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Histological and physico-chemical evaluation of *Buxus wallichiana* Baill

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ABSTRACT: *Buxus wallichiana* Baill, belongs to family Buxaceae. Traditionally *Buxus wallichiana* is used as bittertonic, diaphoretic, anti-rheumatic, vermifuge, antihelmentic, analgesic, purgative, diuretic, antiepileptic, antileprotic and in hemorrhoids. This paper deals with the macroscopic, microscopic and powdered studies of *Buxus wallichiana* wood, along with this physical constants like ash values and extractive values and preliminary phytochemical analysis were studied. Preliminary phytochemical analysis shows the presence of steroids, alkaloids, flavonoids.

Key words: *Buxus wallichiana,* Buxaceae, Antileprotic, Purgative, Diuretic

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INTRODUCTION

Buxus wallichiana Baill, commonly called as Himalayan boxwood, it belongs to family Buxaceae. *B. wallichiana* found at high mounts, shady place and cold climates. Boxwood is an evergreen monoecious shrub or tree growing to height 6 meters with variable form and leave shape. The green branches are initially pubescent, later glabrous, olive green, angular and densely covered with ovate leaves, which are usually opposite. The upper surface of leaves is smooth, coriaceous, dark green and very glossy; the lower surface is lighter in shade and the lamina margin is a smooth (Fleming 1999; Chopra *et al.* 1992). Traditionally *B. wallichiana* was used as bittertonic, diaphoretic, anti-rheumatic, vermifuge, antihelmentic, analgesic, purgative diuretic, antiepileptic, antileprotic and in hemorrhoids. The bark of *B. wallichiana was* used as hair growth stimulant (Chopra et al. 1992; Kritikar & Basu, 1989; Husain *et al.* 1992). Phytochemicals reported are alkaloids buxemenol E (Kvaltinova *et al.*1991), buxaltine H, Buxiramin D, buxatine, buxandrine F, buxidine F (Husain *et al.*1992), (+)-16a, 31-diacetylbuxadine (Ata *et al.* 2002), semperviraminol, buxamine F (Atta-ur-Rahman *et al.*1999) and evaluation of hair growth activity of *B. wallichiana* was reported (Nandeesh *et al.* 2009). Only one biological activity by the steroidal alkaloid buxemenol E from *B. sempervirens* was found to produce hypotensive effect in rat attributed by central and peripheral activation of muscranic receptor and also by partial inhibition of *acetylcholinestrase* enzyme (Kvaltinova *et al.* 1991). Plant was widely used for the treatment of different ailments but there is no data about its pharmacological effect.

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MATERIAL AND METHODS

Collection of plant material and extraction. The wood of *B. wallichiana* was collected from the Doddabetta region of Nilgiris district and identified by Dr. Rajan, Botanist from Government Arts College, Ootcamund, Tamilnadu. The specimen was preserved in college herbarium, voucher no. SKVCP 15. The collected wood was shade dried and grinded to a coarse powder. Successive extraction was done with petroleum ether, chloroform, methanol, and water respectively soxlet extraction.

Chemicals and Instruments. Toluidine blue, tertiary butyl alcohol, ethyl alcohol, acetic acid, formalin, chloral hydrate, ethanol, hexane, petroleum ether, sodium hydroxide, glycerin, Camera Lucida, drawing sheet, glass slides, cover slips, watch glass, rotary microtome. Nikon Labhot 2 Microscopic unit.

Microscopic analysis. The microscopic analysis of root was carried out as described by O'Brien et al., (1964).

Physico-chemical analysis. Physico-chemical values such as the percentage of ash values and extractive values were performed according to official methods prescribed Indian Pharmacopoeia, (1996) and the WHO Guidelines on Quality Control Methods for Medicinal Plant Materials (WHO/QCMMPM guidelines, 1992).

Preliminary phytochemical screening. Preliminary phytochemical screening of different extracts of *Buxus wallichiana* wood was carried out by using standard procedures described by Kokate (1986b).

RESULTS AND DISCUSSION

Macroscopic Studies: *B. wallichiana* wood was yellowish brown in color, powder was yellow in color, wood and powder was bitter in taste, wood possesses no odour, whereas powder had characteristic odor. The petroleum ether, chloroform and aqueous extracts were found to be brown in color; methanol extract was brownish black in color. Petroleum ether, chloroform and aqueous extract were solid, while methanol extract was resinous in consistency. Taste was acrid for pet ether and chloroform extracts, very bitter for methanol and bitter for aqueous extract. No odour for pet ether and chloroform extracts where as characteristic odour for methanol and aqueous extracts were observed (Table 1).

