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Original Article

Pharmacognostic and phytochemical evaluation of *Prunus persica* (L.)

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ABSTRACT:
Objective: To evaluate the morphological, microscopical, physicochemical and phytochemical properties of leaves of *P. persica*. The study also represents the fingerprint profile of *P. persica* using high performance thin layer chromatography (HPTLC) technique.
Materials and Methods: Microscopical photographs of different magnifications were taken with Olympus Microscope, Model Olympus (India), attached to YOKO CCD Camera. Preliminary phytochemical screening was done and HPTLC studies were carried out. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS-4 software were used.
Results: Microscopical studies of the leaf confirmed the presence of polygonal epidermal cells with cuticle and anomocytic stomata, bicollateral vascular bundle, Prismatic shape calcium oxalate crystal, non-lignified multi-cellular trichomes. Mesophyll with 2-3 layered palisade cells. The results from HPTLC finger print scanned at wavelength 400 nm for ethyl acetate extract of *P. persica* leaf revealed the presence of fifteen phytoconstituents with R_f values ranged from 0.06 to 0.99 and ethanol extract of *P. persica* leaf showed the presence of sixteen phytoconstituents with the corresponding ascending order of R_f values ranged from 0.02 to 0.98.
Conclusion: The studies reveals the presence of alkaloids, glycosides, Flavonoids, carbohydrates, fixed oils, steroids, tannins & phenols, amino acids & proteins, all these findings included with HPTLC profile will be useful towards establishing pharmacognostic and phytochemical standards on identification, purity, quality and classification of the plant, which is gaining relevance in plant drug research, the identification and preparation of monograph of plant.

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INTRODUCTION

Prunus persica L. (Peach) is 15 to 25 feet tall tree with an equal or greater spread, Peach trees form a rounded crown with upwardly-reaching branches clothed in three to six-inch-long, dark green, deciduous leaves. The lovely flowers which appear in April before the new leaves unfold are available in single, semi-double, and double forms in colors ranging from pure white to deep red and bi-colors. The flowers are susceptible to damage by late spring frosts or especially cold winter [1].

P. persica L. (Peach) named as *Amygdalus persica* is a perennial & deciduous tree of the subfamily Prunoideae of the family Rosaceae. There are about 100 genera and 3,000 species in Rosaceae family. Prunus has nearly 200 species cultivated for their edible fruits and seeds. The leaves are anthelmintic, insecticidal, sedative, diuretic, demulcent, expectorant, and vermicidal and are used in leucoderma and in piles [2]. Leaf paste is used to kill worms in wounds and fungal infections. The treatment of gastritis, whooping cough and chronic bronchitis is carried out internally with leaves [3].

The flowers are considered as laxative and diuretic and are used to treat constipation and oedema. The fruit is used as a demulcent, an anti-scorbutic and a stomachic. Fruit being aphrodisiac, anti-pyretic, act as a tonic to the brain, enhance the blood, removes bad smell from the mouth, anti-tumor [4]. The seeds are used as an anthelmintic and emmenagogue [5]. The oil extracted from seeds is known as 'kapha', used as an abortifacient, good in deafness, piles, stomach troubles of children and earache. Peach kernels are used for blood diseases, menstrual disorders, coughs and rheumatism in China and Malaysia. The kernel oil is applied to impetigo. The bark is used in leprosy and jaundice [6].

Leaves of *P. persica* have been investigated for their antioxidant and anti-inflammatory activities in the past [7, 8]. The plant is self-fertile. Suitable for: light (sandy), medium (loamy) and heavy (clay) soils and prefers well-drained soil. Suitable pH: acid, neutral and basic (alkaline) soils. It cannot grow in the shade. It prefers moist soil. It is commonly cultivated in West Asia, Europe, Himalayas and India up to an altitude of 1000 ft. *Prunus persica* is a deciduous tree growing to 6 m.

The flowers are pink, red or white in color, hermaphrodite (have both male and female organs) and are pollinated by Bees. The Leaves are green color, alternate**,** simple, serrate margin, elliptic & lanceolate shaped, pinnate venation**,** deciduous persistence, Leaf blade length is 4 to 8 inches. The Fruits are round**,** 3 to 6 inches length, red or yellow color & fleshy covering.

