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**PHYTOCHEMICAL ANALYSIS OF THE BHARANGI ROOTS - *CLERODENDRUM
SERRATUM* (L.) COLLECTED FROM WAYNAD AND PUNJAB**

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ABSTRACT

Bharangi—*Clerodendrum serratum* (L.) is a medicinal plant that is used to treat *Shwasa*-bronchial asthma, *Kasa*-cough, *Shotha*-oedema, and *Jwara*-fever. It has a hot potency that balances Kapha and Vata. To obtain specific drug action and photochemical constituents, location of collection, the season of collection, and the time of collection need to be considered. This study analyzed and compared Bharangi root—*Clerodendrum serratum* (L.) collected from Waynad, Kerala (Sample A), and Punjab (Sample B). In both Samples A and Sample B, the difference in loss on dry (0.573), total ash (0.361), alcohol soluble extract (0.802), and water-soluble extract (2.648) was observed, which were within the standard limits. More drug concentration was observed at UV 254 and 366 nm in Sample A, where as in sample B was observed at UV 660 nm in HPTLC. This could be because of the collection's location and

season. Water extraction was higher in the Sample A which is an Anupa Desha, while alcohol extraction was higher in Sample B which is an Jangala Desha.

Keywords: Bharangi Moola, *Clerodendrum serratum* (L.), Asthma, HPTLC, Desha, Anupa, Jangala

INTRODUCTION

Bharangi—*Clerodendrum serratum* (L.) is a member of the Verbenaceae family. It is one of the most important medicinal plants used in the Indian System of Medicine for the treatment of respiratory diseases. It is a perennial shrub that grows in deciduous forests, peninsular India, and the Western and Eastern Himalayas at elevations of up to 1,500 metres. Mature roots are hard, woody, and cylindrical, with a light brown external surface and elongated lenticels. Ayurvedic drug Bharangi (*Clerodendrum serratum* Linn.) has a hot potency that balances both the Kapha and Vata.¹ It helps with Shwasa-bronchial asthma, Kasa-cough, Sotha-oedema, and Jwara-fever. To obtain specific drug action, the location of collection, the season of collection, and time of collection must be considered. The analysis and comparison of Bharangi root—*Clerodendrum serratum* (L.) collected from Waynad, Kerala (Sample A), and Punjab (Sample B) were performed in this study.

MATERIALS AND METHOD

Collection of the plant samples

Two samples of Bharangi Moola roots were obtained from a Kozhikode pharmacy, Wayanad Kerala, (Sample A), and Punjab, Amrut Kesari Bangalore (Sample B). After receiving the samples raw drug was authenticated by the Department of Dravya Guna- Ayurveda Pharmacology.

Method of Analysis

Loss on drying was observed at 105°C by drying at 105°C for 5 hours in hot air oven. Total Ash was obtained by incinerating in a crucible at temperature not exceeding 450°C. Acid insoluble Ash was obtained by adding 25ml of dilute HCl and by drying on a hot plate. Alcohol soluble extract was obtained by adding 100 ml of distilled Alcohol (approximately 95%). After Shaking occasionally for 6 hours, allowing it to stand for 18 hours and Keeping it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Water soluble extract was by adding 100 ml of distilled water, and drying in a hot air oven at 105°C for 6 hours. Determination of pH was done by dissolving one tablet of pH 4, 7 and 9.2 in 100 ml of distilled water. Refractive index, Specific

gravity, Viscosity of the sample also assessed.

