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Phytochemical Studies on *Bauhinia racemosa* Lam. *Bauhinia purpurea* Linn. and *Hardwickia binata* Roxb

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Abstract: The present paper deals with the phytochemical studies on *Bauhinia racemosa* Lam., *Bauhinia purpurea* Linn. and *Hardwickia binata* Roxb. The phytochemical study of three plants involve preliminary phytochemical studies, physico-chemical studies, quantitative estimation of primary and secondary metabolites, TLC study and HPLC fingerprint study of ethanolic extract of leaves of three plants. In HPLC fingerprint study, the three peaks at a retention time of 15min, 17min and 19min were identical in *B. racemosa* and *B. purpurea* which was confirmed by overlaid spectra. The generated data may be useful in suggesting chemotaxonomical interrelation between three plants.

Keywords: Phytochemical, Chemotaxonomy, *Bauhinia racemosa, Bauhinia purpurea*, *Hardwickia binata.*

Introduction

Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in giving the solution to systematic problems on the one hand, and in the search for additional resources of raw materials for pharmaceutical industry on the other hand[1]. Plant synthesizes a wide variety of chemical compounds, which can be sorted by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. Primary metabolites make up the physical integrity of the plant cell and are involved with the primary metabolic process of building and maintaining of living cells. Secondary metabolites do not seen to be vital to the immediate survival of the organism that produces them and are not an essential

part of the process of building and maintaining living cells. With the development of natural product chemistry, the potential of chemotaxonomy is now becoming increasingly obvious. The application of chemical data to systematics has received serious attention of a large number of biochemists and botanists during the last three decades.

Mukherjee and Laloraya (1977) have studied on the ketoacids and free amino acid pattern during leaf growth in *Bauhinia purpurea*[2]. Diciero *et al.,* (1998) have analyzed the seed of *B. variegata* and its amino acid analysis showed high content of asparatic acid, glutamic acid, serine and glycine[3]. Rehman and Begum (1966) isolated 3-galactoside and 3-rhamnoside of kaemferol from the leaves of *B. variegata*[4]. Preliminary phytochemical studies have revealed that the genus *Bauhinia* is mainly constituted of steroidal glycosides, terpenoids, lactones and flavonoids[5].

In the present study, we have concentrated on phytochemical characters of seeds and leaves of *Bauhinia racemosa* Lam., *Bauhinia purpurea* Linn. and *Hardwickia binata* Roxb. belongs to family Leguminosae.

Experimental

Collection and identification of plants

The plant materials such as seeds and leaves of *Bauhinia racemosa* Lam., *Bauhinia purpurea* Linn. and *Hardwickia binata* Roxb. belongs to the family Leguminosae were collected from the Gulburga University Campus, Gulbarga. The plants were identified with the help of 'The Flora of Presidency of Madras'[6] and 'The Flora of Gulbarga District'[7]. The voucher specimens were deposited in the Herbarium, Department of Botany, Gulbarga University, Gulbarga. The voucher numbers for *B. racemosa, B. purpurea* and *H. binata* are HGUG S325, HGUG S206 and HGUG S154 respectively.

Preliminary phytochemical studies

The shade dried seeds and leaves of each plant were powdered separately using mixer grinder and subjected to soxhlet extraction using 95% ethanol and ethanolic extract was used for preliminary phytochemical tests. In the preliminary phytochemical tests, test for phenols (phenol test and ellagic acid test), test for flavonoids (flavonoid test, shinoda test, ferric chloride test and lead acetate test), test for saponins (foam test), test for glycosides (Killar Killiani test), test for alkaloids (Mayer's test, Wagner's test and Dragendorff's test) and test for tannins (ferric chloride test and gelatin test) were carried out[8-10].

Physicochemical studies of seeds and leaves

The physico-chemical studies such as total ash and acid insoluble ash was carried out for seeds and leaves of three plants[11]. Mineral content of seeds was carried out by atomic absorption spectrophotometer.

