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Powder microscopy and phytochemical screening on stem bark and leaves of *Buxus wallichiana* Baill - Buxaceae

Vandana Bharthi, Kavya B, BM Meghashree and Sulochana Bhat

Abstract
Buxus wallichiana Baill, belongs to family Buxaceae, known worldwide for its manifold uses. Traditionally, the leaves are used as a bitter tonic possessing purgative and diaphoretic property. The wood is diaphoretic and tincture of the bark is employed as febrifuge. The paste of the stem bark is used for proficient fracture healing. *B. wallichiana* is reported to be useful in the treatment of rheumatism, hair growth and syphilis. The wood of the tree are also used for musical and mathematical instruments, cabinet works and in carving. In recent years, there is an emphasis on the Pharmacognostical standardization of medicinal plants for their high therapeutic potential. Hence, the present study deals with the powder microscopy of *B. wallichiana* stem bark and leaves along with physical constants like ash values, extractive values and preliminary phytochemical analysis. Preliminary phytochemical analysis shows the presence of steroids, alkaloids, saponins and glycosides.

Keywords: *Buxus wallichiana*, buxaceae, stem bark, leaves, purgative, diaphoretic

1. Introduction
Buxus wallichiana commonly known as Himalayan boxwood belongs to family Buxaceae. Genus *Buxus* comprises about 70 species that are widely or sparsely distributed in Asia, Europe, Africa, North and Central America. In India two species of *Buxus* have been reported that is *Buxus wallichiana* (Himalaya box tree) and *B. papillosa* C.K. Schneid [1]. *B. wallichiana* is an evergreen shade loving tree which grows upto 15m in height and 2.1m in girth with a clean bole upto 7.5m, found irregularly distributed in some parts of North-Western Himalayan region of India from Kashmir to Nepal and Bhutan at altitudes varying from 1200 to 2700m. The flower appears during March to May and fruit ripens from July to August. The bark of the tree is yellowish grey corky, soft cut into small rectangles on old stems; leaves lanceolate or narrowly elliptic-oblong; flowers unisexual, yellow and green fascicled racemes; capsules chestnut-brown, broadly ovoid with horns [2].
The tree yields good timber. It is grown in the Nilgiris in cinchona plantations as a hedge. In Himachal Pradesh, the tree grows on shale, gneiss and micaschist. It avoids hot aspects and seeks northerly and north-westerly slope. Under unfavorable conditions such as dense shade, it becomes shrubby. Although found pure or nearly so in patches, it is frequently found associated with other Himalayan species, such as *Taxus baccata* sub sp. *wallichiana* (Himalayan Yew), *Rhododendron arboretum*, etc [2].
The leaves are poisonous to cattle though goats eat them sparingly. They are bitter, possess purgative and diaphoretic properties and are reported to be useful in rheumatism and syphilis. The tincture of the bark is employed as febrifuge. The leaves and bark are used as a substitute for tea in Garhwal. The leaves contain the alkaloids Buxpiine-k, Bauxtauitne-M, Cyclobuxine-D, Cyclobuxoxazine-C in addition to Hentriacontanol, β amyrin and Betulinic acid. Aresin and an essential oil are reported from the bark and leaves [2].
The wood is uniformly whitish yellow to brownish yellow, with little difference between sapwood and heartwood; compression wood is sometimes present. It has silky ivory-like luster and is durable, hard, heavy, extremely fine and even textured and close, straight or sometimes irregular grained. The wood is employed for making various musical and mathematical instruments, shuttles, tool handles, boxes for butter, honey and snuff for combs turney, carving, toys, croquet mallet-heads and balls and tinder [2].
In Jammu and Kashmir, the *Buxus* wood sustains a whole cottage industry especially in Rajouri poonch belt.

The artisans have been involved in this craft are mainly located in Shahdra Sharief, Thanamandi and Budhal areas of famous twin district, that is Rajouri poonch variety of articles comprising baby walkers, toys, decoration pieces, photo frames, hangers, snuff boxes, comb, forks, spoons have extensively been manufactured from this species. The wood of the tree is light and has lustrous high calorific value which makes it a good fuel for heating and cooking purposes. Charcoal is made from chikarri wood used in fire post during winter [1].