Following Preliminary phytochemical screening in both the samples of Bharangi moola was carried out (Table 1)

Table 1

Constituents	Tests	Alcoholic extract
Alkaloids	Dragendroff's test	Orange red precipitate
	Wagners test	Reddish brown precipitate
	Mayers test	Dull white precipitate
	Hagers test	Yellow precipitate
Steroids	Liebermann- buchard test	Bluish green colour
	Salkowski test	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer
Carbohydrate	Molish test	Violet ring
	Fehlings test	Brick red precipitate
	Benedicts test	Red precipitate
Tannin	With FeCl ₃	Dark green color
Flavanoids	Shinoda's test	Light Pink color
Saponins	With NaHCO ₃	Stable froth
Triterpenoids	Tin and thionyl chloride test	Faint pink color
Coumarins	With 2 N NaOH	Yellow color
Phenols	With alcoholic ferric chloride	Dark green color
Carboxylic acid	With water and NaHCO ₃	No effervescence
Amino acid	With ninhydrine reagent	No purple color
Resin	With aqueous acetone	No turbidity
Quinone	Conc. sulphuric acid	Yellow color

Analysis of Raw Drug was done using HPTLC

One gram of Bharangi moola root powder was dissolved in 10 mL ethanol and cold percolated for 24 hours before being filtered. 3, 6, and 9l of the above samples were applied to a pre-coated silica gel F254 on aluminium plates with a Linomat 5 TLC applicator to a band width of 7 mm. Toluene: Ethyl acetate was used to create the plate (9.0: 1.0). The developed plates were visualised under short and long UV, then derivatized with vanillin sulphuric acid reagent and scanned under UV 254, 366, and 620nm, with Rf, spot colour, and densitometric scans recorded.

OBSERVATION AND RESULT

Root of the Bharangimoola Consists of loss on dry in Sample A -9.788% and in Sample B 9.788%. In Sample A 2.451% and in Sample B 2.09% of total ash was observed. In Sample A 6.258% and in Sample B 7.06% alcohol soluble extract was observed. In Sample A 20.078 % and in Sample B 17, 43% water soluble extract were observed (Table 2).

Preliminary analysis revealed the presence of alkaloids, steroids, carbohydrates. Tannin, Flavonoids, Saponins, Terpenoid, and Coumarins in both the sample (Table 3).