MATERIALS AND METHODS

Collection and authentication:

The leaves of *P. persica* were collected from the neighbor village of Meerut (U.P.), and authenticated by Department of Botany, Meerut College Meerut. A voucher specimen was submitted for future reference (Ref No. MRTC/4/23/2/2014)

Pharmacognostic studies:

Macroscopic characteristics:

The morphological characteristics of leaves were studied and the photographs were drawn and taken with the help of Sony Corp. DSC-S980, 12.1-megapixel camera. Macroscopic evaluation has done for detailed study of morphology parameters such as color, odor, taste, shape, size, texture and fractures were determined.

Microscopic characteristics:

The microscopical studies including detailed qualitative parameters were carried out on mention part of the plant. Photographs of different magnifications were taken with Olympus Microscope, Model Olympus (India), attached to YOKO CCD Camera. Camera Lucida Mirror Type PHYSILAB was used for quantitative measurements like stomatal number, vein islets number and palisade ratio etc [9].

Physico-chemical parameters:

Various physico-chemical parameters as per W.H.O. guidelines have been determined for different extracts (Petroleum ether, ethyl acetate & ethyl alcohol). Some of them were Moisture content (LOD), Ash values (total ash, acid insoluble ash and water-soluble ash) and extractive values (alcohol and water soluble extractive values) [10].

Phytochemical screening:

Phytochemical compounds were detected in different solvent extracts (Petroleum ether, ethyl acetate & ethyl alcohol) by performing respective chemical tests. The leaves of *P. persica* were soxhlet extracted successively with the solvent in order of increasing polarity. All the extracts of the plant were subjected to phyto-chemical screening [11].

High-performance thin layer chromatography fingerprinting:

Phytoconstituents detection was done by high performance thin layer chromatography (HPTLC) of ethanol and ethyl acetate extracts to substantiate the standardization data of the plants. The standardization of a crude drug was an integral part of establishing its correct identity. The results of this investigation were serving as a basis for proper identification of the plant [12].

Preparation of sample solution:

Ethanol and ethyl acetate extracts were used in preparation of sample solution. 5 mg/ml concentration of each extracts were prepared in respective solvents of chromatographic grade and then filtered by Whatman filter paper No. 1. Prepared samples of all solvent extracts were applied on TLC aluminium sheets coated with silica gel 60 F 254 (Merck, Mumbai, India) using Linomat sample applicator.

Development of chromatograms:

A number of solvent systems were tried, for ethanol and ethyl acetate extracts for better resolution and maximum number of spots, but the satisfactory resolution was obtained in the solvent Chloroform: Methanol (9:1) for ethanol extract and Hexane: Chloroform: Methanol (7:2:1) for ethyl acetate extract [13]. The chromatograms were developed in twin trough glass chamber saturated with solvents for 20 minutes up to the distance of 80 mm. The air-dried plates were viewed in ultraviolet radiation to mid-day light.

Spots were visible without derivatization at 254 and 366 nm wavelengths but best results were shown when TLC plates were sprayed with detection reagent (Anisaldehyde sulfuric acid reagent and plate was heated at 110°C for 5 minutes) and then visualized in visible light range 400-600nm. Scanning was performed by CAMAG HPTLC densitometer Linomat V sample applicator equipped with a 2 µL Hamilton (USA) syringe in absorbance mode at both 254 and 366 nm, both extracts (ethanol and ethyl acetate) were also scanned at 350-600 nm using deuterium and tungsten lamp. The R_f values and color of the resolved bands were noted [14].

RESULTS AND DISCUSSION

Morphology: *Prunus persica* is a perennial, deciduous tree, and grows as rounded crown with upwardly-reaching branches clothed in three to six-inch-long, dark green, deciduous leaves (**fig. 1**). The flowers have double forms in colors ranging from pure white to deep red and bi-colors. The macroscopical characters of leaves of *P. persica* were observed & mention in **Table 1**.

Fig. 1: Photograph of leaves of *Prunus persica*

Table 1: Morphological characters of *P. persica leaves*

S. No.	Parameters	Description of leaves
1	Color	Dark green
2	Odor	Odorless
3	Taste	Characteristic
4	Length	4 cm - 8 cm
5	Width	2 cm- 4 cm
6	Texture	Smooth
7	Surfaces (upper & lower)	Smooth
8	Apex	Acute
9	Base	Symmetrical
10	Venation	Pinnate
11	Shape of lamina	Heart shaped, not lobed
12	Margin	Serrate
13	Arrangement	Alternate

Microscopy: On staining with phloroglucinol-HCl (1:1) T.S of leaf through the midrib, the following characteristics were observed (**figure 2**)**:**

