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PHARMACOGNOSTICAL STUDY OF STEM AND BARK OF BAUHINIA VAHLII (WIGHT & ARN)

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Abstract

Bauhinia is known by its medicinal effect in the body system as because it is used in many disease for their initial treatment with no such sevier side effect. This research based on the testing of the plant Bauhinia vahlii and its wide analysis. Plant was collected mainly from threeregions of India Uttarakhand (Pithoragarh, Kathgodam, Gathiya). Stem and Bark part of the plant is taken for research. The research elaborates transverse and longitudinal section of this plant. Research include analysis and testing of the plant with addition of it chromatography by the HPTLC. The complete research done by study of steam and bark of the plant Bauhinia vahlii. The result obtains by the research in the constituents of steam and bark clearly shows all the characterization and positive approach towards the health benefit of it. As traditionally it is seen to usage of its leaf in the covering of some edible sweets like singauri because of its extraordinary pharmaceutical benefits which seems in the result came by this research. This creeper like medicinal plant with structurally wide leaf is widely grows in the hilly areas of Uttarakhand by itself.

Keywords: Transverse Sections, Chromatography, Micro-anatomical features, β-Sitosterol, Finger printing profile.

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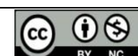

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Introduction

The plants of Bauhinia vahlii is located mostly in the region of high altitude areas of Himalayan belts and is nearly collected from its natural habitats sorrounds to Gethiya.The stem and bark then collected Gethiya(6,500 ft altitude above sea level), Nainital (6,837 ft altitude above sea level) and Pithoragrh (4, 967 ft altitude above sea level), Uttarakhand [1]. The materials were identified at Botanical Survey of India, NRC, and Dehradun for certification of the authenticated plant material under the authority of S.K Srivastva, Scientist E / Deputy Director & Head and have been certified as Bauhinia vahlii [2].

Histological and micro-anatomical features

Transverse Sections and longitudinal section

For anatomical examination of entire stem cut transverse and longitudinal section. For this, soften small pieces of stem without heating in glycerol solution for 1-3 days, depending on their hardness and the section was then cut with the help of razor. First, the thick slices were cut and then from these slice, thin and smaller section were made. The sections were mounted on glass slides in 50% (v/v) glycerin and covered with cover slip. All samples were examined under the microscope and photographs were taken [3].

Transverse and longitudinal section of stem:

1-BHK:

T.S. of stem shows almost quadarangular shape; epidermis is single layered; trichomes abundant in number, apex curved, mostly unicellular, rarely constitutes of two cells; cortex consists of 8 to 15 layers of oval to polygonal, thin walled of parenchymatous cells; cortical cells having prismatic crystals of calcium oxalate; pericycle present in the form of a continuous ring of pericyclic fiberes; phloem composed of usual elements; phloem fiber mostly single or 2 to 3 in groups; xylem consists of usual elements, xylem vessels are round to oval in shape, 2 to 3 in groups and radially arranged; pith composed of oval to polygonal thin walled parenchymatous cells containing rosette crystals of calcium oxalate [4].

Plant collection

Table 1: Details of collection of plants.

S. no.	Name of the Plant	Place of Collection	Accession Number (BSI)
1.	Bauhinia vahlii Wight & Arn	Kathgodam	115897
	Bauhinia vahlii Wight & Arn	Gethiya	115896
3.	Bauhinia vahlii Wight & Arn	Pithoragarh	115898

Transverse section of stem shows simple pitted thickening, medullary rays are not distinct

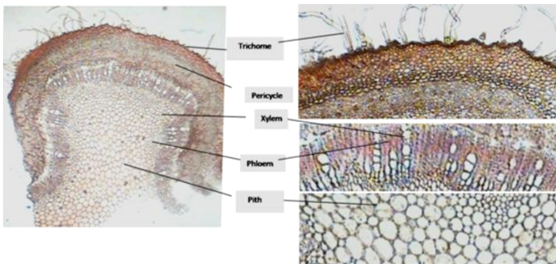


Figure 1: Transverse section of stem and bark of Bauhinia vahlii Kathgodam.

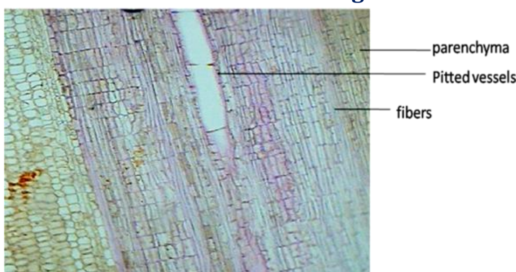


Figure 2: Longitudinal section of stem and bark of (BHK) *Bauhinia vahlii* Kathgodam.