Determination of minerals

Five-gram seeds of each plant were powdered and ashed. The resulting ash was dissolved in 25 ml of dilute HCl (1:1) and incubated for 20-25 min on hot water bath. The solution was filtered. The filtrate was collected and made the volume to 100 ml with water in a volumetric flask and 10 ml of this solution was diluted to 100 ml with distilled water. The diluted solution was used for the estimation of minerals such as iron, copper, manganese, zinc and lead by Atomic Absorption Spectrophotometer (Smith - Hieftie 1000, Thermajarrell Ash Corporation, Franklin, MA).

Physico-chemical properties of seed oil

The physico-chemical studies such as oil content, acid value, iodine value and saponification value of seed oil of three-plant material were carried out[12].

Quantitative estimation of primary and secondary metabolites

Quantitative estimation of proteins by Lowry's method[13], phenols by Folin's Ciocalteu method[14] and flavonoids by spectrophotometry method[15] were carried out.

Thin layer chromatographic study

Silica gel 60 F_{254} – TLC aluminium sheets (Merck, Germany) were used for the thin layer chromatographic study.

TLC study of amino acids and non protein amino acids

For the TLC study of amino acids and non-protein amino acids, the powdered seed material of each plant was extracted with 80% ethanol for 24 hr. This extract was directly used for chromatographic study using the solvent system of n-butanol: acetic acid: water (4:1:1). The amino acids and nonprotein amino acids were detected by spraying with 0.1% ninhydrin in acetone. Sprayed plates were heated at 80°C for 15 min for the development of characteristic colored spots and compared with authentic markers by co-chromatography[16]. The hRf values were calculated and noted.

TLC study of phenols

The powdered seed and leaf material of each plant was extracted with ethanol separately on rotary shaker. The ethanolic extract was condensed and used for chromatographic study using the solvent system of chloroform: methanol (9:1). The phenols were detected by spraying with Folin-Ciocalteu reagent. The characteristic colored spots were compared with authentic markers by co-chromatography[16]. The hRf values were calculated and noted.

TLC study of flavonoids

The powdered seed material was extracted with 10 ml methanol for 15 min on a water bath at 60°C. This extract was filtered and concentrated to 2 ml and 1 ml of water and 10 ml of ethyl acetate were added. The ethyl acetate layer was separated and reduced to one-fourth volume and used for chromatographic study. The solvent system of chloroform: methanol (19:1) was used for chromatography. The flavonoids were detected under UV-365 nm light[16]. The hRf values were calculated and noted.

TLC study of saponins

The powdered seed material was homogenized with 80% ethanol. This suspension was filtered and evaporated and redissolved in chloroform and used for chromatographic study. The solvent system of ethyl acetate: n-hexane (1:9) was used as eluant. The saponins were detected by incubating the plate in glass chamber saturated with iodine vapors. The characteristic colored spots were observed under visible light[17]. The hRf values were calculated and noted.

TLC study of seed oil

The extracted seed oil was redissolved in chloroform and used for chromatographic study. The solvent system used for the separation was chloroform: methanol: ammonia (65:25:4). The spots were detected on TLC by spraying 50% sulphuric acid and heated at 80°C for 15 min[18].

HPLC analysis

Sample Preparation of leaves

4 g of leaf powder was extracted with 100 ml of ethyl alcohol for 4 hr by refluxing at 60°C. The extract was centrifuged at 3000 rpm and then filtered through Whatmann filter paper no. 1 using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The sample was used for HPLC fingerprint study.

Instrumentation

An isocratic HPLC (Shimadzu HPLC Class *VP* series) with two LC- 10 AT *VP* pumps (Shimadzu), variable wave length programmable photo diode array detector SPD-M10A *VP* (Shimadzu), CTO-10AS *VP* column oven (Shimadzu), SCL-10A *VP* system controller (Shimadzu) and reverse phase Luna 5µ C_{18} (2) Phenomenex column (250mm X 4.6mm) was used. The HPLC system was equipped with Class *VP* series version 6.1 software (Shimadzu). The mobile phase components acetonitrile-methanol: water (45:55) were filtered through 0.2µ membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 260-270 kgf / cm^2. The column temperature was maintained at 27°C. 20µl of respective sample was injected by using Rheodyne syringe (Model 7202, Hamilton).

Results and Discussion

The results of preliminary phytochemical study are tabulated in Table-1. The phytochemical study revealed the presence of phenols, saponins, flavonoids, glycosides and tannins. Alkaloids were not detected in any of the tested plants. These findings had confirmed with the previous reports[1]. Thus the preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compounds.