In view of its multiple uses, the population of *Buxus wallichiana* is facing severe threats in many parts of the Himalayan region. So many areas are there, where once the Himalayan box tree was present in abundance, but now there is totally or partially absence of the species. Therefore, it is necessary to develop alternative methods to conserve the species. *B. wallichiana* is widely used in the treatment of different ailments but there are very few evidences or data related to its pharmacognosy and phytochemistry. One such from the literature review has revealed the histological and physico-chemical studies on stem bark of *B. wallichiana* collected from Nilgiris [3]. Hence, this paper presents the powder microscopy and a phytochemical study of *B. wallichiana* stem bark and leaves which proves its therapeutic potential.

2. Regional names in India [2]

Hindi	**:**	Chikri, Papri, Sansadu.
Himachal Pradesh	**:**	Sansad, Shamshad
Jammu & Kashmir	**:**	Chikarri

3. Materials and Methods

Stem bark and Leaves of *Buxus wallichiana* Baill., were collected and authenticated from Survey of Medicinal Plants Unit, Regional Research Institute of Himalayan Flora, Tarikhet, Ranikhet, CCRAS, India. The stem bark and leaves was freed from adulterant by hand picking, shade dried, pulverized by mechanical grinder, passed through 40 mesh sieves and stored in a closed vessel, to carry out powder microscopic studies, physico-chemical and preliminary phytochemical analysis. Stem bark and leaf powder were extracted with different solvents with the help of Soxhlet extraction apparatus. Physicochemical and preliminary phytochemical screening of stem bark and leaves were carried out according to the standard methods and recorded. Photomicrographs were captured with Catcam Image Analyzer.

3.1 Powder Microscopy: The powder microscopy of stem bark and leaves were studied as per the standard procedures by capturing the images of different fragments of tissues and obtained observations through image analyzer [4-6]

3.2 Physico-chemical analysis: The physico chemical parameters like moisture content, loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values were carried out as per the standard procedures [4].

3.3 Phytochemical analysis: The powdered drugs extracted in different solvents were tested for various phytoconstituents present in them. They are generally tested for the presence of alkaloids, flavonoids, tannins, phenols, steroids and saponins using standard procedures [7].

3.4 Thin Layer Chromatography (TLC): Dried stem bark and leaf powder was extracted with Petroleum ether (60-80 ^{o}C), Chloroform and Ethanol by using Soxhlet extraction apparatus. TLC studies of these extracts were carried out by using commercially available precoated plates with standardized adsorption layers, i.e. Silica gel 60 F254, (Merck, Germany) at room temperature as per the standard procedures [8].

3.5 Fluorescence analysis: Fluorescence analysis has been carried out by using different chemical reagents as per the standard procedures. A small quantity of stem bark and leaf powder is placed on clean watch glass and 1-2 drops of freshly prepared reagent solution is added, mixed by gentle tilting of the watch glass and after few minutes, the watch glass is placed inside the UV chamber and observed the colour in visible light, short (254 nm) and long (366 nm) ultra violet radiations. The colour observed by application of different reagents in different radiations was recorded [9].

4. Results

4.1 Powder Microscopy of Stem Bark (Plate I): Powder light brown in color, smooth to touch, smell agreeable and taste is slightly bitter. When powder treated with Chloral hydrate, water and saffranin, following different fragments of tissues was observed under microscope.

- Different fragments of tissues showing rounded to elongated parenchymatous cells with starch grains, reddish tannin content, Xylem Vessel with pitted thickening and tracheids.
- Parenchymatous cells showing starch grains.
- Polygonal Parenchymatous cells.
- Pitted Xylem Vessel.
- Pitted Xylem Vessel in groups.
- Single Prism shaped crystal.
- Single tracheid.
- Reddish Tannin Content.
- Elongated Parenchymatous cells showing group of starch grains.

Diagnostic Characters

- Presence of abundant Prism shaped crystals.
- Presence of simple rounded starch grains in parenchymatous cells.
- Presence of single and groups of tracheid.
- Presence of Xylem vessels with pitted thickenings.
- Presence of reddish tannin content in parenchymatous cells.