2-BHG:

T.S. of stem shows almost quadarangular shape; epidermis single layered; trichomes apex less curved than BHK, constitutes of two or three cells; cortex consists of 13-15 layers of oval to polygonal parenchymatous cells; a few cortical cells having prismatic crystals of calcium oxalate; pericycle present in the form of a continuous ring; phloem composed of phloem fibers mostly single or 2 to 5 radially arranged in groups; xylem consists of usual elements, xylem vessels are oval to elongated in shape, radially arranged and larger than BHK, rosette crystals are present in pith [5].

L.S. of stem shows less developed medullary rays, 1 or 2 cells wide; simple pitted thickening are present.

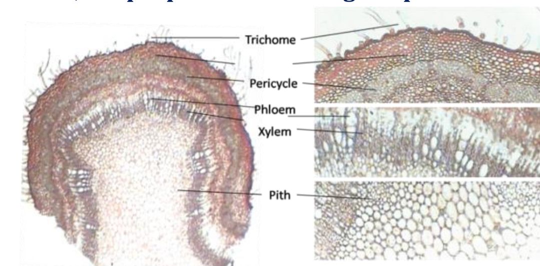


Figure 3: Transverse section of stem and bark of (BHG) *Bauhinia vahlii* Gathiya.

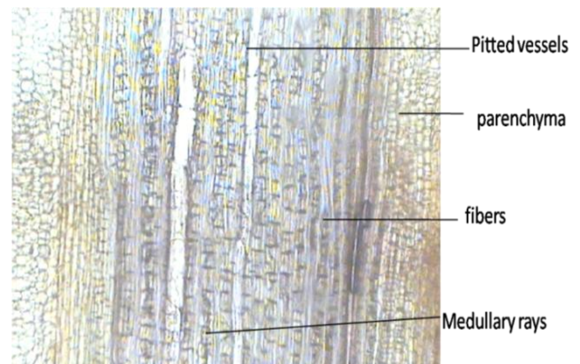


Figure 4: longitudinal section of stem and bark of (BHG) *Bauhinia vahlii* Gathiya

3- BHP:

Fig.: Plant Bauhinia vahlii Pithoragarh

T.S. of stem shows almost quadarangular shape; epidermis single layered; trichomes mostly unicellular; rarely have in two cells, curved in apex, abundant in number; cortex having 15 to 17 layers of oval to polygonal, thin walled parenchymatous cells; a few cortical cells having prismatic crystals of calcium oxalate; pericycle present in the form of continuous ring of pericyclic fibers; phloem composed of usual elements, phloem fibers present in single or 5 to 7 in groups; xylem consists of usual elements, vessels mostly solitary arranged in radial rows; pith composed of oval to polygonal thin walled parenchymatous cells; rosette crystals of calcium oxalate present in pith [6,7].

L.S. of stem shows less developed medullary rays, mostly unisereate; simple pitted thickening are present.

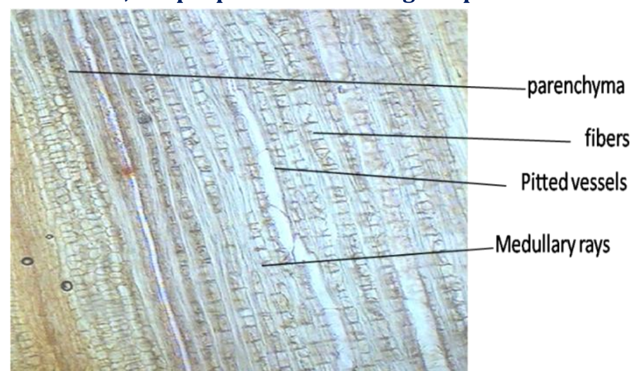


Figure 5: Longitudinal section of stem and bark of *Bauhinia vahlii* (BHP).

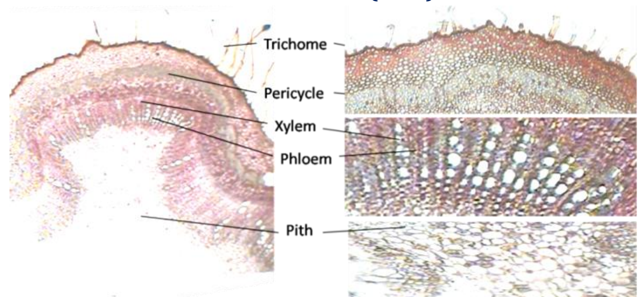


Figure 6: Transverse section of stem and bark of (BHP) *Bauhinia vahlii* Pithoragarh.

