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HPTLC fingerprinting analysis of phytoconstituents from *Bixa orellana* and *Beta vulgaris* plant pigment

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ABSTRACT

Pigments are a type of coloring component which are utilized by humans to enhance colors in their lives. Using synthetic pigments for the purpose of coloring food, clothes, fruit juices, paints are accepted worldwide previously, but due to hazardous impact of synthetic colors on environment, and on human health made to go for alternative sources of the pigments which are safe to use. Isolation of natural pigment is another preference that will increase the supply of pigment from natural sources while minimizing environmental and health risks. Thus, there is a growing necessity for biocolor derived from natural sources that can substitute synthetic colors. Natural colorants are commonly found from plants, animals, and microorganisms. Plant pigments have several benefits, so it seems much of prominence for pigment production. *Bixa orellana* and *Beta vulgaris* were isolated for yellow, orange, and red color pigments from natural ecological source. Pigment extraction from plants requires extract preparation and then isolation of pigments using different solvents. Extracted pigments were analyzed by preliminary screening techniques such as phytochemical assay and various confirmation tests. We found positive results for flavonoid, tannin, carbohydrate, protein, saponin and alkaloid using phytochemical assays. HPTLC fingerprinting was done for each extract and found positive result for alkaloid and phenolic compounds. Thus, it was aimed to develop the extraction analytical methods for determination of *Bixa Orellana* and *Beta Vulgaris* spp. by HPTLC fingerprint approach.

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INTRODUCTION

Plants play a significant part in the survival of life on Earth. Natural colorant production is increasing all over the world. Plant pigments are unique chemicals found in plants that absorb different wavelengths of light and give them a colorful appearance. It play an essential role in photosynthesis, plant growth and development [1]. Plant pigments are metabolic byproducts that give plants their characteristic colors referred to as biochromes [2]. Each pigment category is made up of a group of compounds with their own name, chemical structure, chemical properties, and color.

Bixa orellana L. (family Bixaceae) is a neotropical plant that is known in Mexico as achiote [3]. The predominant orange-red colored component of natural achiote pigments is bixin, which is known as annatto [4]. The aim of this research was to determine the bioactive elements of Bixa orellana through qualitative pharmacognostic and phytochemical analyses of the seeds, as well as to determine the plant part/organ with the highest concentration of phytoconstituents [5].

Plant-derived pigments such as betalains have become popular for use as natural colorants in the food industry [6]. *Beta vulgaris* contains a number of bioactive compounds that can reveal health-promoting effects, including betalains, ascorbic acid, flavonoids, polyphenols, saponins and nitrate [7]. Several in vitro investigations have revealed that betalain pigments protect cellular components from oxidative damage [8]. Extraction is required for the preparation of specific pigments as well as for gaining

natural colorant extracts. Phytochemical screening revealed the presence of tannins, saponins, flavonoid, alkaloid, and phenolic component in plant. A comprehensive assortment of phytoconstituents in different extracts through HPTLC fingerprinting profiles displayed the existence of alkaloids, flavonoids and phenolics compounds.

MATERIALS AND METHODS

The plant material for the proposed study such as *Bixa orellana* was collected from serenity botanical garden and *Beta vulgaris* was collected from local market. Dr. Hitesh solanki, Professor at Department of Botany, Bioinformatics and climate change impact management, University school of sciences, Gujarat University, Ahmedabad, Gujarat has authenticated the plants gathered. The plant authentication number of Bixa *orellana* and *Beta vulgaris* were found GU/BOT/B/O4 and GU/BOT/A/V12 respectively.

Processing of the plant

Washing

To remove the clinging undesirable particles, the gathered healthy leaves were rinsed with water.

Drying of plant material

Because it lowers the moisture content of fresh materials, drying is a crucial step of dried material preparation for subsequent processing. However, drying conditions have been proven to have a considerable impact on sensory quality, bioactive component stability and activity. The plant material was dried in the shade for 7-15 days.

Grinding

Grinding to get a homogeneous sample and to increase the surface contact of the sample with the solvent solution.

Storage

Plant powders are Stored at lower temperature.

Physicochemical parameters

The numerous physicochemical characteristics established in accordance with The Unani Pharmacopoeia of India. Odor, taste, color, moisture content, total ash value and extraction yield were all included.

Determination of moisture content

1.5 gm powdered leaves were measured into a weighted plane & slim Porcelain dish. It was dehydrated in the oven at temperatures ranging from 100^{o} to 105^{o}C. Cooling in desiccators and observing weight loss is commonly measured as 'moisture' [9].

Determination of total ash content

Weigh about 2 grams of the air-dried material in a formerly burned and tarred silica crucible. Spread the material out evenly and progressively raise the temperature to 500 - 600°C until it is white, showing the carbon absence.

Let the remains to cool for 30 minutes in a desiccator before weighing with no time interval. Using air-dried material standards, percentages of 'total ash' were calculated [10].

Total ash value of the sample =100(Z-x)/y %

X= 'weight of empty dish'

Y= 'weight of the drug taken'

Z= 'weight of the dish + ash (after complete incineration)'

Extraction process

The dehydrated plant material of both plant species was grind using mortar and pestle to obtain fine powder and then it was passed through 1 mm sieve. To obtain crude extracts, 10 gram fine powders of both plant materials were soaked in 100 ml of various solvents such as methanol, acetone, chloroform, dichloromethane, ethanol and water separately for 5 to 7 days [11]. The plant material was filtered and the remaining solid was extracted to remove all the remaining liquid. The obtained liquid was purified by filtration. The solvent was extracted using rotatory vacuum evaporator under reduced pressure. The dried extracts were placed in an airtight container and kept at 4°C until further examination [12]. The yields of weighted extracts were kept in small bottles at refrigerator (4°C) (Figure 1). Yield percentages were calculated using the following formula:

Extract yield % = weight of dried extract /weight of dried leave × 100

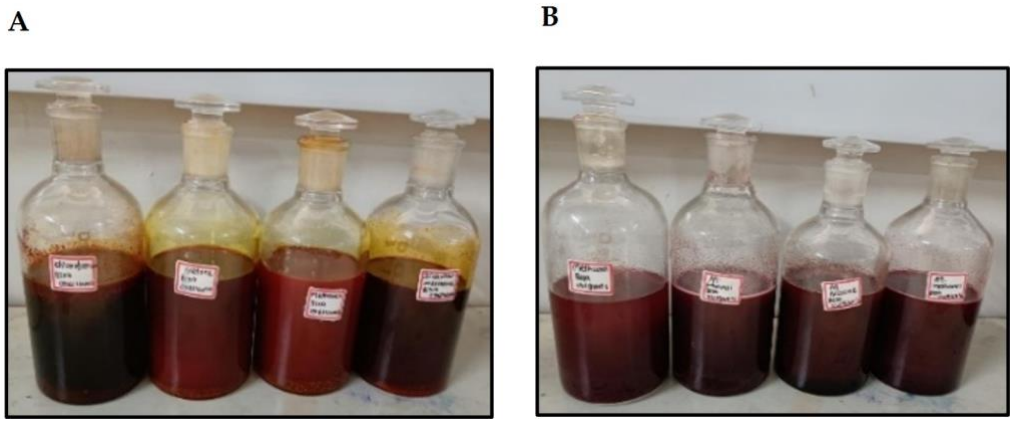

Figure 1. Extraction of (A) *Bixa Orellana,* and (B) *Beta vulgaris.*

Preliminary screening for extracted pigment by using chemical method

To examine the numerous chemical groups found in extracts, qualitative preliminary phytochemical experiments were performed (Table 1). Using recognized techniques, the presence of primary metabolites such as proteins, carbohydrates, fixed oils and fats was determined [13]. Secondary metabolites in *Bixa orellana* and *Beta vulgaris* leaf extracts

included alkaloids, flavonoids, saponins, polyphenols, tannins, terpenoids and glycosides [14].

HPTLC fingerprinting analysis

HPTLC studies were carried out using the standard method described by Wagner *et al*. 10ul of sample were loaded in Silica gel TLC plate [15]. The samples loaded plate was kept in TLC twin trough developing chamber (after being saturated with solvent vapor) with respective mobile phases, namely toluene- acetone-formic acid (4.5 : 4.5 : 1) for flavonoids and Ethyl acetate-methanol-water(10:1.35:1) for alkaloid [16]**.** The plate was developed up to 90mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo documentation chamber and the images were captured under visible light, UV 254 nm and UV 366 nm. The peak table, peak display and peak densitogram were noted [17]**.**

Table 1. Qualitative phytochemical screening of selected plant extract.

Test	Procedure	Positive result indicated by
Mayer's test for Alkaloid	2 mL filtrate was treated with a few drops of Mayer's reagents.	white creamy precipitation
Wagner's test for Alkaloid	2 mL of filtrate mixed with a few drops of Wagner's reagents.	Reddish-brown precipitate
Hager's test for Alkaloid	2 mL filtrate treated with few drops of Hager's reagents.	Bright yellow precipitation
Molisch's test for Carbohydrate	Take 2 ml of filtrate, add two drops of alcoholic solution of α- naphthol, shake well, add 1 ml of concentrated sulphuric acid at side of tube.	Violet ring
Fehling's test for Carbohydrate	1 ml of filtrate was mixed with 1 ml of each of the Fehling solutions A and B. Then a mixture heated on water bath.	Red precipitate
Benedict's test for Carbohydrate	0.5 mL Benedict's reagent was mixed with 0.5 mL filtrate. The mixture was heated in a boiling water bath for 2 minutes.	Specifically colored precipitate
Foam test for Saponin	The extract (50mg) is diluted with distilled water and made up to 20ml. The suspension is shaken in graduated cylinder for 15 min.	Development of foaming
Millon's test for Proteins and amino acids	A few drops of Millon's reagent were applied to 2 ml of filtrate.	White precipitate
Ninhydrin test for Proteins and amino acids	To 2 ml of aqueous filtrate, two drops of ninhydrin solution (10 mg ninhydrin in 200 ml acetone) were added.	Purple color
Libermann-Burchard's test for Phytosteroids and terpenoid	In 2 ml of acetic anhydride, the extract (50 mg) was dissolved. Few drops of concentrated sulphuric acid were progressively added along the side of test tube.	Variety of color changes
Spot test for Fixed oil and fats	A small quantity of extract is pressed between two filter paper.	Oil stain on the paper
Ferric chloride test for Phenolic and flavonoids	The extract (50 mg) was dissolved in 5 mL of distilled water. A few drops of neutral ferric chloride solution were added.	Dark green color
Gelatin test for Phenolic and flavonoids	The extract (50 mg) was dissolved in 5 ml of distilled water and 2 ml of 1% gelatin solution containing 10% sodium chloride was added into it.	White precipitate
Lead acetate test for tannin	The extract (50 mg) was dissolved in distilled water and 3 ml of 10% lead acetate solution was added into it.	Bulky white precipitate
Copper acetate test for Diterpenes	The plant extract was mixed with 3-4 drops of copper acetate solution and dissolved in water.	Bright green color

RESULTS

Collection of plant material

The first phase in this research was to gather and treat plants such as *Bixa orellana* and *Beta vulgaris.* following that, several physicochemical features of plant powder should be noted. The following Table 2 shows the observed outcome:

Table 2. Moisture content and Ash value of *Bixa orellana* and *Beta vulgaris.*

Plants	Moisture content %	Ash value %
Bixa orellana	4.91 %	0.36 %
Beta vulgaris	4.45 %	0.94 %

Extraction of natural colorant

Table 3 displays the extraction yields as well as the physical properties of plant extracts. *Bixa Orellana* extraction yields ranged from 12.34 % to 17.8 % and *Beta vulgaris* extraction yields range from 22.78 % to 30.47 % in various solvent systems. The yields of extracts varied greatly depending on the extraction solvent and plant material utilized. Extraction yields achieved in Methanol, Acetone, Chloroform, Dichloromethane, Aqueous methanol, Aqueous ethanol, Aqueous acetone, and Methanol solvent systems of 17.81 %, 13.71 %, 12.34 %, 13.47 %, 30.47 %, 28.69 %, 27.71 % and 22.78 %, respectively.