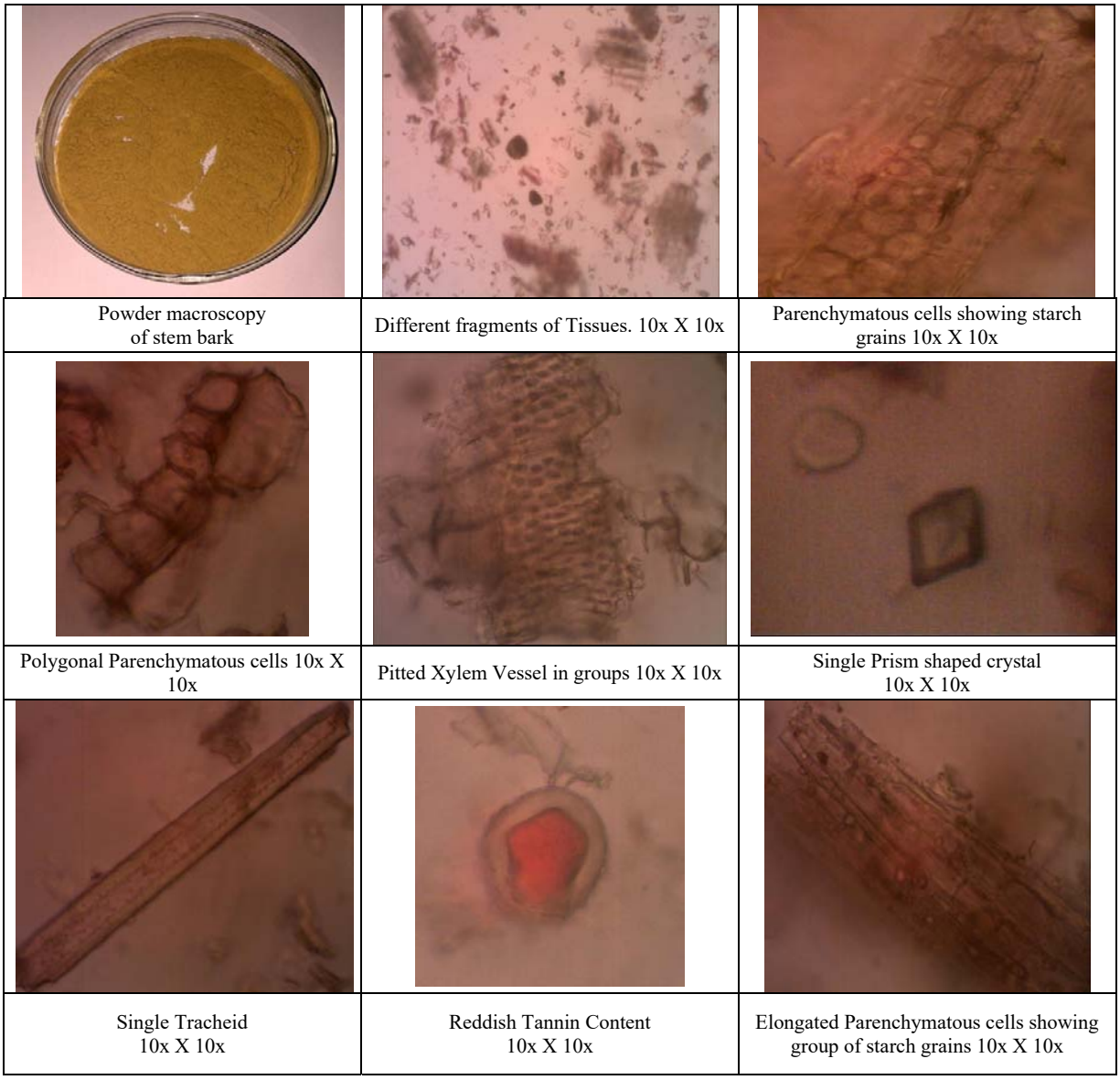


Plate 1: Powder Microscopy of *Buxus wallichiana* - Stem Bark

4.2 Powder Microscopy of Leaves (Plate II): Leaf powder is pale green in color, smooth to touch, smell agreeable and taste slightly sweetish. When treated with chloral hydrate, water and saffranin, following different fragments of tissues were observed under the microscope.

- Epidermal cells in surface view.
- Epidermal cells, parenchymatous cells showing tannin content.
- Parenchymatous cells.
- Single trichomes.
- Xylem parenchyma with simple pits.
- Fibers with prism shaped crystals.
- Group of fibers.
- Epidermal cells with stomata in surface view.
- Group of Helical xylem vessels.
- Spiral Xylem vessel.
- Parenchymatous cells with Calcium oxalate crystals in groups.
- Single prism shaped crystal.

