	-	+	++
13.	Terpenoids	-	-	-	+	+++	+++	+	+++	+++	++
14.	Quinone	-	-	-	-	+++	+++	+++	+++	+++	+++
15.	Resin	-	-	-	+	+	-	-	-	-	+
16.	Starch	-	-	-	-	-	-	-	-	-	-
17.	Diterpenes	-	-	-	-	-	-	-	-	+++	-

'-'= Not Detected, '+' = Low Presence, '++' = Moderate Presence, '+++' = Strong Presence.

Table 2: Phytochemical Screening for Needle Sample of *T. wallichiana*

S. N.	Test	Hexane	Diethyl Ether	Petroleum Ether	Chloroform	Ethyl Acetate	Acetone	50% Ethanol	Methanol	Acetic Acid	Water
1.	Alkaloids	+	++	+	+++	+++	++	+++	+++	+++	+++
2.	Flavonoids	-	-	-	-	++	+++	+++	+++	+++	+++
3.	Glycosides	-	-	+	+	+++	++	+++	++	+++	+++
4.	Phenol	-	+	+	+	+	+++	+++	+++	++	++
5.	Lignin	-	-	-	+	+	++	-	+	++	-
6.	Saponins	+	+	+	+	++	-	-	-	-	-

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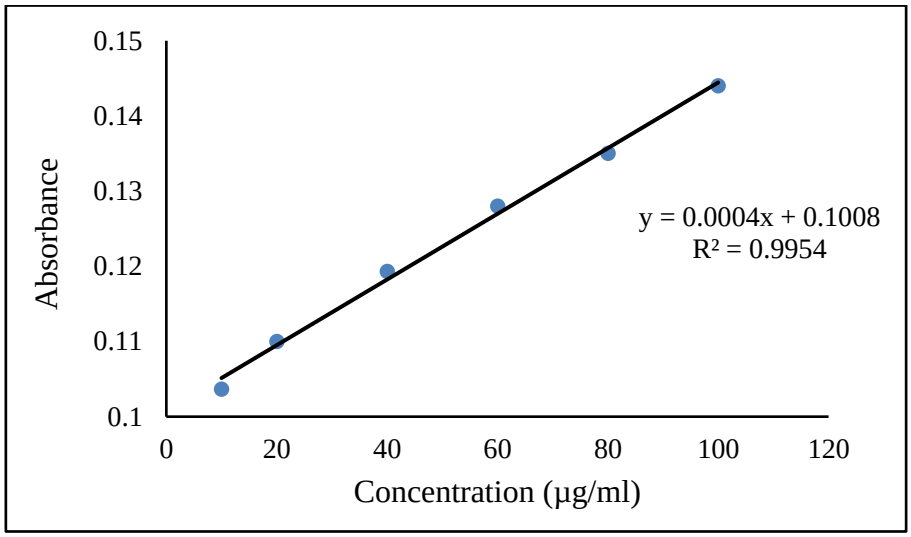
7.	Sterols & Triterpenes	-	-	-	-	-	+	+++	-	-	++
8.	Tannins	-	-	-	-	+	+++	+++	+++	-	+
9.	Proteins/Amino Acids	-	-	-	-	+++	+++	+	-	+++	++
10.	Carbohydrates	-	-	-	-	-	+++	+++	+++	-	+++
11.	Coumarin	++	++	+++	+++	++	+++	+	+	++	++
12.	Steroids	-	-	-	-	-	+	+++	-	-	++
13.	Terpenoids	-	+	+	+	+++	+++	+	+++	+++	+
14.	Quinone	-	-	-	-	+++	+++	+++	+++	+++	+++
15.	Resin	-	-	-	+	+	-	-	-	+	+
16.	Starch	-	-	-	-	-	-	-	-	-	-
17.	Diterpenes	-	-	+	+	+	++	-	-	+++	-

'-'= Not Detected, '+' = Low Presence, '++' = Moderate Presence, '+++' = Strong Presence.