The results of physicochemical study are tabulated in Table-2. The highest percentage of iron and copper content was noticed in the seeds of *Bauhinia purpurea*, whereas zinc, lead and manganese in the seeds of *Hardwickia binata* and copper was not detected in *H. binata*. Rajaram and Janardhanan (1991) reported that the seeds of *B. purpurea* and *B. vahlii* were rich in calcium and iron[19].

The data of physicochemical study of seed oil of the three plants is tabulated in Table-3. Out of the three plants, the highest oil content was yielded from the seeds of *B. purpurea*. The physicochemical parameters such as acid value, iodine value and saponification values of two species of *Bauhinia* are more or less similar to the data reported in the literature[20].

The results of quantitative estimation of proteins, phenols and flavonoids are tabulated in Table-4. Naturally the amount of proteins is more in the seeds when compared to the leaves. It was evident that the protein content was more in the seeds of *B. purpurea* followed by *B. racemosa* and *H. binata*. The leaf protein was more in *B. racemosa* followed by *H. binata* and *B. purpurea*. The amount of phenolic content was more in leaves than the seeds. The maximum amount of phenols in leaves was detected in *B. racemosa* and in seeds it was detected in *H. binata*. The phenolic compounds play an important role in giving protection to the plants against deleterious effects of UV rays and also against certain phytopathogenic microorganisms. The flavonoid content was slightly more in seeds than leaves. The quantitative estimation of proteins, phenols and flavonoids in *B. racemosa*, *B. purpurea* and *H. binata* gives an insight into their chemical nature quantitatively, which can provide us a rich data in understanding certain basic pattern of growth and metabolism. At the same time, protein, phenols and flavonoids can be used as chemical markers in taxonomic studies[1].

Table 1. Data of preliminary phytochemical analysis

Tests	*B. racemosa*		*B. purpurea*		*H. binata*	
	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves
Phenolics:						
Phenol test	+ve	+ve	+ve	+ve	+ve	+ve
Ellagic acid test	+ve	+ve	+ve	+ve	+ve	+ve
Flavonoids:						
Flavomid test	+ve	+ve	+ve	+ve	+ve	+ve
Shinoda test	+ve	+ve	+ve	+ve	+ve	+ve
Ferric chloride test	+ve	+ve	+ve	+ve	+ve	+ve
Lead acetate test	+ve	+ve	+ve	+ve	+ve	+ve
Saponins						
Foam test	+ve	+ve	+ve	+ve	+ve	+ve
Glycosides						
Killar Killani test	+ve	+ve	+ve	+ve	+ve	+ve
Alkaloids						
Mayer's test	-ve	-ve	-ve	-ve	-ve	-ve
Wagner's test	-ve	-ve	-ve	-ve	-ve	-ve
Dragendorff's test	-ve	-ve	-ve	-ve	-ve	-ve
Tannins						
Ferric chloride test	+ve	+ve	+ve	+ve	+ve	+ve
Gelatin test	+ve	+ve	+ve	+ve	+ve	+ve

Table-2. Data of the physico-chemical parameters

Parameters	*B. racemosa*		*B. purpurea*		*H. binata*	
	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves
Total ash(%)	2.85±0.49	2.34±0.44	3.75±0.36	2.42±0.32	2.65±0.88	2.30±0.79
Acid insoluble ash(%)	1.60±0.12	1.50±0.13	1.80±0.11	1.23±0.12	1.52±0.18	1.02±0.21
Iron(ppm)	0.21±0.07	-	0.35±0.05	-	0.34±0.05	-
Copper (ppm)	0.01±0.03	-	0.02±0.03	-	ND	-
Manganese(ppm)	0.03±0.01	-	0.03±0.02	-	0.05±0.05	-
Zinc(ppm)	0.08±0.09	-	0.02±0.07	-	0.125±0.08	-
Lead(ppm)	0.04±0.06	-	0.03±0.01	-	0.06±0.03	-

Where, ND – Not detected

Results are given as the average of triplicate determination ± standard deviation.