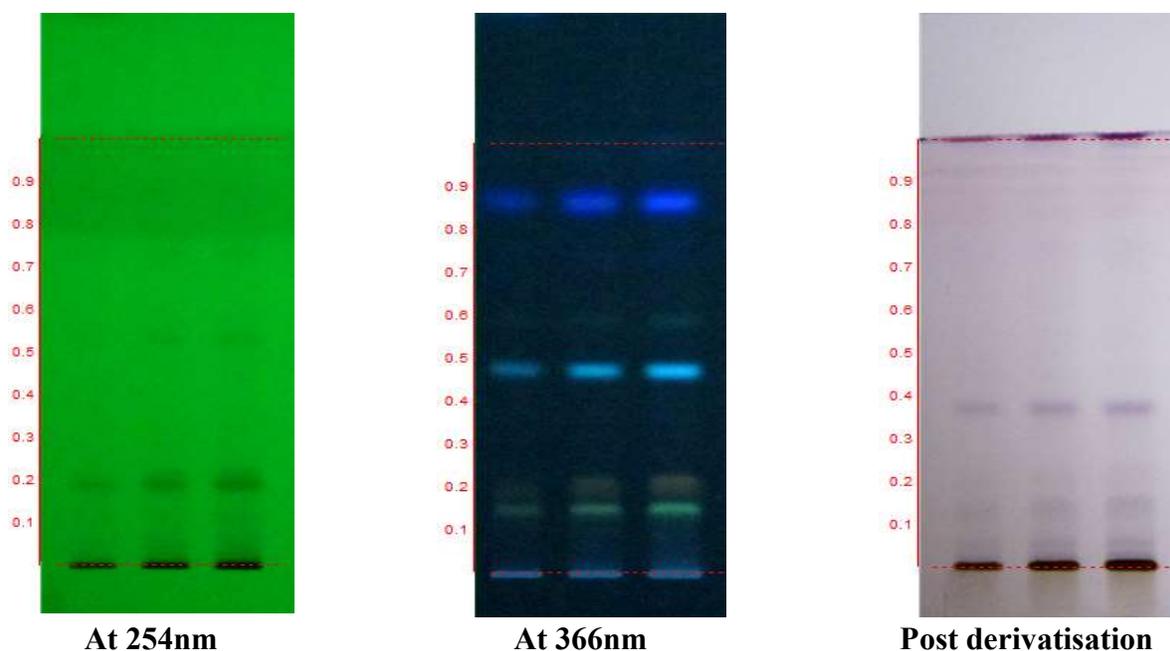
Table 2: Standardization parameters

Parameters	<i>Bharangi moola</i> Sample B	<i>Bharangi moola</i> Sample A
	Results n = 3 %w/w	Results n = 3 %w/w
Loss on drying	9.215 ± 0.015	9.788 ± 0.012
Total ash	2.09 ± 0.007	2.451 ± 0.003
Acid insoluble ash	0.0 ± 0.0	0.0983 ± 0.01
Water soluble ash	0.60 ± 0.01	-
Alcohol soluble extractive	7.06 ± 0.01	6.258 ± 0.01