Total Phenolic Content: The study was done for three parameters. First parameter was different solvent system, second parameter was 11 sampling sites, and finally, the third parameter was sex that is male and female plant. In all the cases, the study was done using two sample types: Bark and Needle. The results were determined from the standard curve for gallic acid **(Graph 1)** and were expressed as gallic acid equivalent (mg/gm dry weight of extracted sample).



Graph 1: Gallic Acid Standard Curve for Phenolic Content

10 solvents with different polarity were used starting from non-polar hexane to highly polar water as polarity of extracting solvent and the chemical constituent in the extract influence the TPC value **(Table 3)**. Out of these, non-polar solvents including hexane, diethyl ether, and petroleum ether showed very low phenolic content with hexane giving the lowest one, 7.74±0.99 mg GAE/gm DW for bark and 5.46±0.29 mg GAE/gm DW for the needle. Acetone, 50% ethanol and methanol proved to be the best solvents for with acetone giving the highest one, 177.02±0.19 mg GAE/gm DW for bark and 173.72±1.7mg GAE/gm DW for the needle. This is because of their higher polarity which gives better

solubility for the phenolic compounds which are usually semi-polar in nature. Significant difference in TPC value was seen between the bark and the needle sample with the former one giving higher TPC value. For further study, out of these best solvents, methanol was used, as acetone in one hand evaporates more rapidly and 50% ethanol on the other hand takes long time to evaporate due to its water percentage. Hence, all the remaining tests were carried out in methanolic extracts.

Table 3: Total Phenolic Content in Bark and Needle of *T. wallichiana* with different solvent system

S.N.	Solvent Used	Total Phenolic Content (mg gallic acid eq./ gm dry wt. sample)	
		For Bark	For Needle
1.	Hexane	7.74±0.99	5.46±0.29
2.	Diethyl Ether	28.42±1.99	5.76±0.26
3.	Petroleum Ether	10.59±2.51	7.08±3.48
4.	Chloroform	54.19±0.45	45.48±3.83
5.	Ethyl Acetate	99.73±3.15	93.86±1.38
6.	Acetone	177.02±0.19	173.72±1.7
7.	50% Ethanol	138.51±1.06	131.42±2.67
8.	Methanol	165.79±2.71	124.53±1.25
9.	Acetic Acid	108.46±1.27	75.59±2.45
10.	Water	74.98±1.02	67.48±4.83

Bark and needle samples were collected from 11 different sampling sites of Nepal and by random selection, one sample each for bark and needle (both from the same tree) was selected for each site. **Table 4** shows the result for TPC value for each sampling site. There is clear difference in TPC value between bark and needle samples with former ones having higher value than later, the case as seen in the previous test **(Table 3).** Regarding the change in site, here Mustang's bark sample showed the highest TPC value 424.51±2.89 mg GAE/gm DW followed by Parbat's bark sample with 422.51±0.99 mg GAE/gm DW. ACAP site has the lowest TPC value of 371.08±1.07 mg GAE/gm DW. However, the difference between the highest and the lowest value is not so high (~50) to be considered as significant difference. The case is slightly different in case of needle samples as the difference margin is slightly higher comparatively (~75) with highest of Mustang with 317.48±1.04 mg GAE/gm DW and lowest of Lamjung 241.24±0.59 mg GAE/gm DW. Minimal variation may be because the samples were mostly collected from similar altitude ranges with matching climate and surroundings. Slight difference brought about may be due to the difference in sampling time resulting in seasonal variation and as samples were selected randomly their maturity level might be different i.e. some samples might be from old trees and some from young ones which work as one of the variables.

The difference in bark and needle sample is clear as usual **(Table 5),** however not much variation was observed between the bark samples of male and female species. The difference that was slightly repetitive was in case of needle samples where female species showed high TPC value than male ones in all the 4 cases **(Table 5).** This minimal variation might be because in Taxus species, the sex difference is determined only by the presence of seed present in females enclosed in a fleshy aril and here the explant

being tested are bark and needle, which gives us avenues to consider that the chemical constituent of bark and needle might be nearly similar in case of both the sexes.