Table-3. Data of physico-chemical analysis of seed oil

S.No	Parameters	Seed Oil		
		B. racemosa	*B. purpurea*	*H. binata*
1	Oil content (%)	5.0	17.5	4.5
2	Color	Yellow	Golden yellow	Green
3	Acid value	4.29	0.8	10.4
4	Iodine value	98.3	82.2	26.39
5	Saponification value	209	192.3	240

Table-4. Data of quantitative estimation of proteins, phenols and flavonoids

Plant constituents	*B. racemosa*		*B. purpurea*		*H. binata*	
	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves
Proteins (%)	8.9±0.88	0.63±0.98	18.9±1.03	0.48±0.78	8.1±0.98	0.57±0.79
Phenols (%)	0.12±0.22	0.55±0.36	0.11±0.43	0.48±0.57	0.19±0.91	0.39±0.43
Flavonoids(%)	0.07±0.13	0.04±0.09	0.11±0.17	0.09±0.11	0.06±0.23	0.05±0.05

Results are given as the average of triplicate determination ± standard deviation.

The data of thin layer chromatographic study of amino acids and non-protein amino acid is tabulated in Table-5. The amino acids are the basic building blocks of proteins. Apart from being bound as proteins, amino acids are also exists in the free form in many tissues. A few non-protein amino acids are also reported from the plants. In the present study the amino acid pattern of the seeds of three plants showed remarkable homology, of which lysine was detected in *B. racemosa* and *H. binata*; phenylalanine, methionine and leucine in *B. racemosa* and *B. purpurea*. Proline was detected in *H. binata*. In the present study the spots detected in the seeds of three plants were not identical with the referral standards of non-protein amino acids. Rajaram and Janardhanan (1991) have studied the chemical composition of seeds especially amino acids, minerals and antinutritional factors and reported that in *B. purpurea*, *B. racemoca* and *B. valhii* the amino acids lysine, tyrosine and phenylalanine were fairly high in all the tested plants[19].

The data of thin layer chromatographic study of phenolic compounds is tabulated in Table-6. The seeds of *B. racemosa* showed six spots and leaves showed sixteen spots. The spots detected in leaves at hRf value 46.9, 65.7 and 82.3 were identical with standards such as hydroquinone, catechol and 4-nitrophenol respectively. The seeds and leaves of *B. purpurea* showed three and ten spots. The spots detected in leaves at hRf values 46.9 and 65.7 identical with hydroquinone and catechol. The seeds and leaves of *H. binata* showed four and nine spots. The spots detected in leaves at hRf values 46.9 and 65.7 identical with hydroquinone and catechol. The spots detected in seeds at hRf values 40 and 73 were common in *B. racemosa* and *B. purpurea,* whereas single spot at hRf value 93 was common in *B. racemosa* and *H. binata*. The spots detected in leaves of three plants at hRf values 9.7, 24.2, 46.9, 65.7 and 94.2 were common, of which the spots at hRf values 46.9 and 65.7 were matched with hydroquinone and catechol. The phenolic compounds have immense help in assessing relationship and also play an important biological activity[1].

The data of thin layer chromatographic study of flavonoids is tabulated in Table-7. Seven prominent fluorescent spots were detected in seeds of *B. racemosa*, six in *B. purpurea* and four in *H. binata*. The spots detected at hRf values 7.8 and 25 were common in three plant seeds. Flavonoids normally occur in plants as glycosides or aglycones. A wide range of flavonoids present in higher plants is useful in chemotaxonomic studies.

The data of thin layer chromatographic study of saponins is tabulated in Table-8. The qualitative separation of saponins by TLC revealed the presence of 8 spots in *B. racemosa* and 6 spots in *B. purpurea* and *H. binata.* Spot at hRf value 40 was common in all three plants. Small *et al.,* (1990) reported that the saponins proved to be exceptionally useful in valuation of the relationship of the sub-species of the most economically important species, *Medicago sativa*[21].