Ash Values: The ash value of *B. wallichiana* wood for total ash was found to be 1.2 % and 1.1, 0.4 and 1.4 % w/w for acid insoluble, water-soluble and sulfated ash respectively (Table 2).

Extractive Values: The extractive values of Buxus wallichiana wood were found to be 1.7, 2.6 and 3.7 % for cold water, hot water and ethanol respectively (Table 2).

Phytochemical Analysis: From preliminary qualitative phytochemical analysis, the results revealed the presence of alkaloids, carbohydrates and flavonoids for methanol and aqueous extract of *B.wallichiana.* Steroids were present only in pet ether and chloroform extracts (Table 3).

Anatomy of the bark: The bark is wide and measures about 1 mm thick, the bark is differentiated in to outer bark or periderm and inner bark or secondary phloem (Fig. 1). Periderm is 350 µ thick; it consists of radial files of tabular cells with thin suberised walls. Fairly deep irregular fissures are seen in the periderm. The inner bark is broad and homogenous. It consists of small rectangular cells arranged in regular radial files; phloem rays and phloem sclerenchyma are absent. The secondary phloem is 650 µ in radial width (Fig. 2). Intact sieve tube members are seen in the innermost zone of the secondary phloem outer to the intact phloem zone phloem parenchyma cells contain large rhomboidal crystals of calcium oxalate (Fig. 3); these crystals are clearly seen in polarized light microscope, the crystals appear bright (Fig. 4).

Wood (secondary xylem): The wood shows fairly distinct growth rings, the growth rings are marked by narrow thick walled fibers. The wood is diffuse porous with the vessels uniform in size and distribution with in

Table 1. Organoleptic properties of wood, powder and extracts of *B. wallichiana* wood.

Material	Color	Consistency	Taste	Odour
Wood	Yellowish brown	Solid	Bitter	None
Powder	Yellow	Solid	Bitter	Characteristic
Extract				
Pet ether	Brown	Solid	Acrid	None
Chloroform	Brown	Solid	Acrid	None
Methanol	Brownish black	Resinous	Very bitter	Characteristic
Aqueous	Brown	Solid	Bitter	Characteristic

Table 2. Ash values and extractive values (% w/w) of *B. wallichiana* wood.

Type of ash	% yield (w/w)	Method of Extraction	% yield (w/w)
Total ash	1.2	Cold water	1.7
Acid insoluble	1.1	Hot water	2.6
Water soluble	0.4	Ethanol	3.7
Sulfated ash	1.4	-	-

Table 3. Phytochemical analytical test of various extracts of *Buxus wallichiana* wood.

Phytochemical constituents	*Extract of* Buxus wallichiana, *wood*			
	Petroleum ether	Chloroform	Methanol	Aqueous
Carbohydrate			+	+
Alkaloids			+	+
Steroids and sterols	+	+		
Glycosides				
Saponins				
Flavonoids			+	+
Tannins				
Proteins and Amino acids				

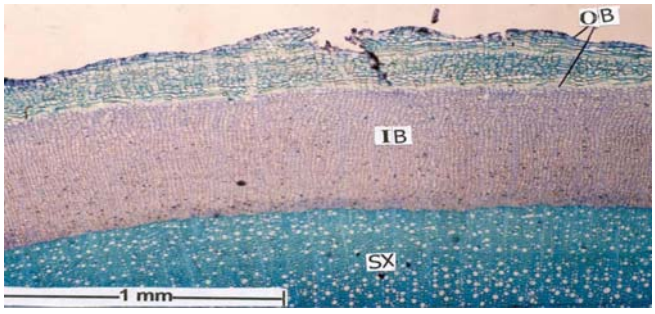

Fig.1. Transverse section of *Buxus wallichiana* (Baill.) bark showing outer and inner bark. (OB-outer Bark; IB-inner Bark and SX - Secondary Xylem).

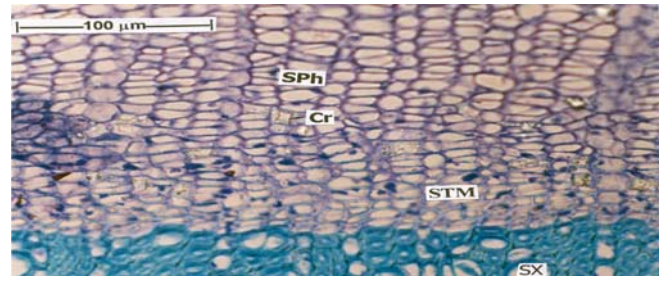

Fig.3. Transverse section of the vascular bundle of *Buxus wallichiana* (Baill.) bark (SPh - Secondary Phloem; SX - Secondary Xylem and STM-Sieve Tube Member).