Macroscopic Study

In some cases, general appearance of the herb is similar to related species. Detailed study of the morphological characters can be helpful in differentiating them. The macroscopy of a drug includes its visual appearance to the

naked eye. It depends to a large extent on the part of the plant from which the drug is obtained [8]. For stem the following organoleptic parameters were studied: type of stem, colour, surface texture and appearance, odour and taste. For bark colour as seen in bulk, surface condition, fracture, odour and taste were studied [9,10].

Colour: Dark brown
Odour: No characteristic odour
Taste: Not any taste
Shape: Curved
Texture: Rough
Fracture: Fibrous

Powder Study

Further, microscopic examination of epidermal trichomes and calcium oxalate crystals is extremely valuable, especially in powdered drugs the cells are mostly broken expect lignified cells. The cell content such as starch, calcium oxalate crystal aleurone, etc, are scattered in the powder. Some fragments are specific for each powder which may consist of part of cells or group of cells [11].

Powder Miscroscopy

BHG:

Light brown coloured powder, with fibrous texture having no specific odour and taste. Abundant prismatic crystals of calcium oxalate; round to oval, single or in groups having average diameter is 16.875µm; starch grains having 2 to 4 components, average diameter is10.625 µm; stone cells in singles or in groups with wide lumen, fragments of aseptate fibers having narrow lumen & average diameter is16.25 µm.Groups of polygonal cork cell in surface view. The observed diagnostic features are illustrated below [12,13].

BHP:

Light brown coloured powder, with fibrous texture having no specific odour and taste. Abundant prismatic crystals of calcium oxalate having average diameter is17.875 µm; round to oval, single or in groups starch grains having 2 to 4 components, average diameter is8.375 µm; stone cells in singles or in groups with wide lumen, fragments of aseptate fibers having narrow lumen & having average diameter is 13.00 µm; Groups of polygonal cork cell in surface view. The observed diagnostic features are illustrated below [14,15].

BHK:

Light brown coloured powder, with fibrous texture having no specific odour and taste. Abundant prismatic crystals of calcium oxalate having average diameter is16.25 µm; round to oval, single or in groups starch grains having 2 to 4 components, average diameter is11.375 µm; stone cells in singles or in groups with wide lumen, fragments of aseptate fibers having narrow lumen & having average diameter is 12.625 µm; Groups of polygonal cork cell in surface view. The observed diagnostic features are illustrated below [16,17].

Fluorescence analysis:

Fluorescence of powder of stem and bark of the Bauhinia plant was observed in day light and in UV light under the wave length of 254 and 366 nm. The drug powders were treated with different solvents in different slides. The solvents used were 1N sodium hydroxide (aqueous), conc. hydrochloric acid, conc. sulphuric acid, conc. nitric acid, acetic acid, iodine, 50% potassium hydroxide and 1N sodium hydroxide (alcoholic). They were subjected to fluorescence analysis in day light and in UV light [18,19].

Table 2: Estimation of Fluorescent Analysis of different sample of stem and bark of *Bauhinia vahlii.*

S. No.	Sample	Treatment	Visible Light	254 nm	366 nm
1(i)	BHK	Powder+ Ethnolic NaOH	Light brown	Dark greenish Brown	Dark brown
(ii)		Powder+ Aq. Solution of NaOH	Brown	Greenish blue	Greenish blue
(iii)		Powder+HNO3	Dark orangish brown	Dark brown	Blakish blue
(iv)		Powder + HCl	Purplish Brown	Light purple	Light purple
2 (i)	BHG	Powder+ Ethanolic NaOH	Brown	Light greenish Brown	Light brown
(ii)		Powder+ Aq. NaOH	Dark brown	Yellow at the edge and brown at centre	Dark greenish blue
(iii)		Powder+HNO3	Orangish Brown	Light brown	Light brown
(iv)		Powder + HCl	Light purplish brown	Dark purple	Dark purple
3(i)	BHP	Powder+ Ethanol	Dark Brown	Dark greenish brown	Light orangish brown
(ii)		Powder+ Aq. NaOH	Light Brown	Light greenish blue	Light greenish blue
(iii)		Powder+HNO3	Light Brown	Dark Brown	Blackish Brown
(iv)		Powder + HCl	Dark Purplish Brown	Purple	Purple

Determination of Foreign Matter

Weighed 100–500 g of the drug sample to be examined or the 1-2g quantity taken and spread it out in a thin layer in a suitable dish or tray. The foreign matter was detected by inspection with the unaided eye or by the use of a lens (10 x). Separated and weighed it and calculated the percentage present [2.]