Table 4: Total Phenolic Content in Bark and Needle of *T. wallichiana* collected from different Sampling site

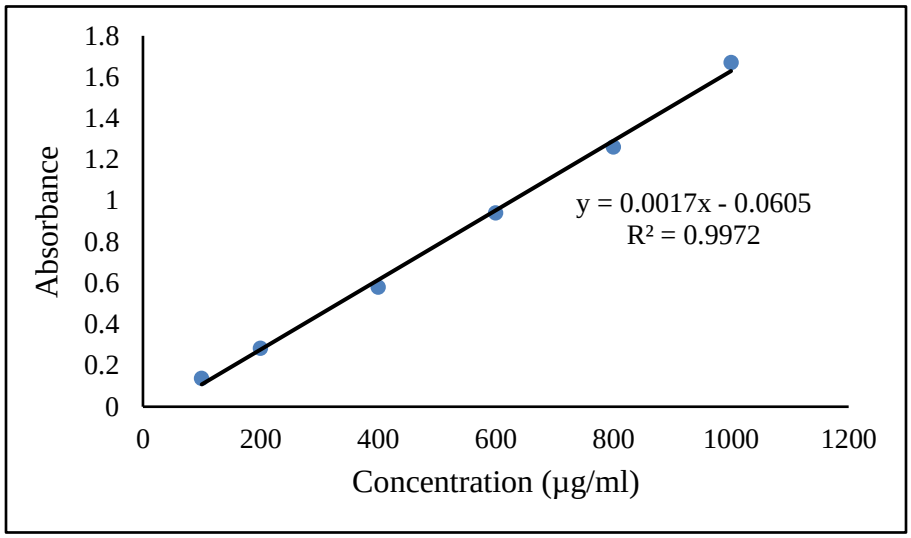
S.N.	District	Total Phenolic Content (mg gallic acid eq./ gm dry wt. sample)	
		For Bark	For Needle
1.	Gorkha	400.68±0.04	287.73±2.98
2.	Taplejung	419.54±3.54	309.39±0.19
3.	Rasuwa	391.11±1.11	269.96±1.69
4.	Sindhupalchok	411.05±0.56	281.25±3.02
5.	Mustang	424.51±2.89	317.48±1.04
6.	Manang	381.53±0.04	258.11±2.74
7.	ACAP	371.08±1.07	243.75±2.73
8.	Solukhumbu	385.69±0.72	248.82±1.13
9.	Parbat	422.51±0.99	318.11±0.57
10.	Sikles	417.61±1.93	296.33±1.45
11.	Lamjung	376.57±1.72	241.24±0.59

Table 5: Total Phenolic Content in Bark and Needle of *T. wallichiana* with variance in the sex

S.N.	District	Sex	Total Phenolic Content (mg gallic acid eq./ gm dry wt. sample)	
			For Bark	For Needle
1.	Lamjung	Male	376.57±1.72	241.24±0.59
		Female	367.57±1.57	243.74±0.56
2.	Mustang	Male	424.51±2.89	317.48±1.04
		Female	422.51±0.99	338.23±1.09
3.	ACAP	Male	371.08±1.07	243.75±2.73
		Female	379.08±1.07	253.95±0.36
4.	Manang	Male	381.53±0.04	258.11±2.74
		Female	380.28±1.85	261.02±1.07

Total Flavonoid Content: As in the phenolic test, here also the parameters were the same. The results were determined from the standard curve for quercetin **(Graph 2)** and were expressed as quercetin equivalent (mg/gm dry weight of extracted sample). In the case of the first test for TFC value with solvent system as a variable **(Table 6),** the result was identical with that for TPC value **(Table 3).** Non-polar solvents showed lowest TFC value with hexane at the bottom (1.98±0.05 mg quercetin/gm DW for bark and 2.72±0.02 mg quercetin/gm DW for needle). The three best solvents were once again acetone, methanol and 50% ethanol. The reason might be the medium polarity of flavonoids making its extraction highly feasible in medium polar solvents. The difference between bark and needle samples were visible

but the differences are not as vague as in the case of TPC. Highest value for TFC in bark sample is 48.69±0.78 mg quercetin/gm DW and highest TFC value for needle sample is significant as 31.03±0.72 mg quercetin/gm DW, once again proving bark to be a better candidate. Further tests were done with the methanolic extracts.