Diagnostic Characters

- Presence of parenchymatous and epidermal cells with tannin content.
- Presence of group of well developed fibers.
- Presence of fibers with prism shaped crystals.
- Presence of calcium oxalate crystals in the parenchymatous tissue.
- Presence of ranunculaceous type of stomata.

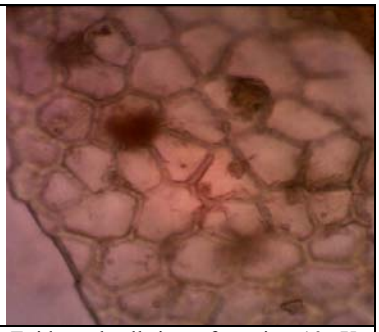
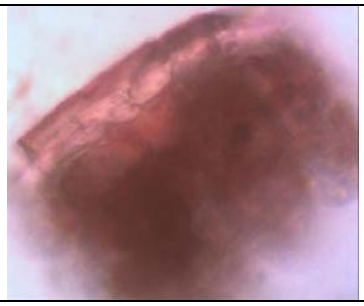
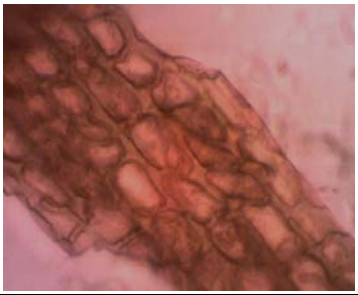
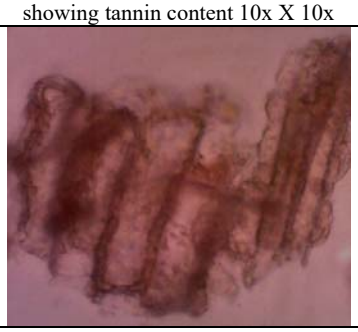
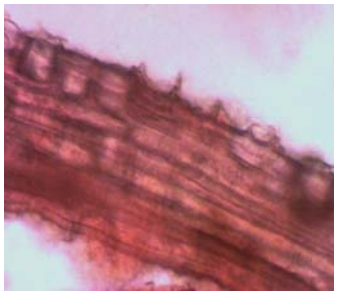
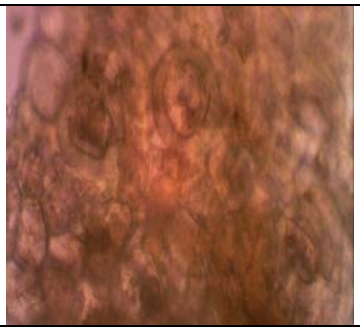
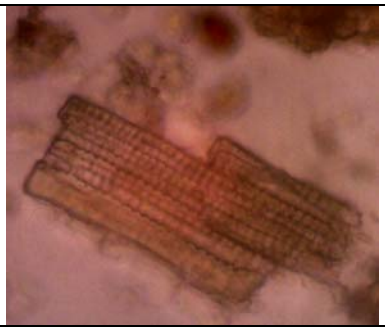
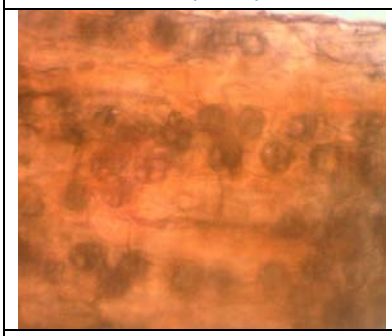
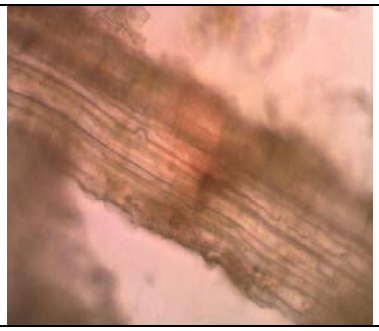
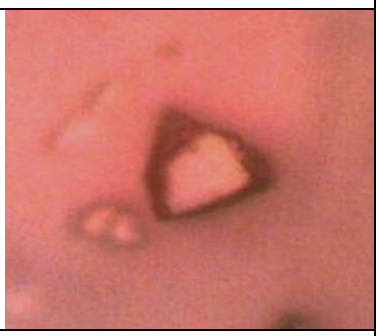
		
Powder macroscopy of leaves	Different fragments of tissue 10x X 4x	Epidermal cells in surface view 10x X 10x
		