The color of extract from the solvents Methanol, Acetone, Chloroform, Dichloromethane were discovered to be Orange and the sense of touch was found to be sticky. While the extract from Aqueous methanol, Aqueous ethanol, Aqueous acetone, and Methanol were intense red in color and sticky to the sense of touch.

Table 3. Physical characteristics and % yield of extract: *Bixa orellana* and *Beta vulgaris.*

Plant	Solvent	Colour of Extract	Sense of Touch	% Yield
Bixa orellana	Methanol	Orange	Sticky	17.81
	Acetone	Orange	Sticky	13.71
	Chloroform	Dark orange	Sticky	12.34
	Dichloro methane	Dark orange	Sticky	13.47
Beta vulgaris	Aqueous methanol	Intense red	Sticky	30.47
	Aqueous ethanol	Intense red	Sticky	28.69
	Aqueous acetone	Intense red	Sticky	27.71
	Methanol	Intense red	Sticky	22.78

Preliminary screening for extracted pigment by using chemical method

The phytochemicals found in plant samples are the focus of natural product biological activity. A small amount of the dry extract was used for qualitative phytochemical screening. The presence of significant phenolic compounds, saponins, tannin, Flavonoid and other substances has been identified in the plants. (Table 4).

Table 4. Preliminary tests for *Bixa orellana* and *Beta vulgaris* extract.

Phytoconstituents		BVAE	BVAA	BVM	BVAM	BOD	BOM	BOA	BOC
Alkaloid	Mayer's test	+	+	-	++	+	-	-	+
	Wagner's test	+	+	-	+	++	+	+	+
	Hager's test	-	++	-	-	++	+	++	-
Carbohydrates	Molisch's test	++	++	+	+++	+	++	+	++
	Fehling's test	+++	++	+	+	++	++	++	+++
	Benedict's test	++	++	++	+	+	++	++	+
Saponins	Foam test	++	++	+	+	+	++	+	+
Proteins and amino acids	Millon's test	-	-	-	+	-	-	-	-
	Ninhydrin test	++	+	-	+	+	-	+	+
Phytosteroids and terpenoid	Libermann-Burchard's test	++	++	++	+	+	++	++	+
Fixed oil and fats	Spot test	++	+	++	+	+	+	-	-
Phenolic and flavonoids/Tannin	Ferric chloride test	+++	-	+	++	++	+	+	++
	Gelatin test	+	-	+	+	+	-	+	-
	Lead acetate test	+++	++	++	++	+	+	+	++
Diterpenes	Copper acetate test	++	-	-	++	-	++	++	-

HPTLC fingerprinting of extracted pigments

Alkaloid profile

HPTLC fingerprint profile, chromatogram and densitogram for Alkaloid is presented in figure 2, 3, and 4, respectively. A variety of extracts like methanol extract, acetone extract, chloroform extract, dichloromethane extract, aqueous methanol extract, aqueous ethanol extract, aqueous acetone extract and methanol extract of *Bixa orellana* and *Beta vulgaris* were used for HPTLC Alkaloids profile that represented the presence of bands with Rf values ranged from 0.03 to 0.93, 0.09 to 0.92, 0.04 to 0.94, 0.03 to 0.75, 0.03 to 0.92, 0.09 to 0.91, 0.04 to 0.83, 0.09 to 0.90, respectively.

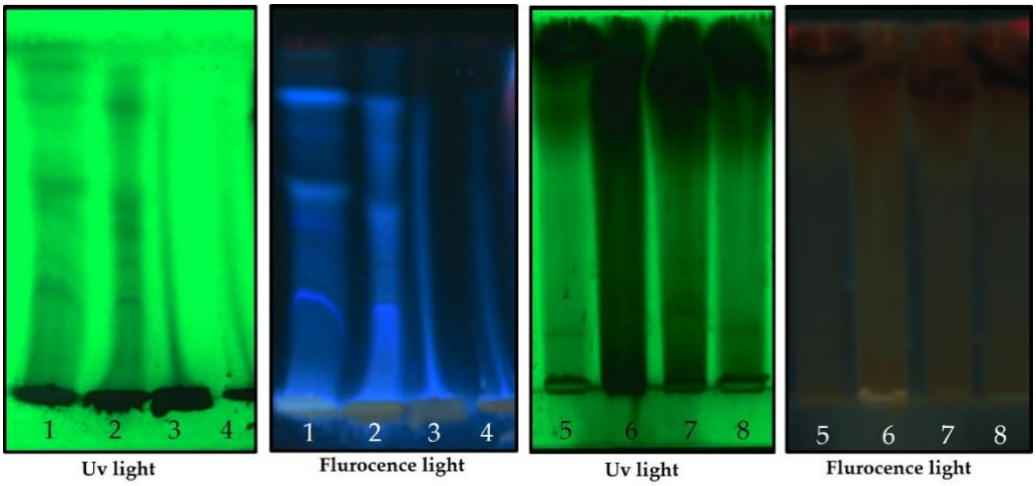

Figure 2. Chromatograms of plant extract in HPTLC analysis. Alkaloid profile of (1. BVAE- Aqueous ethanol extract of *Beta vulgaris*, 2. BVAA- Aqueous acetone extract of *Beta vulgaris*, 3. BVM- Methanol extract of *Beta vulgaris*, 4. BVAM- Aqueous methanol extract of *Beta vulgaris*, 5. BOD- Dichloromethane extract of *Bixa orellana*, 6. BOM- Methanol extract of *Bixa orellana*, 7. BOA- Acetone extract of *Bixa orellana*, 8. BOC- Chloroform extract of *Bixa orellana*).