Water soluble extractive	17.43 ± 0.01	20.078 ± 0.01

Table 3: Results of preliminary phytochemical screening

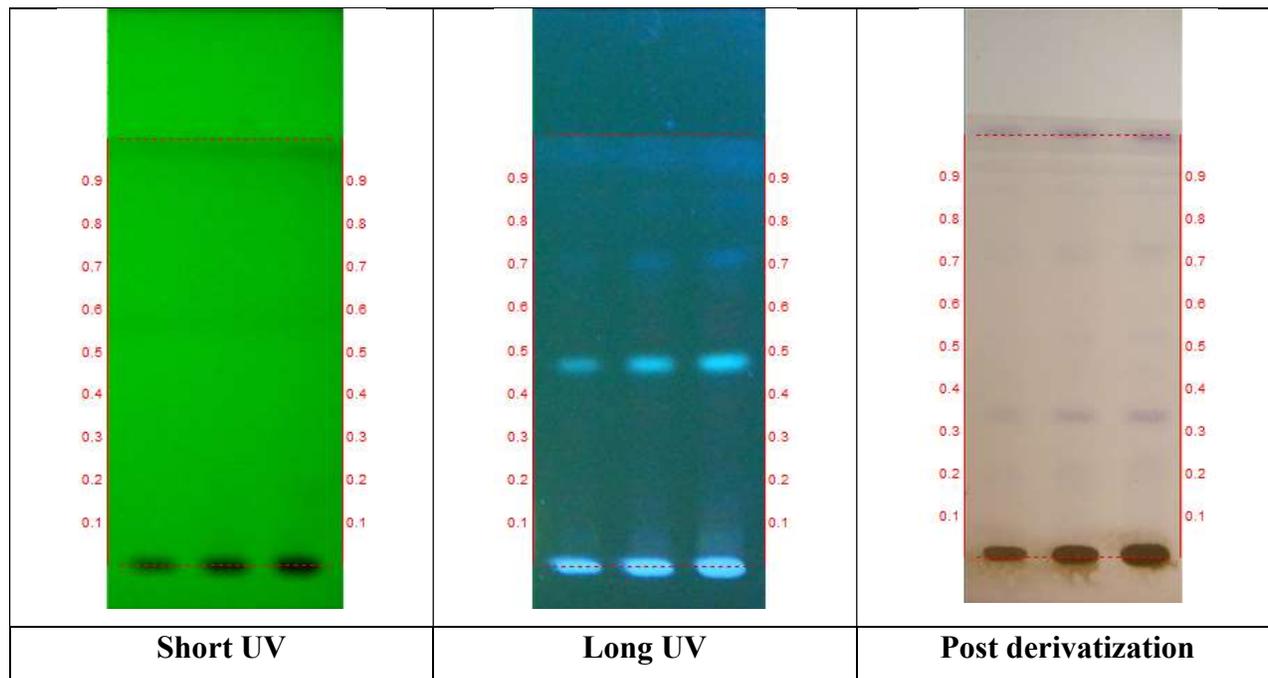
Test	<i>Bharangi moola</i> Sample B	<i>Bharangi moola</i> Sample A
	Inference	Inference
Alkaloid	+	+
Steroid	+	+
Carbohydrate	+	+
Tannin	+	+
Flavanoids	+	+
Saponins	+	+
Terpenoid	+	+
Coumarins	+	+
Phenols	-	-
Carboxylic acid	-	-
Amino acids	-	-
Resin	-	-
Quinone	-	-

present; (-) – negative(+)