Epidermal & parenchymatous cells showing tannin content 10x X 10x	Parenchymatous cells 10x X 10x	Single Trichome 10x X 10x
		
Xylem parenchyma with simple pits 10x X 10x	Fibers with prism shaped crystals 10x X 40x	Group of fibers 10x X 10x
		
Epidermal cells with stomata in surface view 10x X 10x	Group of Helical xylem vessels 10x X 40x	Spiral Xylem vessel 10x X 40x
		
Parenchymatous cells with Calcium oxalate crystals in groups 10x X 40x	Group of fibers 10x X 10x	Single prism shaped crystal 10x X 40x

Plate 2: Powder Microscopy of *Buxus wallichiana* – Leaves

4.3 Physico-chemical analysis: The physico chemical parameters like moisture content, loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values were carried out and recorded the values in Table 1 & 2.

Table 1: Physicochemical parameters of Stem bark and Leaves of *Buxus wallichiana*

S. No	Name of the parameter	Values (%) w/w	
		Stem Bark	Leaves
1.	Description	Yellowish	Dark green
2.	Foreign matter	Not less than 1.0	Not less than 1.0%
3.	Total ash	3.69	4.86
4.	Acid-insoluble ash	0.42	0.76
5.	Water-soluble extractive	14.8	24.7
6.	Alcohol- soluble extractive	26.6	10.8
7.	Loss on drying at 105 ^{0}C	1.43	6.76
8.	pH (1%w/v of aqueous solution)	5.26	5.88

Table 2: Extractive values for Soxhlet extraction of Stem Bark and Leaves

S. No.	Solvent	Values(%)w/w	
		Stem Bark	Leaves
1.	Petroleum ether (40-60 ^{0}C)	0.58	14.9
2.	Chloroform	0.70	1.90
3.	Ethanol	0.51	8.15

4.4 Phytochemical analysis: The phytochemical parameters in different solvents were tested for the presence of various phyto-constituents such as proteins, carbohydrates, saponins, starch, phenols, flavonoids present in them by standard procedures and recorded the values in Table 3.

Table 3: Preliminary Phytochemical Tests for *Buxus wallichiana* Stem Bark & Leaves

S. No	Natural product group	Test for natural products	Stem Bark		Leaves	
			Extract used for the test	Presence (+) / Absence (-)	Extract used for the test	Presence (+) / Absence (-)
1	Alkaloids	Dragendr off test	Alcoholic	+	Aqueous	+
		Hager's test	Alcoholic	+	Alcoholic	+
		Wagner's test	Alcoholic	+	Alcoholic	+
		Mayer's test	Alcoholic	+	Aqueous	+
2	Carbohydrates	Benedict test	Alcoholic	+	Alcoholic	+
		Fehling's test	Aqueous	-	Alcoholic	+
		Molisch's test	Aqueous	-	Aqueous	+
		Anthrone test	Aqueous	-	Aqueous	-
3	Fixed oil	-	Petroleum ether	+	Petroleum ether	-
4	Glycosides	-	Alcoholic	+	Aqueous	+
5	Phenols	Ferric chloride test	Aqueous	-	-	
6	Proteins	Biuret's test	-		Alcoholic	-
		Million's test			Aqueous	-
7	Saponin	Foam test	Aqueous	+	Aqueous	+
8	Starch	Iodine test	Aqueous	-	Aqueous	-
9	Steroids	Liebermann Burchard test	-		Alcoholic	+
		Salkowski reaction			Aqueous	-
10	Tannins	Ferric chloride test	Alcoholic	+	-	

4.5 Thin Layer Chromatography

For Stem Bark: The TLC was carried out for three different solvent extracts i.e. Toluene: Ethyl acetate (7:3) for Petroleum ether (PE) extract; Hexane: Ethyl acetate (6:4) for Chloroform extract and Hexane: Ethyl acetate (6:4) for Ethanol extract.

For Leaves: The TLC was carried out for three different solvent extracts i.e. Hexane: Ethyl acetate (9:1) for Petroleum ether (PE) extract; Hexane: Ethyl acetate (3:7) for Chloroform extract and Hexane: Ethyl acetate (3:7) for Ethanol extract.
After developing, the plates were dried under room temperature for 5-10 minutes and observed under UV-254 & UV-366. Photographs were taken and the R_f values were recorded (Plate III & IV).