Table 5. Peak table with retention factor (Rf) values of alkaloid compounds of *Bixa orellana* extract.

BOD		BOM		BOA		BOC	
RF value	**Assigned substance**	**RF value**	**Assigned substance**	**RF value**	**Assigned substance**	**RF value**	**Assigned substance**
0.03	Unknown	0.09	Nicotine		Unknown	0.03	Unknown
0.13	Unknown	0.21	Unknown	0.06	Unknown	0.14	Unknown
0.24	Unknown	0.31	Strychnine	0.2	Unknown	0.46	Alkaloid 1
0.26	Unknown	0.4	Unknown	0.31	Strychnine	0.52	Unknown
0.31	Strychnine	0.92	Unknown	0.53	Unknown	0.75	Unknown
0.5	Unknown			0.89	Unknown		
0.58	Unknown			0.94	Unknown		
0.81	Unknown						
0.93	Unknown						

BOD- Dichloromethane extract of *Bixa orellana*, BOM- Methanol extract of *Bixa orellana*, BOA- Acetone extract of *Bixa orellana*, BOC- Chloroform extract of *Bixa orellana*.

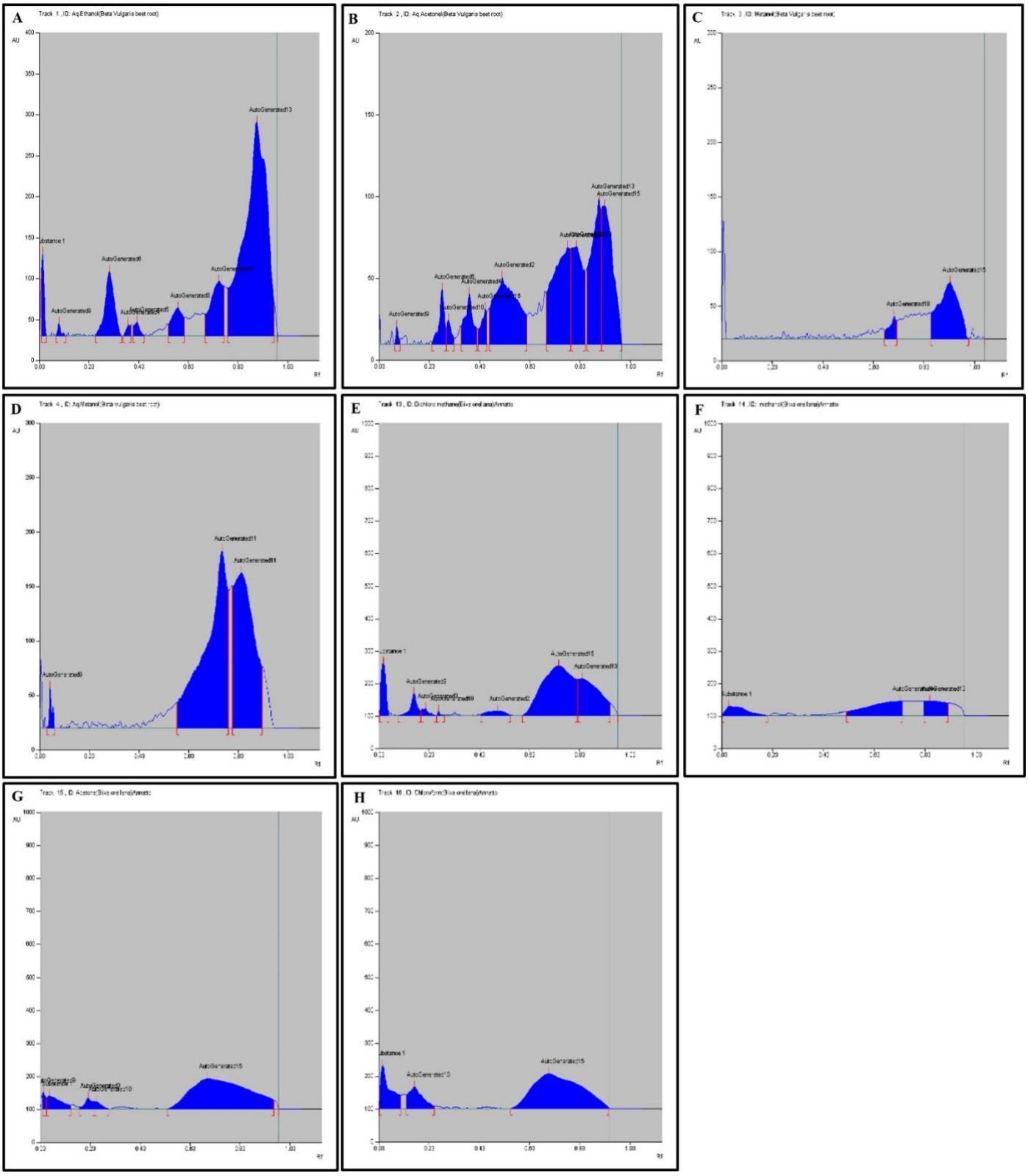

Figure 3. HPTLC peak densitogram of alkaloid profile (at 366 nm) of *Beta vulgaris* and *Bixa orellana* plant extract (A. BVAE- Aqueous ethanol extract of *Beta vulgaris*, B. BVAA- Aqueous acetone extract of *Beta vulgaris*, C. BVM- Methanol extract of *Beta vulgaris*, D. BVAM- Aqueous methanol extract of *Beta vulgaris*, E. BOD- Dichloromethane extract of *Bixa orellana*, F. BOM- Methanol extract of *Bixa orellana*, G. BOA- Acetone extract of *Bixa orellana*, H. BOC- Chloroform extract of *Bixa orellana*).