Table 3: Foreign Matter

Sr.no.	Sample	Foreign Matter
1	BHK	Not more than 1%
2	BHG	Not more than 1%
3	BHP	Not more than 1%

Extractive value

Extractive value is a measure of the content of the drug extracted by solvents. Extractive value can be water soluble, ethanol soluble and ether soluble extractives. Extractive value is unless and otherwise prescribed, carried out by maceration [21].

Water soluble extractive:

Water soluble Extractive Value of different sample of Bauhinia vahlii stem and bark is tabulated in the Table 4.

Table 4: Water soluble extractive value.

Sr. no.	Sample	Rang(percent w/w)	Mean(percent w/w)*
1	BHK	6.51-6.61	6.58
2	BHG	6.76-6.97	6.90
3	BHP	7.22-7.47	7.43

* mean value of three readings

Alcohol soluble extractive: Procedure followed by the reference of (Anonymous, 2008)

Alcohol soluble Extractive value different sample of Bauhinia vahlii

Table 5: Alcohol soluble extractive value.

Sr. No.	Sample	Range(percentage w/w)	Mean(percentage w/w)*
1	BHK	13.45-13.7	13.65
2	BHG	12.69-12.92	12.89
3	BHP	12.66-12.87	12.78

* mean value of three readings

Table 6: Total moisture percentage.

Sr. no.	Sample	Range(percentage w/w)	Mean(percentage w/w)*
1	BHK	1.95-2.09	2.05
2	BHG	2.58-2.86	2.77
3	BHP	2.48-2.67	2.56

* mean value of three readings

Ash Values

Ash value is an important characteristic of a drug and with the help of this parameter we can detect the extent of adulteration as well as establish the quality and purity of the drug. There is a considerable difference in the ash values of different drugs but mostly the difference varies within narrow limits in case of the same drug. The acid insoluble ash is the part of the total ash, which is insoluble in dilute hydrochloric acid. It consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthly material. The water-soluble ash is the part of total ash, which is soluble in hot water. It is used to estimate the amount of inorganic elements [22].

Total ash: About 2g of the powdered drugs were accurately weighed in a clean, dried, pre-weighed silica crucible. The crucible was incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled in desiccators and weighed. The procedure was repeated till a constant weight was observed [23]. The percentage of the total ash was calculated in triplicate with reference to the airdried drug total ash was calculated by using this formula:

Total ash %=(Fw- Pw)x100/w

Where, Fw = Final weight of crucible with total ash; Pw = Pre weight of crucible; w = Total weight of powdered plant material. **(Anonymous, 2008)**

Percent Ash value of different sample of Bauhinia vahlii stem and bark is tabulated in the table below.

Table 7: Total ash.

Sr. no.	Sample	Range(% w/w)	Mean(% w/w)
1	BHK	3.76-4.05	3.87
2	BHG	3.44-3.78	3.64
3	BHP	3.82-4.07	4.05

* mean value of three readings

Acid insoluble ash: The ash obtained as described in the determination of total ash was boiled in a crucible with 25 ml of 6N hydrochloric acid for 5 min. The insoluble ash was collected on an ash less filter paper by filtration and it was washed with hot distilled water unless normal pH was reached. The insoluble ash was transferred into a tared silica crucible, ignited at a temperature not exceeding 450°C, cooled and weighed. The procedure was repeated until a constant weight was observed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug [24].

Acid Insoluble Ash % = (FwII - FwI) x 100/w

Where FwII= Final weight of crucible with acid insoluble ash; FwI = Preweight of crucible; w = Total weight of powdered plant material. **(Anonymous, 2008)**

Percent Acid insoluble ash value of different sample ofBauhinia vahlii stem and bark is tabulated in the table below.

Table 8: Acid Insoluble ash.

Sr.no.	Sample	Range	Loss on drying (%)*
1	BHK	0.70-0.82	0.79
2	BHG	0.75-0.89	0.83
3	BHP	0.69-0.80	0.77

*mean value of three readings

Water soluble ash Procedure followed by the reference of (Anonymous, 2008)

Percent water soluble ash value of different sample of Bauhinia vahlii.

Table 9: Water soluble ash.