R_f values for Stem Bark

PE extract: under 254nm: 0.97, 0.78, 0.68, 0.51 and 0.41; under 366nm: 0.13, 0.15, 0.25, 0.37, 0.43, 0.55 and 0.95.

Chloroform extract: under 254nm: 0.97, 0.44, 0.40, 0.26 and 0.17; under 366nm: 0.12, 0.16, 0.31, 0.37, 0.62, 0.75 and 0.87.

Ethanolic extract: under 254nm: 0.80, 0.68, 0.32 and 0.23; under 366nm: 0.28, 0.41, 0.43, 0.48, 0.5, 0.75 and 0.81.

R_f values for Leaves

PE extract: under 254nm: 0.98, 0.88, 0.65, 0.37, 0.32 and 0.22; under 366nm: 0.14, 0.17, 0.21, 0.28, 0.34, 0.57 and 0.82.

Chloroform extract: under 254nm: 0.96, 0.84, 0.73, 0.68 and 0.54; under 366nm: 0.5, 0.64, 0.7, 0.81 and 0.85.

Ethanolic extract: under 254nm: 0.96, 0.87 and 0.75; under 366nm: 0.14, 0.72 and 0.82.

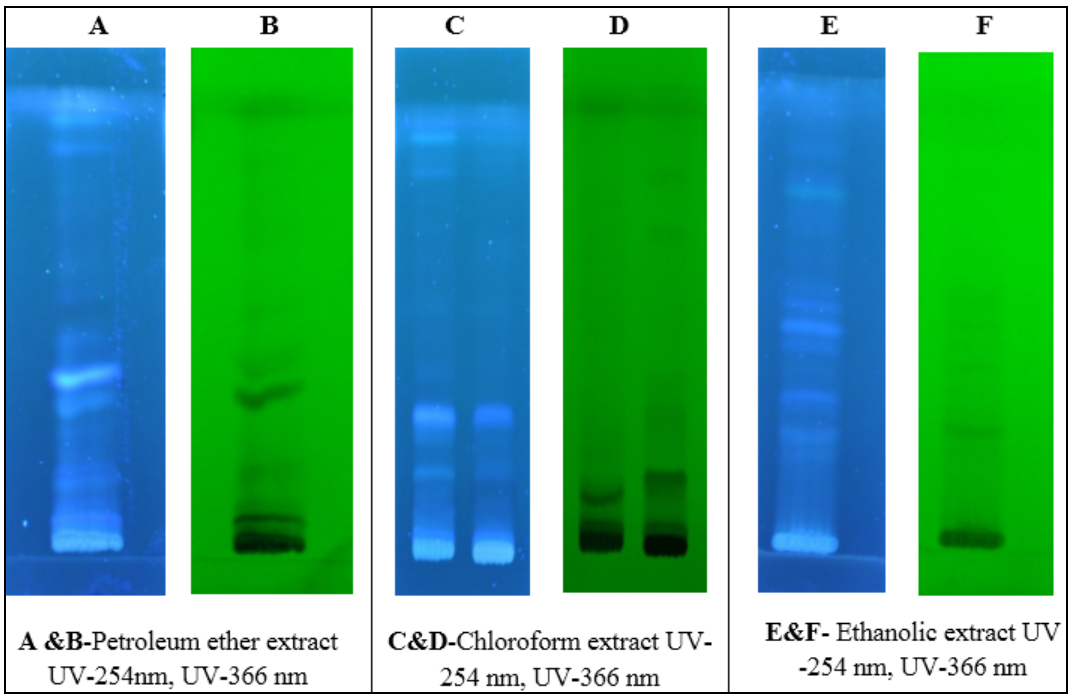


Plate 3: TLC Fingerprint of the *Buxus wallichiana Baill* Stem Bark

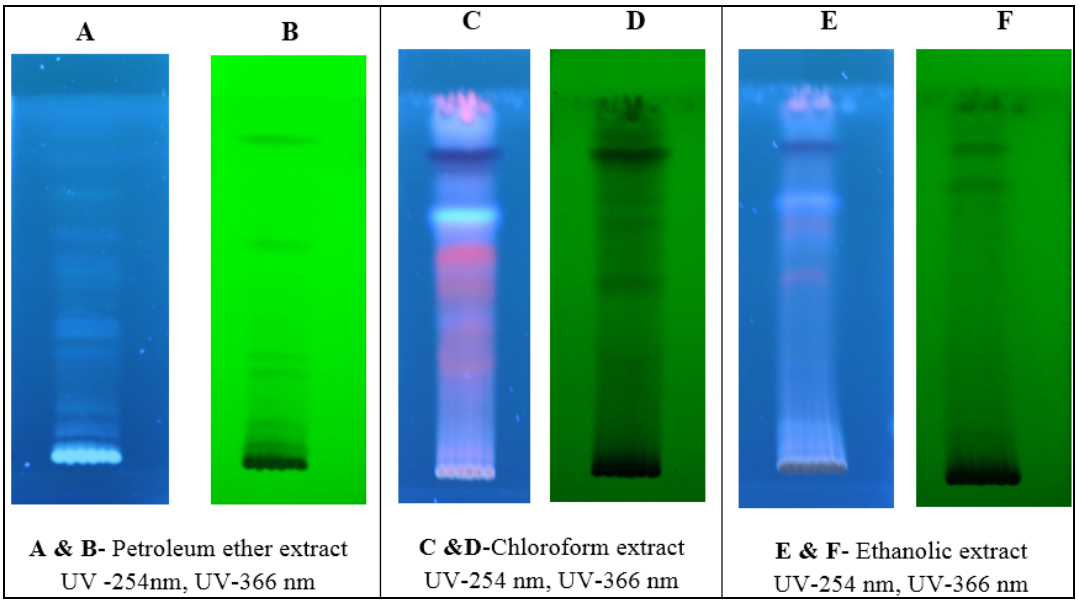


Plate 4: TLC Fingerprint of *Buxus wallichiana* Leaves

4.6 Fluorescence studies: The fluorescence analysis has been carried out by treating with different chemical reagents. The different colour reactions were observed under day light, UV-254 andUV-366nm and recorded the colour reactions in Table 4 & 5.

Table 4: Fluorescence analysis of *Buxus wallichiana* Stem Bark

Sl. No.	Powder + Reagent	Ordinary light	U.V long wavelength 366 nm	U.V short wavelength 254 nm
1.	Powder as such	Yellowish	Yellowish purple	Green
2.	Powder + 1N NaOH	Dark green	Pale yellow	Green
3.	Powder + 1N NaOH in water	Yellowish	Yellowish	Light green
4.	Powder + 50% HCl	Yellow	Yellowish	Green
5.	Powder + 50% HNO_3	Orange	Dark yellow	Dark green
6.	Powder + 50% H_2SO_4	Yellowish	Yellowish	Dark green
7.	Powder + Petroleum ether	Yellow	Yellow	light green
8.	Powder + 5% $FeCl_3$	Light green	Light green	Light green
9.	Powder + Picric acid	Yellow	Yellow	Yellow