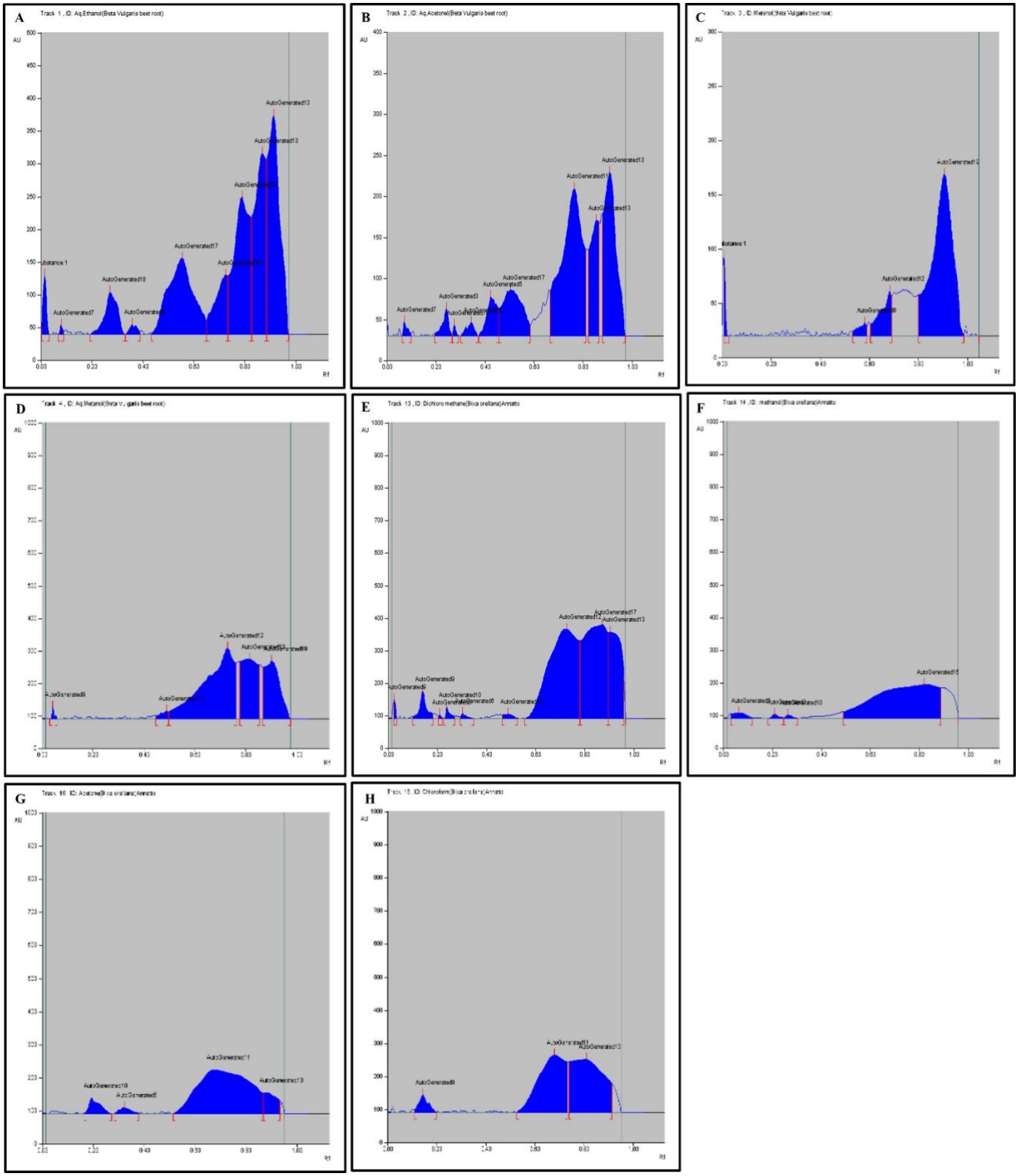

Figure 4. HPTLC peak densitogram of alkaloid profile (at 254 nm) of *Beta vulgaris* and *Bixa orellana* plant extract (A. BVAE- Aqueous ethanol extract of *Beta vulgaris*, B. BVAA- Aqueous acetone extract of *Beta vulgaris*, C. BVM- Methanol extract of *Beta vulgaris*, D. BVAM- Aqueous methanol extract of *Beta vulgaris*, E. BOD- Dichloromethane extract of *Bixa orellana*, F. BOM- Methanol extract of *Bixa orellana*, G. BOA- Acetone extract of *Bixa orellana*, H. BOC- Chloroform extract of *Bixa orellana*).

Table 6. Peak table with retention factor (Rf) values of alkaloid compounds of *Beta vulgaris* extract.

BVAE		BVAA		BVM		BVAM	
RF value	**Assigned substance**	**RF value**	**Assigned substance**	**RF value**	**Assigned substance**	**RF value**	**Assigned substance**
0.03	Unknown	0.09	Nicotine	0.04	Unknown	0.09	Nicotine
0.1	Unknown	0.23	Unknown	0.64	Unknown	0.48	Unknown
0.22	Colchicine	0.3	Strychnine	0.83	Unknown	0.53	Unknown
0.36	Unknown	0.33	Unknown			0.81	Unknown
0.46	Alkaloid 1	0.41	Chelidonine			0.9	Unknown
0.68	Unknown	0.49	Alkaloid 1				
0.77	Unknown	0.7	Unknown				
0.88	Unknown	0.85	Unknown				
0.92	Unknown	0.91	Unknown				

BVAE- Aqueous ethanol extract of *Beta vulgaris*, BVAA- Aqueous acetone extract of *Beta vulgaris*, BVM- Methanol extract of *Beta vulgaris*, BVAM- Aqueous methanol extract of *Beta vulgaris*.