Track 1: *Bharangi moola*– 3 µl; Track 2– *Bharangi moola* – 6 µl; Track 3– *Bharangi moola* – 9 µl
Solvent system: Toluene: Ethyl Acetate (9:1)

Figure 1: HPTLC Photo documentation of sample of *Bharangi moola* Sample A



Track 1: *Bharangi moola* - 3 μ l, Track 2: *Bharangi moola* - 6 μ l, Track 3: *Bharangi moola* - 9 μ l
Solvent system: Toluene: Ethyl acetate (9:1)

Figure 2: HPTLC Photodocumentation of sample of *Bharangi moola* Sample B

Table 4: R_f values of the sample *Bharangi moola* Raw drug Sample A

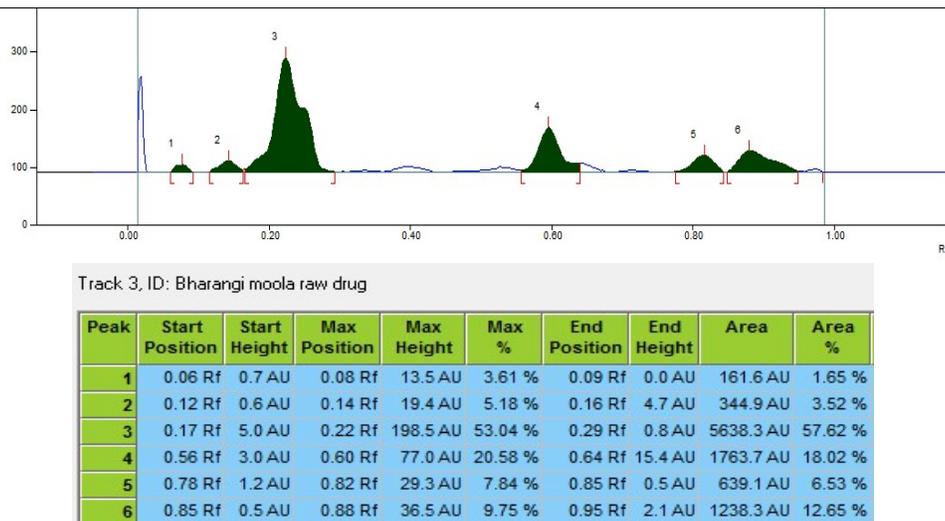
At 254nm	At 366nm	Post derivatisation
-	-	0.07(L Violet)
0.20(Green)	0.16(F Green)	0.16(L Violet)
-	0.23(F Brown)	-
-	-	0.38(L Violet)
-	0.48(F Violet)	-
0.54(L Green)	-	-
-	0.60(F L Violet)	-
0.74(L Green)	0.74(F L Violet)	-
-	0.88(F D Blue)	-