10.	Powder + Glacial acetic acid	Yellowish	Yellowish green	Light green

Table 5: Fluorescence analysis of *Buxus wallichiana* Leaves

Sl. No.	Powder + Reagent	Ordinary light	U.V long wavelength 366 nm	U.V short wavelength 254 nm
1.	Powder as such	Dark green	Green	Green
2.	Powder + Conc. HCl	Dark green	Light green	Light green
3.	Powder + Conc.HNO_3	Yellowish	Yellowish red	Yellowish red
4.	Powder + Conc.H_2SO_4	Yellow	Yellowish brown	Yellowish brown
5.	Powder + Glacial acetic acid	Greenish yellow	Greenish yellow	Greenish yellow
6.	Powder + 5%NaOH	Yellowish green	Yellowish green	Yellowish green
7.	Powder + 5% KOH	Light green	Light green	Light green
8.	Powder + 5% $FeCl_3$	Green	Light green	Light green
9.	Powder+ Picric acid	Yellow	Yellow	Yellow

5. Conclusion

In recent times, medicinal plants occupy an significant position for being the paramount sources of drug discovery, irrespective of its categorized groups as herb, shrub or tree and they have been indispensable in treating diverse forms of diseases. In terms of ecological, *Buxus wallichiana* has a great role in biodiversity of woody species and treatment of different ailments. It is also used in number of other purposes like wood craft, fuel, fodder, musical instruments, carving and so on. Due to its over exploitation, the natural populations of this species are being depleted fast. Therefore, it is necessary to conserve this species using alternative methods for propagating such high value multipurpose tree species and create awareness among the common populace. The parameters of the present study could be useful in identification, authentication, ensuring quality, purity and efficacy of the drug for its conservation in the future.

6. Acknowledgement

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