Phenolic profile

HPTLC fingerprint profile, chromatogram and densitogram for Phenolic compounds is presented in figure 5, 6 and 7, respectively. A variety of extracts like Methanol extract, Acetone extract, Chloroform extract, Dichloromethane extract, Aqueous methanol extract, Aqueous ethanol extract, Aqueous acetone extract and Methanol extract of *Bixa orellana* and *Beta vulgaris* were used for HPTLC Phenolics profile that represented the presence of bands with Rf values ranged from 0.04 to 0.83, 0.05 to 0.43, 0.05 to 0.76, 0.05 to 0.72, 0.16 to 1.01, 0.13 to 1.04, 0.05 to 1.01 and 0.13 to 1.04, respectively.

Chromatograms of plant extract for phenolic profile (*Beta vulgaris* and *Bixa orellana*) in HPTLC analysis

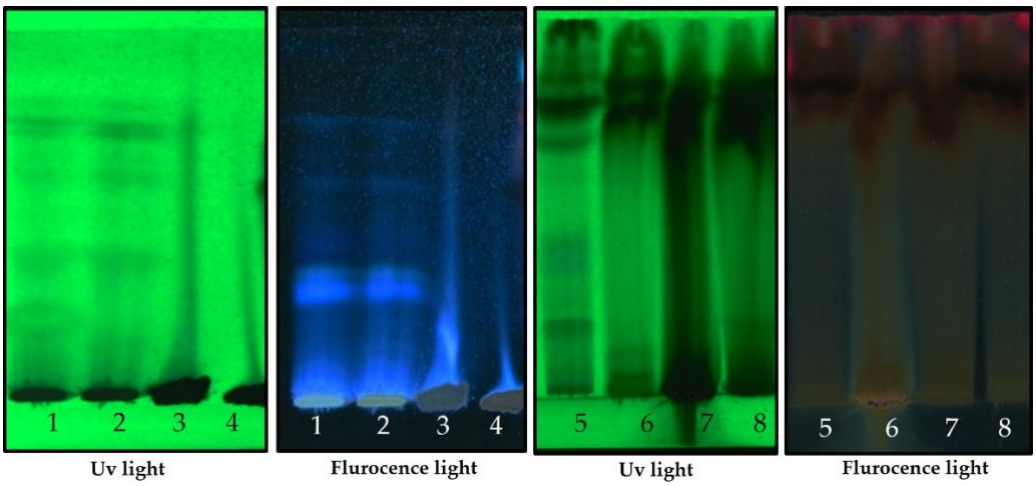

Figure 5. Chromatograms of plant extract in HPTLC analysis. Phenolic profile of (1. BVAE- Aqueous ethanol extract of *Beta vulgaris*, 2. BVAA- Aqueous acetone extract of *Beta vulgaris*, 3. BVM- Methanol extract of *Beta vulgaris*, 4. BVAM- Aqueous methanol extract of *Beta vulgaris*, 5. BOD- Dichloromethane extract of *Bixa orellana*, 6. BOM- Methanol extract of *Bixa orellana*, 7. BOA- Acetone extract of *Bixa orellana*, 8. BOC- Chloroform extract of *Bixa orellana*).