*L-Light,D-Dark,F-Fluorescence

Table 5: R_f values of sample of *Bharangi moola* Sample B

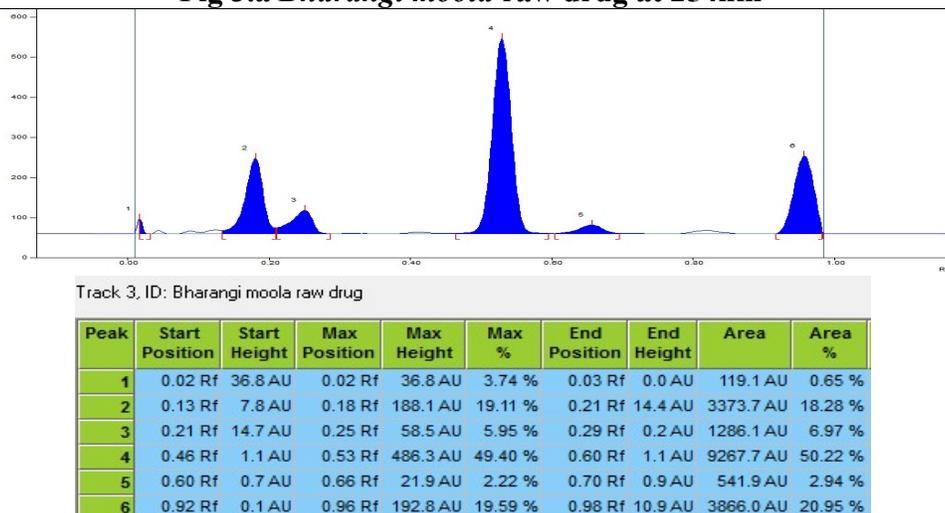
At 254nm	At 366nm	Post derivatisation
-	-	0.23 (Purple)
-	0.48 (F. aqua blue)	-
-	-	0.53 (Purple)
-	0.72 (F. blue)	0.72 (Purple)
-	0.84 (F. blue)	-

*F - fluorescent



Six peaks were detected during densitometric scan of Bharangi root at 254nm and it was found that peak 3rd had higher concentration of chemical composition covering an area of 53.04%

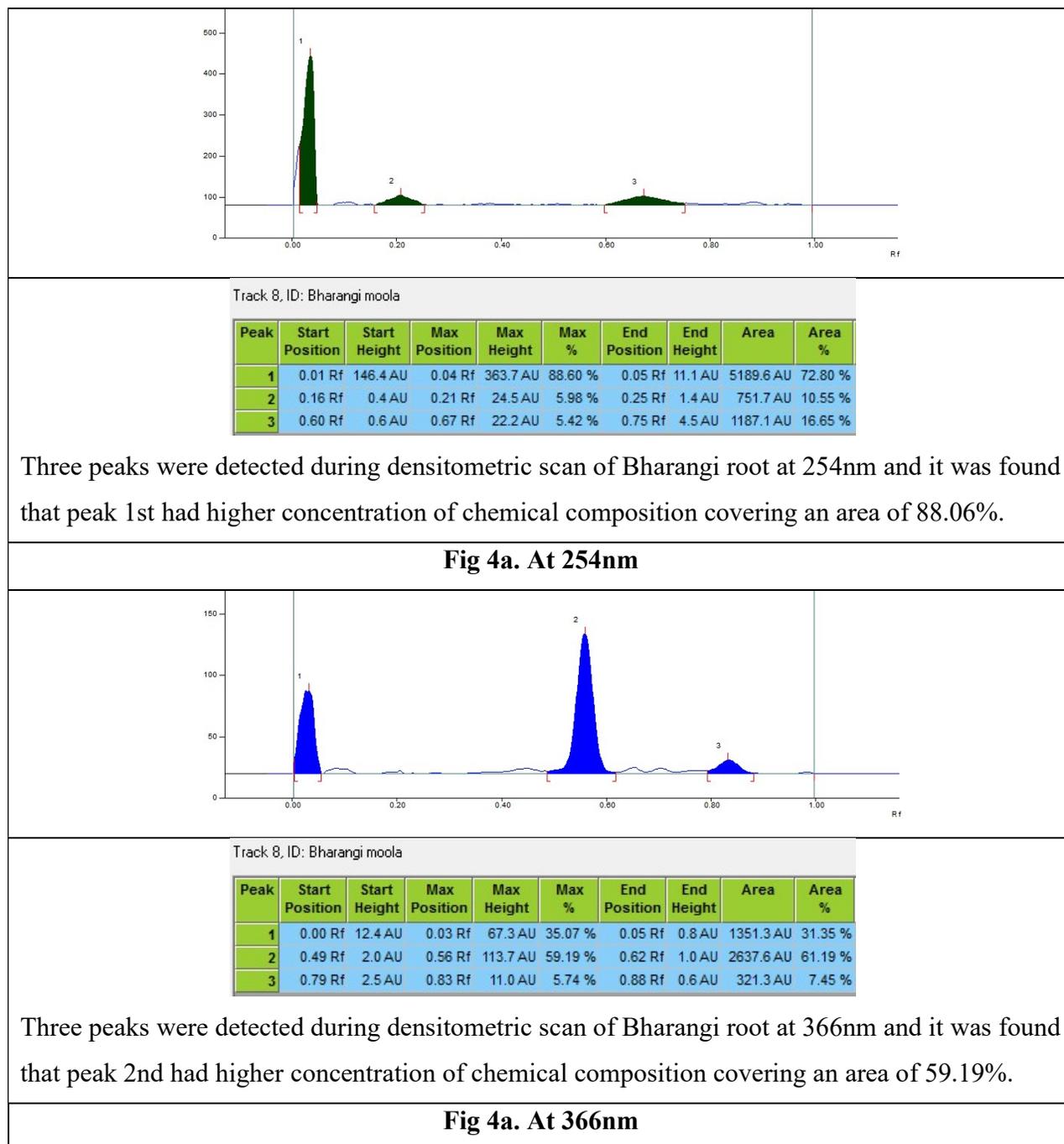
Fig 3.a Bharangi moola raw drug at 254nm