Sr. no.	Sample	Range(% w/w)	Mean (% w/w)*
1	BHK	1.62-1.90	1.82
2	BHG	0.75-0.92	0.87
3	BHP	1.95-1.15	1.09

*mean value of three readings

Chromatography: HPTLCFinger Printing Profile

A Cammag (Switzerland) HPTLC system equipped with a sample applicator Linomat V, Twin trough glass Chamber (20x10 cm2) with SS lid, TLC Scanner III, Reprostar III and Wincats an integrated Software 4.02 (Switzerland),

Rotavapour was used for qualitative and quantitative analysis of constituents mixture [25].

Chemical & Reagents

Analytical grade; Toluene, ethyl acetate, Formic acid, Chloroform, Methanol, Alcohol, Anisaldehyde and Sulphuric Acid were used; obtained from S.D. Fine Chem. Ltd. (Mumbai, India). HPTLC Aluminium pre coated plate with Silica gel 60 GF254 (20x10 cm2; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India) and Reference standard betasitosterol, quercetin, lupeol, rutin, serulicacid, galicacid and ferulic acid.

Sample preparation: 1g of coarsely powdered crude drugs was extracted with 10 ml Methanol for 24 hours by cold extraction method. The extracts were filtered by Whatmann no. 42 filter paper and make up to 10 ml in a volumetric flask and used for H.P.T.L.C.

Procedure

HPTLC Aluminium pre coated plate with Silica gel60 GF254 (20x10 cm2; 0.2 mm thick) was used with Toluene: Ethyl acetate: Formic acid (5:4:1) v/vas mobile phase. Methanolic extract of samples and β- Sitosterol standard solution applied on plate by using Linomat V applicator. Cammag Twin TroughGlass Chamber (20x10 cm2) with SS lid was used for development of HPTLC plate. The Twin Trough Glass Chamber was saturated with mobile phase for 30 minutes. HPTLC plate was developed to 8 cm distance above the position of the sample application. The plate was removed from the chamber and air dried at room temperature. This plate was sprayed (derivatized) with 0.5% Anisaldehyde – Sulphuric acid reagent and HPTLC finger print profile was snapped by CammagReprostar III, before deivatization under UV 254 nm, 366 nm and after derivatization. The plate was scanned after derivatization using Camag TLC Scanner III at wavelength 550nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data [26].

Table 10: HPTLC details of Methanolic extract of Bauhiniavahlii Stem and bark.

Sr. No.	Detection/ Visualization	*Bauhinia vahlii*stem and bark (Track T1, T2 & T3)		Standard- β- Sitosterol (Track S1, S2 & S3)	
		Rf. values	Colour of band	Rf. Values	Colour of band
1.	Under UV 254 nm	0.55 0.61 0.65 0.95	grey grey grey dark grey	-	-
2.	Under UV 366 nm	0.11 0.34 0.45 0.51 0.57 0.80 0.95	sky blue sky blue sky blue sky blue bright sky blue green sky blue	-	-
3.	After derivatization	0.31 0.51 0.61 0.65 0.75 0.95	light violet light violet violet violet dark violet violet	0.75	dark violet

Linearity of Detector Response and Assay:

In order to establish linearity, standard solution of β- Sitosterol (1mg/ml) applied on HPTLC Aluminium pre coated plate with Silica gel60 GF254 (20X10 cm2; 0.2 mm thick), 2ml, 4ml, 6ml on Track No. S1, S2 & S3 respectively and for assay, 12ml of Methanolic extract of samples applied on Track No.BHP, BHG & BHK on the same plate. HPTLC plate was developed to 8 cm distance above the position of the sample application and removed from the chamber and air dried at room temperature. This HPTLC finger print profile was snapped by CammagReprostar III, before derivatization under UV Light 254 nm, 366 nm and after derivatization. The plate was scanned immediately after derivatization using Camag TLC Scanner III at wavelength 550nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data. It was observed that β- Sitosterol appeared at Rf. 0.75 (dark violet colour)12. The peaks, graph and spectra obtained were given in Fig. 2 and 3 and Rf. values, colour of bands, quantity of β- Sitosterol, linearity, standard deviation & regression coefficient found via graph.

Table 11: Quantity applied on plate and values found via graph.