The data of thin layer chromatographic study of oil is tabulated in Table-9. The chromatogram of *B. racemosa* seed oil showed twelve spots, *B. purpurea* and *H. binata* showed six spots. The spots at hRf values 10, 17 and 27 were identical with that of standards

such as phosphotidylinositol, lysophosphotidylethanolamine and phosphotidylcholine respectively. Phosphotidylinositol was common in *B. racemosa* and *B. purpurea,* lysophosphotidylethanolamine in *B. racemosa* and *H. binata* and phosphotidylcholine was present in all the three plants. Similar studies were done on the seed oil of *Digella sativa*, *Coriandrum sativum* and *Guizotia abyssinica*[18].

Table-5. Qualitative separation of amino acids and non protein amino acids

S. No.	Standard amino acids & Non-protein amino acids	hRf values	*B. racemosa*	*B. purpurea*	*H. binata*
1.	--	7.1	-	+	-
2.	Lysine	10.9	+	-	+
3.	Ornithine monohydrochloride*	11.6	-	-	-
4.	Arginine	13.4	-	-	-
5.	Histidine	14.5	-	-	-
6.	Aspartic acid	16.3	-	-	-
7.	--	18.0	+	-	-
8.	Dihydroxyphenyl alanine	20.0	-	-	+
9.	Serine	22.9	-	-	-
10.	Phenylalanine	25.7	+	+	-
11.	Alanine	28.0	-	-	-
12.	--	30.3	-	+	-
13.	Threonine	33.1	-	-	-
14.	Glycine	34.9	-	-	-
15.	--	36.8	-	-	+
16.	Hydroxyproline*	37.2	-	-	-
17.	--	40.0	+	-	-
18.	Glutamic acid	43.6	-	-	-
19.	Isoleucine	47.7	-	-	-
20.	Valine	47.8	-	-	-
21.	Methionine	49.7	+	+	-
22.	Nor leucine*	51.2	-	-	-
23.	Cystine HCl*	51.4	-	-	-
24.	2-amino n-butyric acid*	51.7	-	-	-
25.	Tyrosine	54.5	-	-	-
26.	Proline	55.4	-	-	+
27.	Tryptophan	57.9	-	-	
28.	Leucine	62.7	+	+	-
29.	--	68.4	-	-	+
30.	--	70.0	+	-	-
31.	--	75.0	-	+	-
32.	--	77.1	-	-	+
33.	--	81.0	+	-	-
34.	--	92.8	-	+	-

Where, * = Non protein amino acids

Table-6. Qualitative separation of phenols

S.No	Color of spot	hRf value	*B. racemosa*		*B. purpurea*		*H. binata*		Standards
			Seed	Leaves	Seed	Leaves	Seed	Leaves	
1	Grey	3.1	-	+	-	-	-	-	--
2	Blue	4.6	-	+	-	-	-	+	--
3	Blue	8.0	+	-	-	-	-	-	--
4	Blue	9.7	-	+	-	+	-	+	--
5	Blue	13.0	+	-	-	-	-	-	--
6	Blue	15.2	-	+	-	+	-	-	--
7	Blue	16.0	-	-	-	-	+	-	--
8	Blue	24.2	-	+	-	+	-	+	--
9	Blue	25.0	+	-	-	-	-	-	--
10	Blue	29.0	-	-	-	-	+	-	--
11	Blue	30.5	-	+	-	+	-	-	--
12	Blue	31.0	-	-	+	-	-	-	--
13	Grey	39.3	-	+	-	+	-	-	--
14	Blue	40.0	+	-	+	-	-	-	--
15	Blue	46.9	-	+	-	+	-	+	Hydroquinone
16	Blue	50.0	-	-	-	-	+	-	--
17	Grey	54.6	-	+	-	-	-	+	--
18	Blue	60.0	-	+	-	+	-	-	--
19	Blue	65.7	-	+	-	+	-	+	Catechol
20	Blue	73.0	+	-	+	-	-	-	--
21	Blue	75.7	-	+	-	+	-	-	--
22	Blue	82.3	-	+	-	-	-	-	4-Nitrophenol
23	Blue	87.5	-	+	-	-	-	+	--
24	Blue	93.0	+	-	-	-	+	-	--
25	Blue	94.4	-	+	-	+	-	+	--
26	Blue	98.4	-	+	-	-	-	+	--