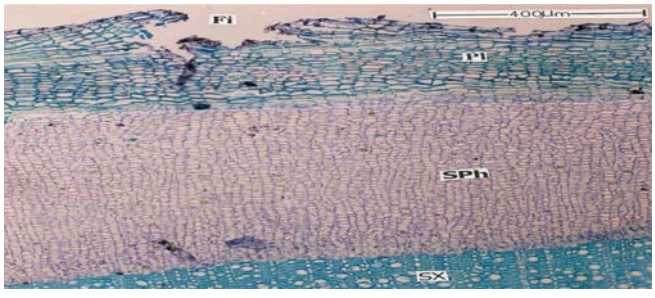

Fig.2. Magnified transverse section of *Buxus wallichiana* (Baill.) bark (Fi-Fissure; PL-Phellogen; SPh - Secondary Phloem and SX-Secondary Xylem).

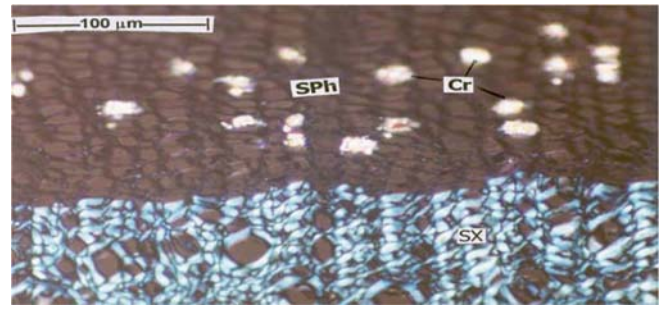

Fig.4. Transverse section of the vascular bundle of *Buxus wallichiana* (Baill.) bark showing calcium oxalate crystals - Cr. (SPh-Secondary Phloem; SX-secondary Xylem).

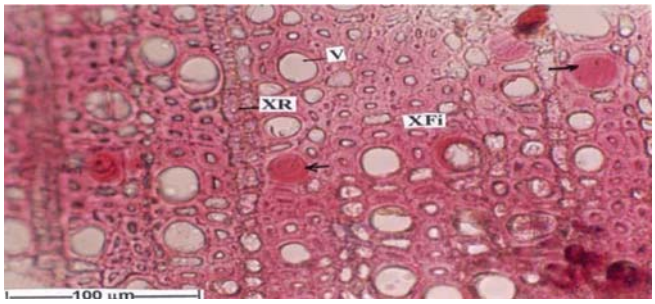

Fig.5. Transverse section of *Buxus wallichiana* (Baill.) wood showing secondary xylem under low magnification. (XR-Xylem Ray; V-Vessel; GR-Growth Ring).

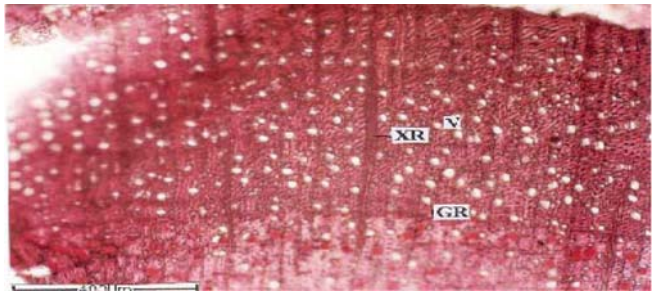

Fig.6. Transverse section of *Buxus wallichiana* (Baill.) wood showing secondary xylem under high magnification. (XR-Xylem Ray; Xfi-Xylem Fibre)