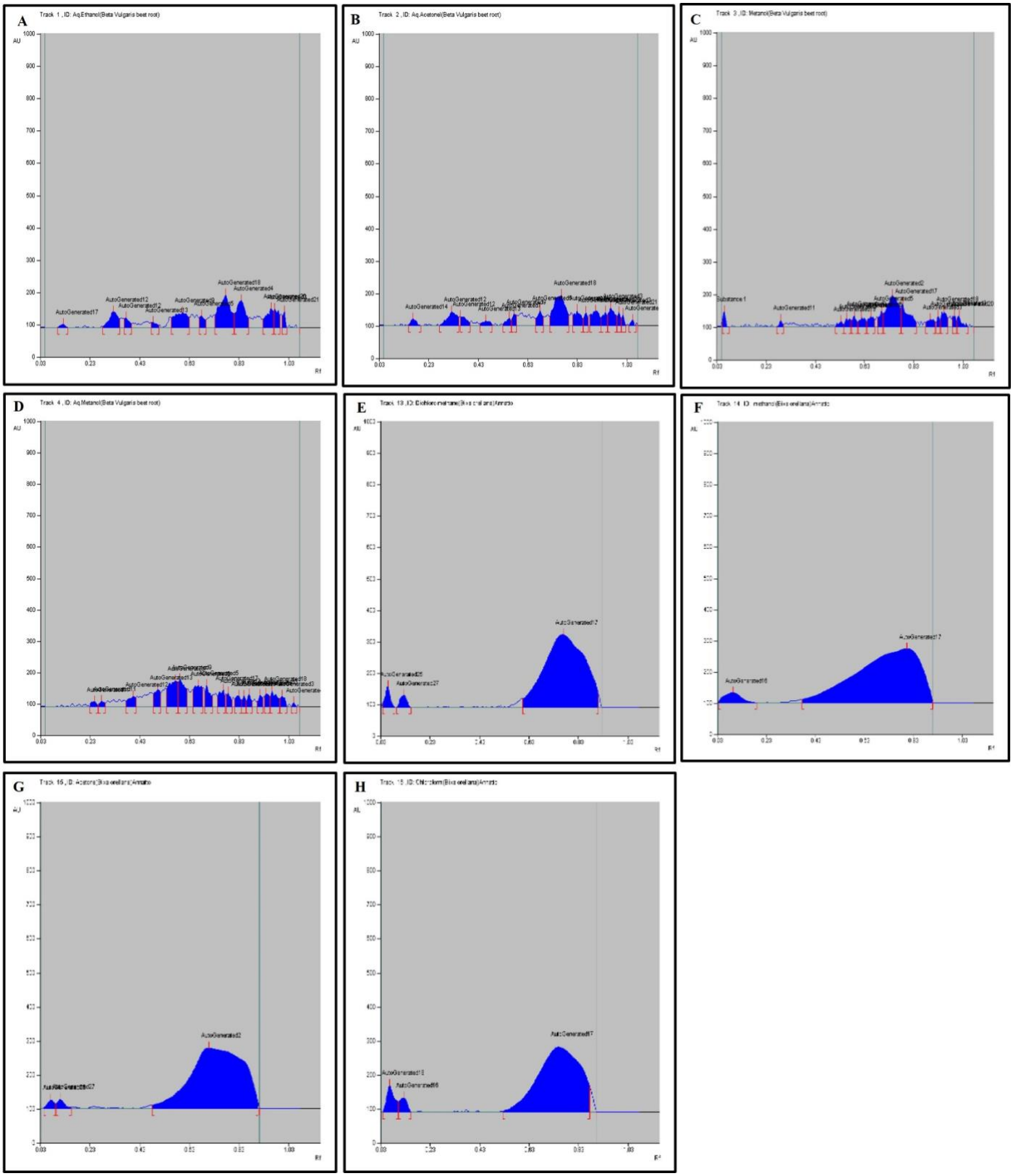

Figure 6. HPTLC peak densitogram of Phenolic profile (at 366 nm) of *Beta vulgaris* and *Bixa orellana* plant extract (A. BVAE- Aqueous ethanol extract of *Beta vulgaris*, B. BVAA- Aqueous acetone extract of *Beta vulgaris*, C. BVM- Methanol extract of *Beta vulgaris*, D. BVAM- Aqueous methanol extract of *Beta vulgaris*, E. BOD- Dichloromethane extract of *Bixa orellana*, F. BOM- Methanol extract of *Bixa orellana*, G. BOA- Acetone extract of *Bixa orellana*, H. BOC- Chloroform extract of *Bixa orellana*).

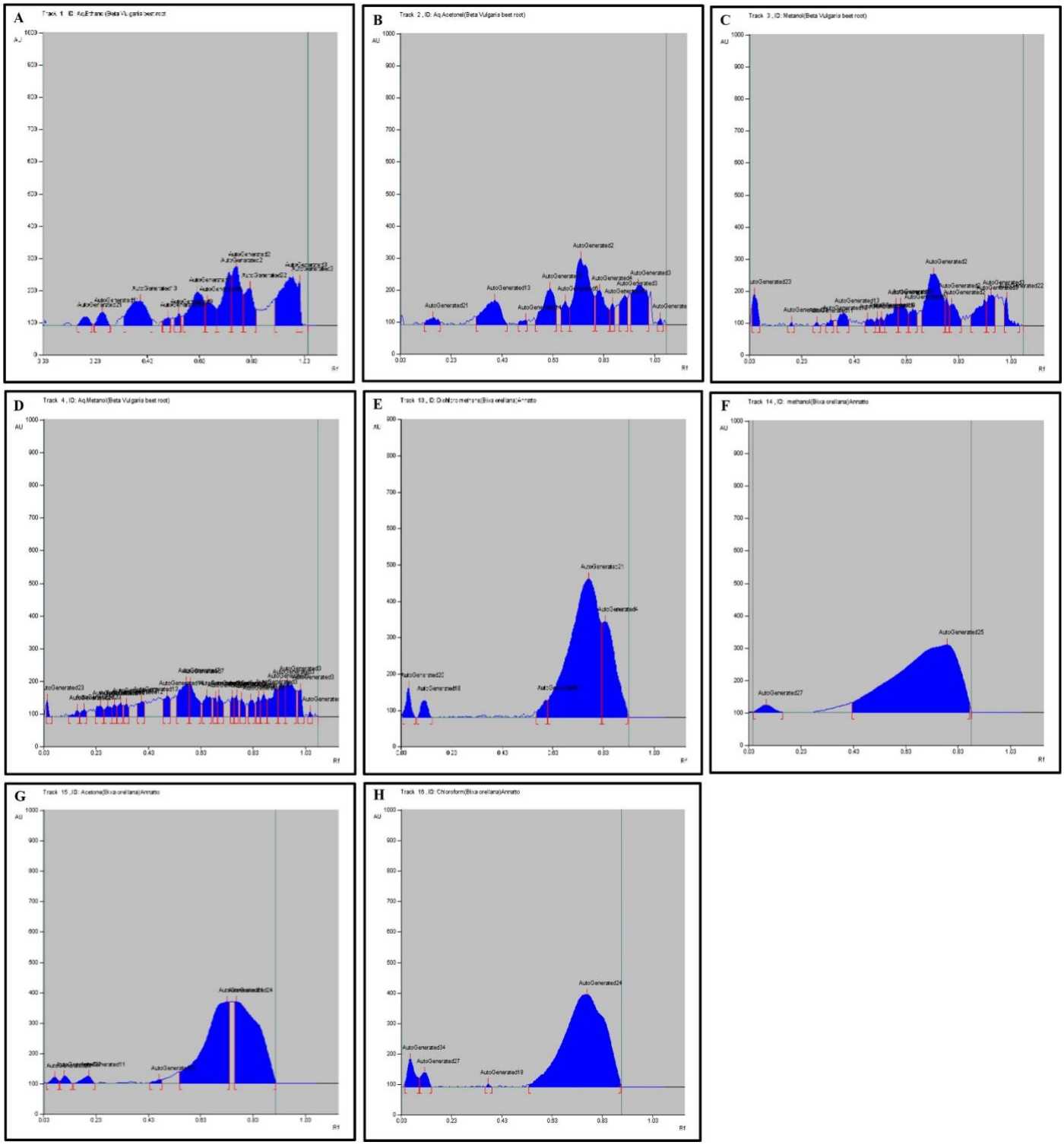

Figure 7. HPTLC peak densitogram of phenolic profile (at 254 nm) of *Beta vulgaris* and *Bixa orellana* plant extract (A. BVAE- Aqueous ethanol extract of *Beta vulgaris*, B. BVAA- Aqueous acetone extract of *Beta vulgaris*, C. BVM- Methanol extract of *Beta vulgaris*, D. BVAM- Aqueous methanol extract of *Beta vulgaris*, E. BOD- Dichloromethane extract of *Bixa orellana*, F. BOM- Methanol extract of *Bixa orellana*, G. BOA- Acetone extract of *Bixa orellana*, H. BOC- Chloroform extract of *Bixa orellana*).