Table-7. Qualitative separation of flavonoids

S.No	Color of spot	hRf values	*B. racemosa*	*B. purpurea*	*H. binata*
1	Fluorescent yellow	7.8	+	+	+
2	Fluorescent blue	13.7	+	-	+
3	Fluorescent blue	17.6	+	-	-
4	Fluorescent blue	25.0	+	+	+
5	Fluorescent blue	28.0	-	+	-
6	Fluorescent blue	31.3	-	-	+
7	Fluorescent blue	38.0	-	+	-
8	Fluorescent blue	44.0	+	+	-
9	Fluorescent blue	74.5	+	-	-
10	Deep fluorescent blue	82.0	-	+	-
11	Fluorescent blue	86.2	+	-	-

Table-8. Qualitative separation of saponins

S.No.	Color of spot	hRf values	*B. racemosa*	*B. purpurea*	*H. binata*
1	Yellow	9.4	-	+	-
2	Yellow	16.4	-	+	-
3	Yellow	24.5	-	-	+
4	Yellow	34.1	+	-	-
5	Brown	36.7	+	-	-
6	Brown	40.0	+	+	+
7	Yellow	43.0	+	+	-
8	Yellow	45.1	+	-	+
9	Yellow	50.4		+	+
10	Brown	51.6		-	+
11	Yellow	66.4	+	-	-
12	Dark brown	72.7		+	-
13	Dark brown	88.6	+	-	-
14	Dark brown	98.7	+	-	+

Table-9. Qualitative separation of seed oil

S.No.	hRf values	*B. racemosa*	*B. purpurea*	*H. binata*	Standards
1.	5.3	Pink	-	-	--
2.	10	Pink	Pink	-	Phosphotidylinositol
3.	17	Pink	-	Pink	Lysophosphotidyl ethanolamine
4.	21	Pink	-	-	--
5.	27	Pink	Pink	Pink	Phosphotidylcholine
6.	41	D. brown	Pink	-	--
7.	46	L. brown	-	L brown	--
8.	49	L. brown	-	-	--
9.	51	L. brown	-	-	--
10.	59	Pink	-	L. brown	--
11.	61	Pink	-	-	--
12.	65	-	Pink	-	--
13.	71	-	-	L. brown	--
14.	73	-	Pink	-	--
15.	90	D. brown	-	-	--
16.	93	-	D. brown	D. brown	--

The qualitative HPLC fingerprint profile of ethanolic extract of leaves of *Bauhinia racemosa* and *Bauhinia purpurea* was selected at a wavelength of 230nm due to sharpness of the peaks and proper baseline (see Figure 1-2). The profile showed the compounds separated at a retention time of 15min, 17min and 19min were identical in both the species, which were confirmed by the overlaid spectra. The qualitative HPLC fingerprint profile of ethanolic extract of leaves of *Hardwickia binata* (Figure 3) was selected at a wavelength of 254nm due to sharpness of the peaks and proper baseline. The profile showed six prominent peaks at a retention time of 1.547min, 2.293min, 2.699min, 2.987min, 4.725min and 6.005min.

Further advanced spectroscopic studies are required for structural elucidation and identification of compounds detected in three plants. The generated data may be useful in suggesting chemotaxonomical interrelationship of three plants.