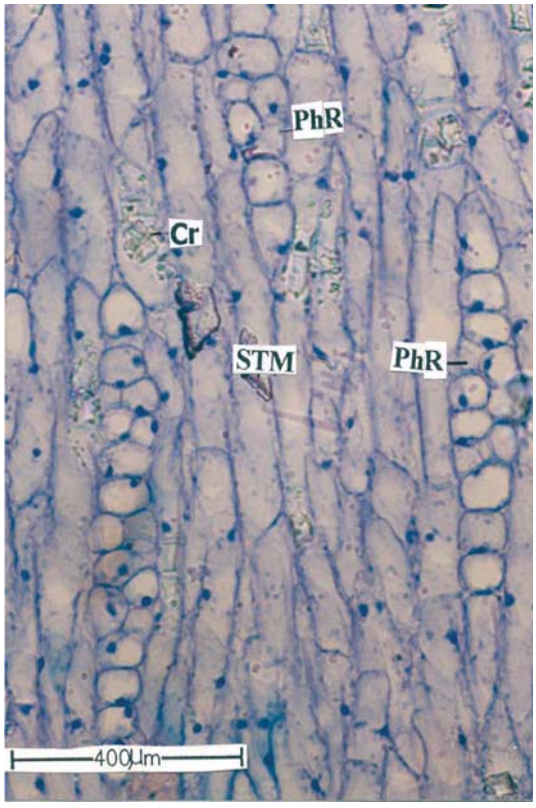

Fig.7. Tangential longitudinal sections of *Buxus wallichiana* (Baill.) wood showing secondary xylem with medullary rays. (PhR - Phloem Ray; Cr-Crystals; STM-Sieve Tube Member).

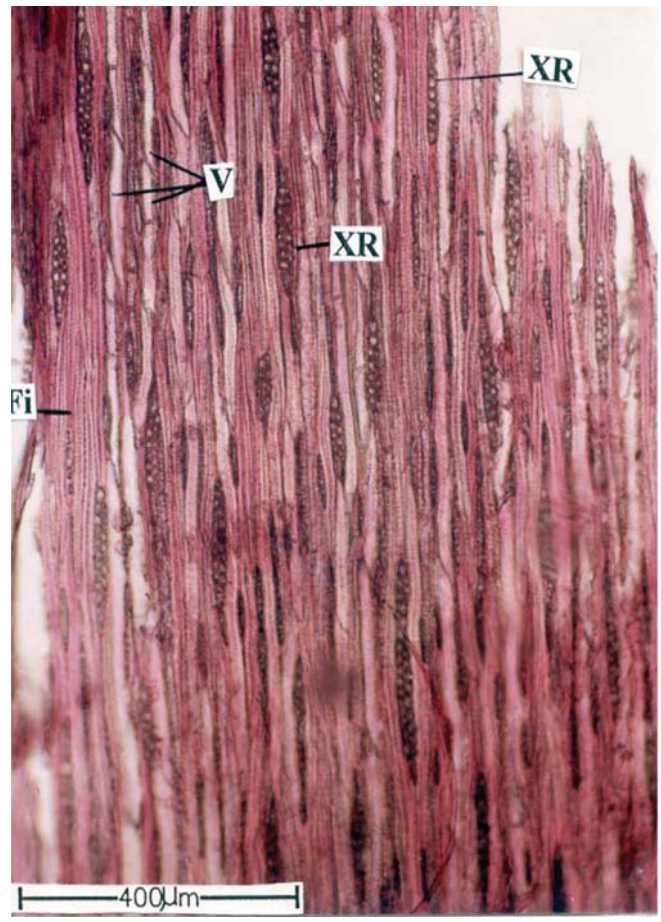

Fig.8. Tangential longitudinal sections of *Buxus wallichiana* (Baill.) wood showing secondary phloem with medullary rays. (XR-Xylem Ray; V-Vessel; Fi-Fibre).

a growth ring. The vessels are circular to elliptical, mostly solitary; vessel walls are thick (Fig. 5). Some of the vessels are filled with dark amorphous gummy substance (Fig. 6). The vessel is 20 µ in diameter; xylem fibers have very thick lignified walls and narrow lumen. In transverse section, the xylem rays appear narrow and straight.

Tangential longitudinal sections of wood and phloem was omitted: In TLS view, the xylem rays appear narrow and light, the xylem rays are biseriate with two vertical rows of cells, and some of the rays are also uniseriate. The rays range from 100-300 µ in height (Fig. 7). Secondary phloem in TLS shows phloem rays, sieve tube members and phloem parenchyma cells. The phloem rays are biseriate in the middle and uniseriate at the ends (Fig. 8). Sieve tube members are narrow and cylindrical; Calcium oxalate crystals are located in the parenchyma cells.