Graph 2: Quercetin Standard Curve for Flavonoid Content

Table 6: Total Flavonoid Content in Bark and Needle of *T. wallichiana* with different solvent system

S.N.	Solvent Used	Total Flavonoid Content (mg quercetin eq./ gm dry wt. sample)	
		For Bark	For Needle
1.	Hexane	1.98±0.05	2.72±0.02
2.	Diethyl Ether	3.53±0.03	3.61±0.07
3.	Petroleum Ether	2.25±0.05	3.34±0.12
4.	Chloroform	15.71±0.13	7.6±0.2
5.	Ethyl Acetate	23.66±0.61	9.71±0.35
6.	Acetone	48.69±0.78	31.03±0.72
7.	50% Ethanol	28.10±1.14	20.05±0.19
8.	Methanol	47.96±1.9	26.02±0.13
9.	Acetic Acid	24.93±0.1	10.38±0.07
10.	Water	16.92±1.05	9.16±0.68

Out of the 11 sampling sites, **(Table 7)** Mustang's bark and needle sample showed highest TFC value of 108.87±1.63 and 80.51±1.27 mg quercetin/gm DW respectively. Lowest TFC value was that of samples from Lamjung, 55.6±1.58 and 27.51±0.18 mg quercetin/gm DW for bark and needle samples respectively. The reasoning for this variation must be the same as in the case of TPC unless other reasons

are explored and studied. Barks' and needles' TFC value were significantly different as always with former ones having higher values than latter ones.

Table 7: Total Flavonoid Content in Bark and Needle of *T. wallichiana* collected from different sampling site

S.N.	District	Total Flavonoid Content (mg quercetin eq./ gm dry wt. sample)	
		For Bark	For Needle
1.	Gorkha	74.51±0.45	48.64±0.29
2.	Taplejung	97.32±0.15	75.32±0.42
3.	Rasuwa	72.76±0.68	48.41±0.27
4.	Sindhupalchok	83.61±1.12	55.06±0.45
5.	Mustang	108.87±1.63	80.51±1.27
6.	Manang	58.69±2.19	29.75±0.77
7.	ACAP	58.09±0.4	28.65±0.42
8.	Solukhumbu	71.17±0.69	32.03±0.68
9.	Parbat	106.54±2.13	79.42±0.35
10.	Sikles	91.11±0.98	65.56±0.87
11.	Lamjung	55.6±1.58	27.51±0.18

Table 8: Total Flavonoid Content in Bark and Needle of *T. wallichiana* with variance in the sex

S.N.	District	Sex	Total Flavonoid Content (mg quercetin eq./ gm dry wt. sample)	
			For Bark	For Needle
1.	Lamjung	Male	55.6±1.58	27.51±0.18
		Female	53.5±0.2	26.62±0.23
2.	Mustang	Male	108.87±1.63	80.51±1.27
		Female	110.31±2.52	83.03±0.37
3.	ACAP	Male	58.09±0.4	28.65±0.42
		Female	60.25±0.1	31.3±0.61
4.	Manang	Male	58.69±2.19	29.75±0.77
		Female	61.01±0.13	29.99±0.21

In 3 out of 4 cases, female bark samples showed higher TFC value than the male ones even though the difference margin was minimal, whereas the ratio was near to equal in case of needle samples. As usual, bark samples showed higher TFC value than the needle samples which is hence the common result obtained out of all the tests performed.

4. CONCLUSION

Taxus wallichiana being anti-cancerous, medicinal plant undoubtedly had to comprise of bioactive compounds which is clearly verified by our study. The phytochemical screening clearly showed the major biochemical constituent of the species whereas the availability of phenolic and flavonoid content proved and maintained its importance as an anti-cancerous and medicinal plant. Therefore, this present study

provides a horizon to explore the *T. wallichiana* species of Nepal, additionally this study sorts out best methods feasible to execute further research options.

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