Sr. No.	Track No.	Volume applied on plate	Quantity applied on plate	Quantity of β-Sitosterol via graph	Linearity & Regression Coefficient and Standard deviation via graph
1.	T1	12µl	1200µg	2.212µg	Y = 13282.449 + 2806.067 * X r = 0.99520 sdv = 3.18%
2.	T2	12µl	1200µg	2.292µg	
3.	S1	2µl	2µg	2.000µg	
4.	S2	4µl	4µg	4.000µg	
5.	S3	6µl	6µg	6.000µg	
6.	T3	12µl	1200µg	2.497µg	

S1-β-Sitosterol Std. alcoholic solution(1mg/ml) T1-Methonolic extract of BHP
S2-β- Sitosterol Std. alcoholic solution(1mg/ ml) T2-Methonolic extract of BHG
S3-β-Sitosterol Std. alcoholic solution (1mg/ml) T3-M

ethonolic extract of BHK

T1 T2 S1 S2 S3 T3

UV-366 nm

T1 T2 S1 S2 S3 T3

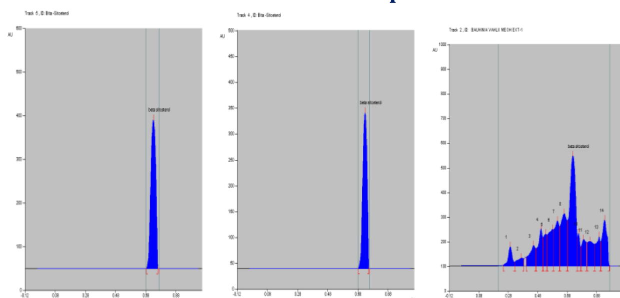
UV-366 nm

T1 T2 S1 S2 S3 T3

UV-254 nm

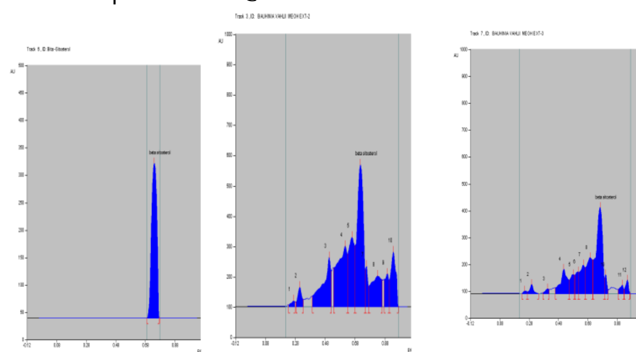
After derivatization

Figure 7: H.P.T.L.C. Finger print of Bauhinia vahlii stem and bark sample.



(Track S1) (Track S2) (Track S3)

Peaks of β- Sitosterol @ 550nm



(Track T1) (Track T2) (Track T3)

Peaks of *Bauhinia vahlii stem* and bark @ 550nm

Figure 8: Peaks of *Bauhinia vahlii* in all Tracks.

Table 12: Percentage of β- Sitosterol present in different sample of *Bauhinias vahlii.*

Sr. No.	Sample from →	BHP	BHG	BHK
1.	Quantity of β-Sitosterol in 1g	1.843mg	1.910mg	2.080mg
2.	% β-Sitosterol	0.1843%w/w	0.1910%w/w	0.2080%w/w

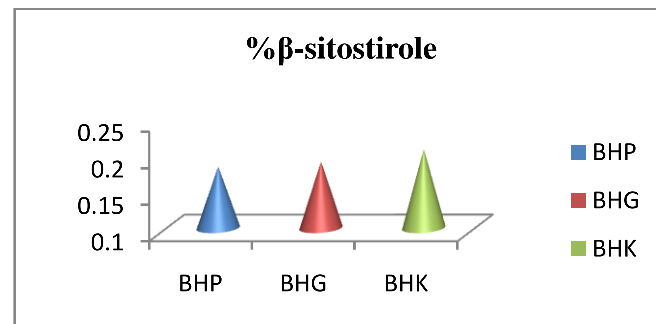


Figure 9: Comparative percentage of β-Sitosterol present in deferent sample of Bauhinia vahlii stem and bark.

Conclusion

Bauhinia Vahlii is used as a traditional medicine in across the himalayan regions and specially in Uttarakhand. There is a reason behind its usage and it is its various health benefits revealed in this research. Research include analysis and testing of the plant with addition of it chromatography by the HPTLC. The complete research done by study of steam and bark of the plant Bauhinia vahlii. The result obtain by the research in the constituents of steam and bark clearly shows all the characterization and positive approach towards the health benefit of it. As traditionally it is seen to usage of its leaf in the covering of some edible sweets like singauri because of its extraordinary pharmaceutical benefits which seems in the result came by this research.

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