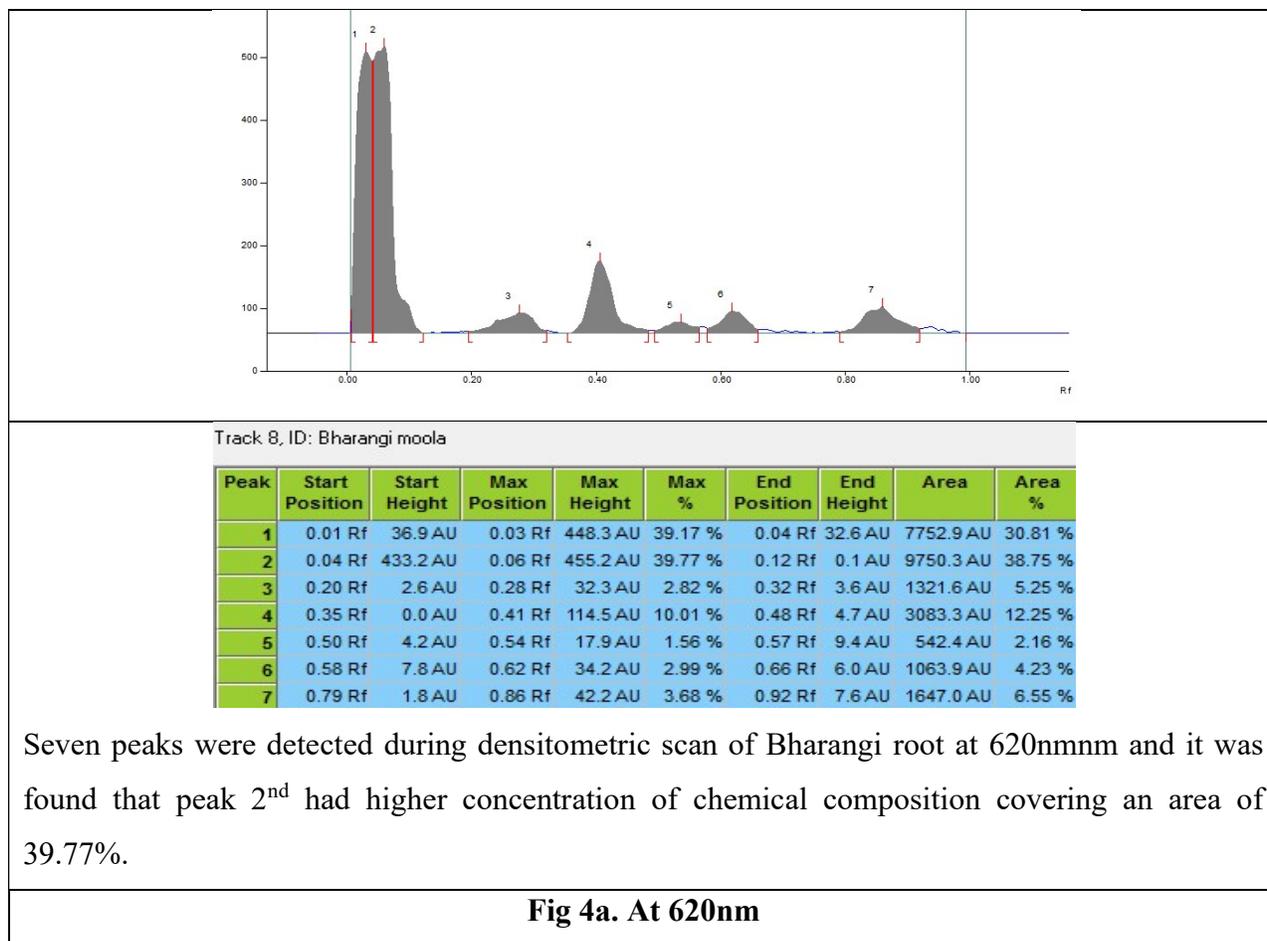


Six peaks were detected during densitometric scan of Bharangi root at 366nm and it was found that peak 4th Peak had higher concentration of chemical composition covering an area of 49.40%.

Fig 3.b Bharangi moola raw drug at 366nm

Figure 3: Densitometric Scan of the sample Bharangi moola raw drug Sample A



Figure 4: Densitometric scan of the sample of *Bharangi moola* Sample B

DISCUSSION

Root of the Bharangi consists of loss on dry in Sample A -9.788% and in Sample B 9.215% which determine the moisture content of a drug. It aids to prevent the decomposition of the drugs either due to chemical change or microbial contamination. In Sample A 2.451% and in Sample B 2.09% of total ash were observed. The ash value is useful in determining the authenticity and purity of a sample, and it is also an important qualitative standard. This percentage clearly

shows that the root is the most effective for drug action and effects. In Sample A 1.258% and in Sample B 2.06% alcohol soluble extract was observed respectively. Similarly in Sample A 8.078 % and in Sample B 5.43% water soluble extract was observed which were in accordance with the standard limits. The presence of Alkaloid, Steroid, and Carbohydrate was discovered during preliminary analysis. Tannin, flavanoids, saponins, terpenoids, and coumarins were found in both samples. Bharangi roots have

shown 6.8091% Carbohydrates were discovered during preliminary testing. D-mannitol was commonly found in the root. Terpenes isolated from plants in other studies include betulin, oleanolic acid, clerodermic acid, betulinic acid, friedelin, and monomelittoside.

Previous research has revealed that the root contains Saponins are Stigmasterol, oleanolic acid, Queretaroic acid, Serratagenic acid, and other compounds. Sitosterol, Clerosterol (also known as 5, 25-stimastadien-3 o), Clerodone (3-hydroxyl- lupan-12-one), B-sitosterol, Lupeol, A steroidal glycoside Phytosterols, Ferulic acid, Arabinose, Scutellaricin, Baicalein, Serratin, and Ursolic acid.² Phenolic compounds have been widely reported, with phenyl propanoids and flavonoids being the most common [3].