Table 7. Peak table with retention factor (Rf) values of phenolic compounds of *Bixa orellana* extract.

BOD		BOM		BOA		BOC	
RF value	**Assigned substance**	**RF value**	**Assigned substance**	**RF value**	**Assigned substance**	**RF value**	**Assigned substance**
0.04	Unknown	0.05	Phenolic 2	0.05	Phenolic 2	0.05	Phenolic 2
0.1	Unknown	0.43	Unknown	0.09	Unknown	0.1	Unknown
0.59	Phenolic 7			0.15	Unknown	0.37	Unknown
0.72	Phenolic 8			0.44	Unknown	0.72	Phenolic 8
0.83	Unknown			0.55	Unknown		
				0.76	Unknown		

BOD- Dichloromethane extract of *Bixa orellana*, BOM- Methanol extract of *Bixa orellana*, BOA- Acetone extract of *Bixa orellana*, BOC- Chloroform extract of *Bixa orellana*.

Table 8. Peak table with retention factor (Rf) values of phenolic compounds of *Beta vulgaris* extract.

BVAE		BVAA		BVM		BVAM	
RF value	**Assigned substance**	**RF value**	**Assigned substance**	**RF value**	**Assigned substance**	**RF value**	**Assigned substance**
0.16	Unknown	0.13	Unknown	0.05	Unknown	0.13	Unknown
0.23	Unknown	0.33	Unknown	0.18	Unknown	0.17	Unknown
0.34	Unknown	0.5	Unknown	0.27	Unknown	0.23	Unknown
0.49	Phenolic 6	0.56	Unknown	0.32	Unknown	0.26	Phenolic 4
0.54	Unknown	0.66	Unknown	0.37	Unknown	0.29	Unknown
0.57	Unknown	0.75	Quercetin	0.49	Phenolic 6	0.31	Unknown
0.65	Unknown	0.8	Unknown	0.51	Unknown	0.34	Unknown
0.7	Unknown	0.86	Unknown	0.53	Unknown	0.39	Unknown
0.75	Quercetin	0.89	Unknown	0.55	Unknown	0.49	Unknown
0.8	Unknown	0.94	Unknown	0.6	Unknown	0.54	Unknown
0.92	Unknown	1.04	Unknown	0.64	Unknown	0.59	Phenolic 7
1.01	Unknown			0.69	Unknown	0.64	Unknown
				0.78	Unknown	0.68	Unknown
				0.8	Unknown	0.7	Unknown
				0.88	Unknown	0.75	Quercetin
				0.94	Unknown	0.76	Unknown
				1.01	Unknown	0.78	Unknown
						0.81	Unknown
						0.84	Unknown
						0.86	Unknown
						0.89	Unknown
						0.93	Unknown
						0.96	Unknown
						1.00	Unknown
						1.04	Unknown

BVAE- Aqueous ethanol extract of *Beta vulgaris*, BVAA- Aqueous acetone extract of *Beta vulgaris*, BVM- Methanol extract of *Beta vulgaris*, BVAM- Aqueous methanol extract of *Beta vulgaris*.

DISCUSSION

The purpose of this study was to evaluate the HPTLC fingerprinting analysis of extracted pigments from plants for usage in a variety of industries. The extraction yield of chloroform extract of *Bixa orellana* was the lowest at 12.34 %. Methanol extract had the highest extraction yield of 17.81 %. The extraction yield of chloroform extract of *Beta vulgaris* was the lowest at 22.78 %. Aqueous methanol extract had the highest extraction yield of 30.47 %. Physicochemical properties of Plant extract such as colour and feeling of touch were observed.

The phytochemical screening of different extracts of plant samples of *Bixa orellana and beta vulgaris* revealed the presence of some secondary metabolites such as alkaloids, phenolics, flavonoids, steroids, and terpenoids.

HPTLC can be used as a phytochemical marker and is more effective in the field of plant taxonomy for secondary metabolite identification. Alkaloids are amino acids like lysine, ornithine, phenyl alanine, tyrosine, tryptophan, and histidine that are present in plants and responsible for detection and analytical analysis of phytoconstituents or pigments. The HPTLC results determined the presence of 16 different types of alkaloids bands and validated 11 different Rf values ranged from 0.03 to 0.93 (Table 5 and 6). The alkaloid band with Rf value 0.22 confirmed the presence of Colchicine, 0.31 confirmed the Strychnine, 0.09 confirmed the nicotine and 0.41 confirmed the Chelidonine in the BVAE, BOD, BOM, BVAA extract, respectively.

The HPTLC results determined the presence of 16 different types of phenolics bands and validated 21 different Rf values ranged from 0.05 to 1.04 (Table 7 and 8). The phenolic band with Rf value 0.75 confirmed the presence of quercetin in the BVAA, BVAM and BVAE extract.

CONCLUSION

Considering the need for alternative synthetic pigment in food products, pharmaceutical science, and cosmeceutical application, it was thought interesting to analyze the natural plant extracts. This survey suggests that all the extracts derived from the *Beta vulgaris* and *Bixa orellana* plants include numerous phytochemicals such as phenol, tannin, flavonoid, saponin and others. The more phytochemicals were identified in the methanolic extract due to higher polarity of the solvent. Natural pigments have a lower stability than synthetic colorants, which poses certain issues in terms of color loss during food processing, storage, and marketing. HPTLC fingerprinting confirmed the presence of some flavonoid and alkaloid compound in different extract of selected plants which might be responsible for stability properties for plant pigment.

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AUTHOR CONTRIBUTIONS

PP; Conceived and designed the experiments. PP and MP; analyzed the data and drafted the manuscripts. PP and MP; reviewed and corrected the manuscripts. All authors read and approved the final version of the article.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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