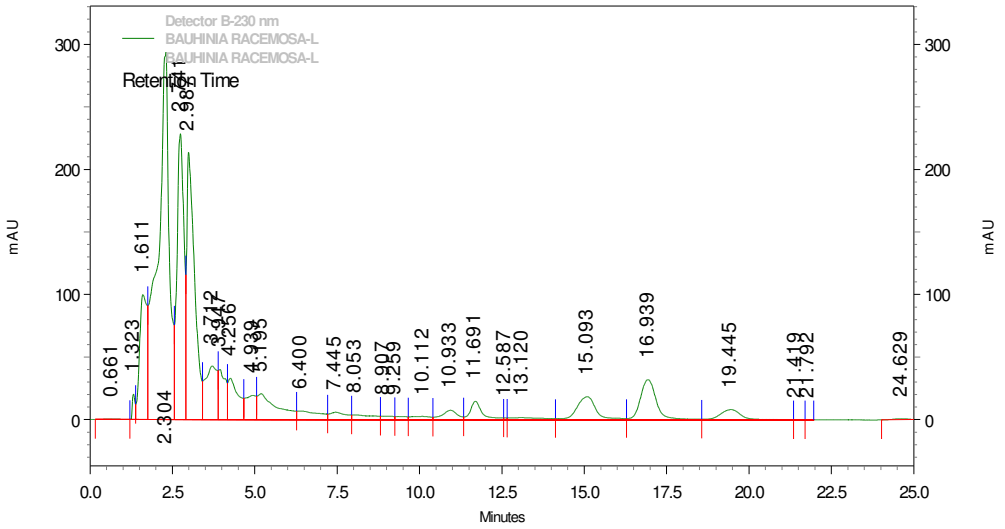

Figure 1. HPLC finger print profile of ethanolic extract of *Bauhinia racemosa* leaves

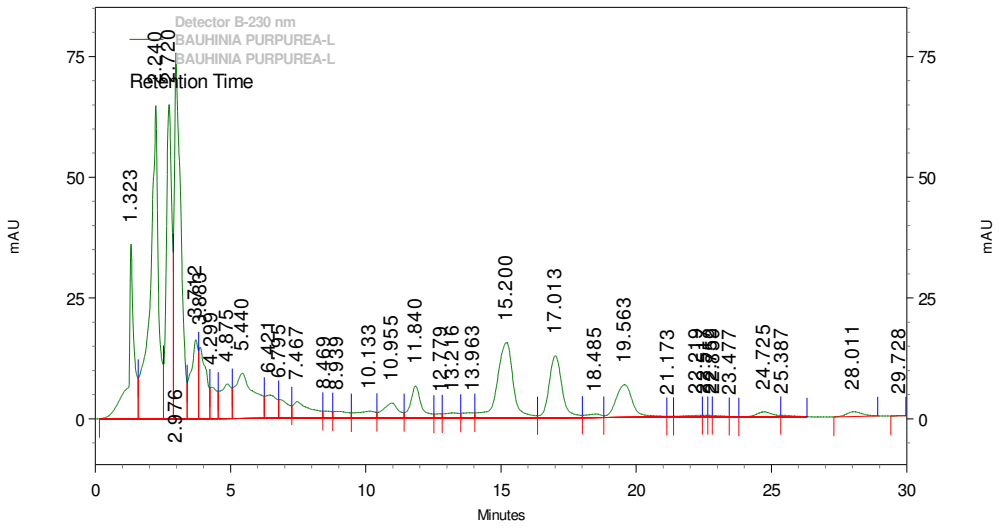

Figure 2. HPLC finger print profile of ethanolic extract of *Bauhinia purpurea* leaves

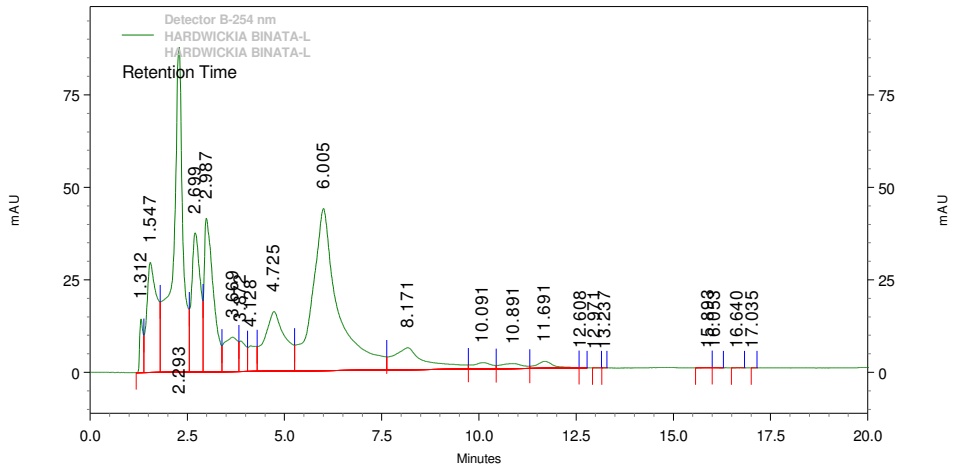

Figure 3. HPLC finger print profile of ethanolic extract of *Hardwickia binata* leaves

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