Diagnostic microscopic characters of powder: In the powdered wood and macerated samples the three different types of wood elements were observed (Fig. 9). There are vessel elements, fibers and tracheids. The vessel elements are narrow and cylindrical measuring 400-425 µm long. The lateral walls of the vessel elements have scalariform

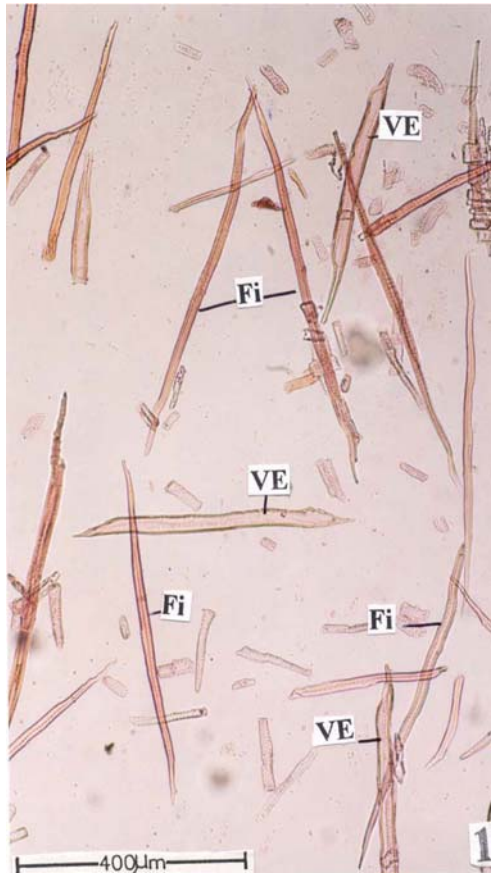

Fig.9. Powder microscopy of *Buxus wallichiana* (Baill.) wood elements under low magnification. (VE-Vessel Elements; Fi-Fibre).

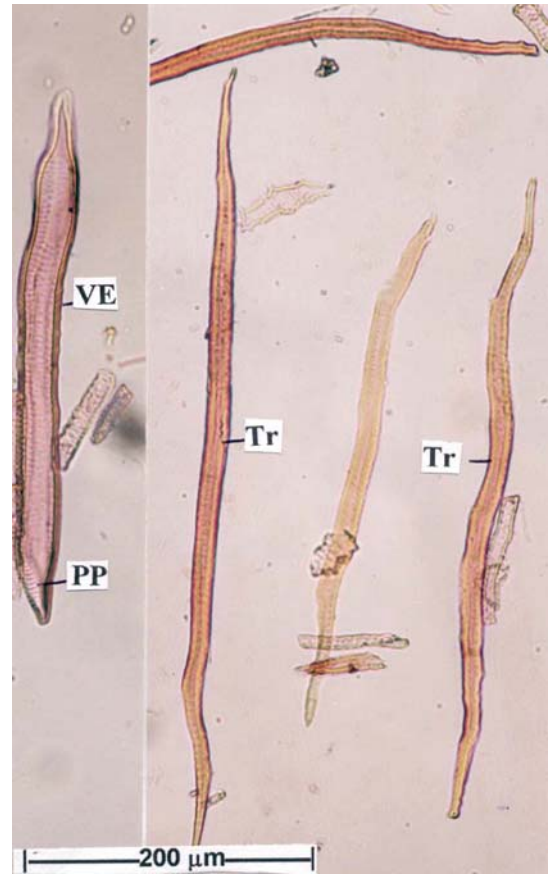

Fig.10. Powder microscopy of *Buxus wallichiana* (Baill.) wood elements under high magnification. (VE-Vessel element; Fi-Fibre; Tr-Trachied; PP-Perforation Plate).

pits; the end walls have scalariform perforation plats. The tracheids are long, narrow and thick walled, they do not have end wall perforation plate, the lateral walls have scalariform thickenings (Fig. 10), the tracheids are 550 µm long. Apart from the tracheids, there are also fibers, which are longer, thicker and narrower than the vessel elements and tracheids, but the fibers do not have lateral wall pits and perforation plates.

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REZIME

Histološka i fizičko-hemijska ispitivanja *Buxus wallichiana* Baill

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Buxus wallichiana Baill pripada familiji Buxaceae. Tradicionalno, *Buxus wallichiana* se koristi kao gorki tonik, dijaforetik, antireumatik, protiv glista, kao antihelmintik, kao analgezično, pirgativno, diuretsko, antiepileptično, antieprotično sredstvo te protiv hemoroida. U ovom radu date su makroskopske, mikroskopske karakteristike i osobine pudera drveta *Buxus wallichiana*, zajedno sa fizičkim parametrima kao što su vrdnosti pepela, vrednosti ekstrakata i rezultati preliminarne fitohemijske analize koja pokazuje prisustvo steroida, alkaloida i flavonoida.

Ključne reči: *Buxus wallichiana,* Buxaceae, antileprotik, purgative, diuretik