The anti-inflammatory action of phenolic compounds, tannins, and terpenoids in asthma has previously been demonstrated [4]. The ability of phenolic compounds to modulate the expression of pro-inflammatory genes such as NOS, cyclooxygenase, and lipoxygenase, as well as acting through nuclear factor (NF- κ B) signalling and mitogen-activated protein kinase and activating the Nrf2 / Keap1 pathway, is linked to their anti-inflammatory effects [5]. At concentrations of 50, 100, and 200 mg/kg,

the ethanolic root extract of *C. serratum* demonstrated significant anti-inflammatory activity in carrageenan-induced oedema in rats, as well as in the cotton pellet model in experimental mice, rats, and rabbits [6]. Terpenoids are also responsible for anti-inflammatory activity; however, they can occasionally cause allergic reactions. Icosahdropicenic Acid (IHPA), a new pentacyclic triterpenoid saponin, was also isolated from Bharangi roots for the first time. IHPA at 100mg/kg showed significant protection against mast cell degeneration (59.62%) when compared to standard sodium cromoglycate (64.48%). The compound also inhibited histamine-induced gout tracheal chain preparation significantly [7].

Saponin and D-mannitol present in Bharngi Moola produce antihistamine and antiallergic effects, respectively. Apigenin-7-glucoside(flvaonide) acts as an anti-inflammatory and antimicrobial agent [8].

In Ayurveda, Also Studies are conducted to establish the various pharmacological action of various form of bharangimoola in various diseases. In comparison to Salbutamol Sulphate, Bharangimoola Arka was found to have a more sustained effect in reducing bronchial asthma in the acute stage, with no adverse effects such as tremor or mouth dryness [9, 10].

In Sample A, six peaks were detected during a densitometric scan of Bharangi root at 254nm, with peak 3rd having a higher concentration of chemical composition covering an area of 53.04%, and six peaks were detected during a densitometric scan of Bharangi root at 366nm, with peak 4th having a higher concentration of chemical composition covering an area of 49.40%.

In Sample B, three peaks were detected at 254nm, with peak 1st having a higher concentration of chemical composition covering an area of 88.06%, three peaks were detected at 366nm, with peak 2nd having a higher concentration of chemical composition covering an area of 59.19%, and seven peaks were detected during a densitometric scan of Bharangi root at 620nm, with peak 2nd having a higher concentration of chemical composition covering an area.

When 1gm of powder was dissolved in 10 ml ethanol, more drug concentration was observed in HPTLC at UV 254 and 366 nm. In comparison, when 1gm of powder was dissolved in 10 ml ethanol, more drug concentration was observed in HPTLC at UV 660 nm.

Anupa Desha can use Sample A, which was collected in Waynad, Kerala, where there is more rain. Furthermore, the drugs collected

from Anupa Desha contain more water. Hence more water extraction was found in Sample A. Sample B was collected from Punjab, Jangala Desha with low rainfall, resulting in less water content which yielded less water extraction. Also, alcohol extraction was comparatively more in the sample which was collected from Jangala Desha than that of Sample A which is collected from Anupa Desha. This mostly indicates Sample which is collected from Anupa Desha is useful for preparing the Preparation like Arka, whereas the Sample which is collected from Jangala Desha is useful to prepare Asava, Arista etc. Similarly, Ayurveda explains how the qualities of drugs change with the seasons. According to the text Charaka Samhita, tender leaves and branches should be collected during the autumn and spring seasons, roots during the summer and late winter seasons, bark, latex, and tuber during the late autumn and early winter seasons, and flowers and fruits during the early winter season. However impact of Season on Drug was not observed in this study.

Changes in the concentration of Phychemical values in samples A and B may be due to the above reasons.

CONCLUSION

The difference in loss on dry (0.573), total ash (0.361), alcohol soluble extract (0.802),

and water soluble extract (2.648) was within standard limits in Both Sample A and Sample B. More drug concentration was observed in HPTLC at UV 254 and 366 nm after dissolving 1gm of powder in 10 ml ethanol. In comparison, more drug concentration was observed in HPTLC at UV 660 nm when 1gm of powder was dissolved in 10 ml ethanol. During preliminary testing, the presence of alkaloid, steroid, carbohydrate, Tannin, flavanoids, saponins, terpenoids, and coumarins were discovered in both samples. This could be due to the location and season of Bharangimoola collection. Anupa Desha had higher levels of water extraction, while Jangala Desha had higher levels of alcohol extraction.

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