- Presence of upper and lower epidermis: Polygonal epidermal cells with cuticle
- Bi-collateral vascular bundle (xylem cells covered with phloem cells by both side)
- Absence of pericycle at outside of phloem
- Presence of parenchyma cells and collenchyma cell

On staining and after glycerin mounting following powder characters were observed (**figure 3**):

- Prismatic shape calcium oxalate crystal
- Anomocytic stomata with epidermal cells
- Lignified spiral xylem vessels
- Starch gains
- Non- lignified multi-cellular trichomes
- Non- lignified unicellular & lignified multi-cellular fibres
- Mesophyll with 2-3 layered palisade cells and spongy parenchyma cells

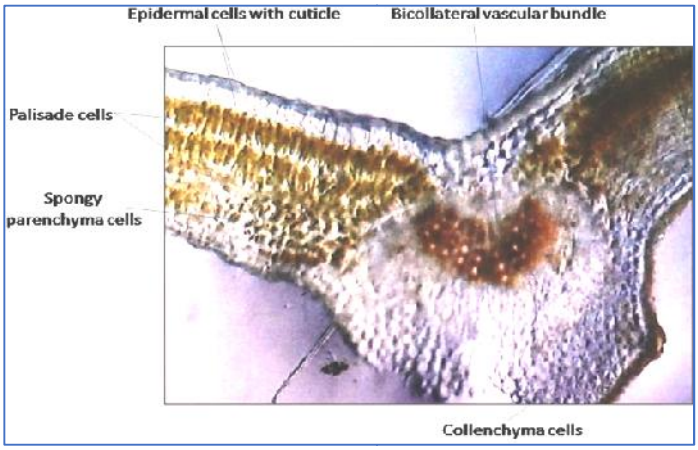

Fig. 2: T.S. of *P. persica* leaf through midrib

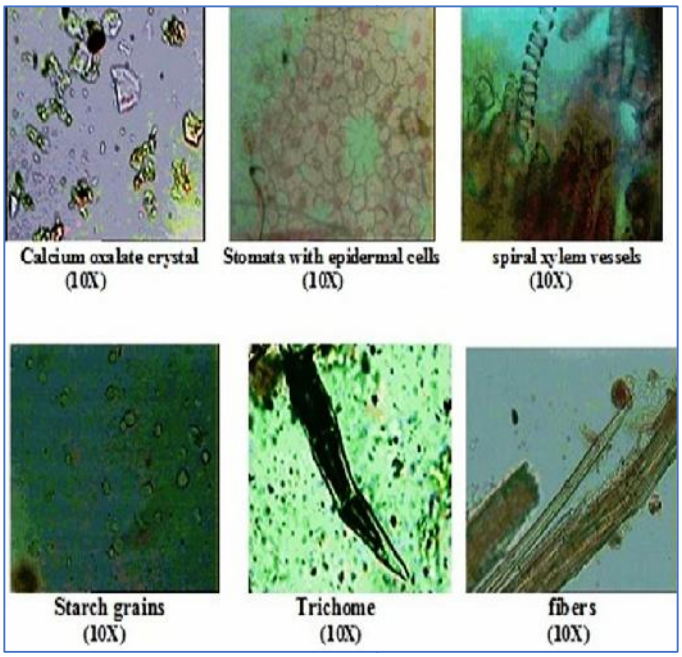

Fig. 3: Photographs of powder microscopic characteristics

As a part of quantitative microscopy (Leaf constants), stomatal number (Upper & lower surface), stomatal index (Upper & lower surface), vein islet number, Palisade ratio were determined for the epidermis of leaves of *P. persica* (**Table 2**).

Physico-chemical evaluation: Various physico-chemical parameters were analyzed by observations of three samples for each parameter and an average value of each parameter was determined as reported in **Table 3**.

Table 2: Leaf constants of *P. persica*

Parameter	Observation
Stomatal number (Upper surface)	262-344 per mm^2
Stomatal number (Lower surface)	402-443per mm^2
Stomatal index (Upper surface)	18
Stomatal index (Lower surface)	29
Vein islet number	59-64 per mm^2
Palisade ratio	6-8

Table 3: Physicochemical parameters of *P. persica* leaves

Parameters	Values (% w/w)
Total Ash	10.43%*
Acid Insoluble Ash	2.52%*
Water Soluble Ash	6.09%*
Loss on Drying	18.73%*
Ether Soluble Extractive Value	2.09%*
Alcohol Soluble Extractive Value	13.31%*
Water Soluble Extractive Value	13.62%*

*n=3, (Mean value)

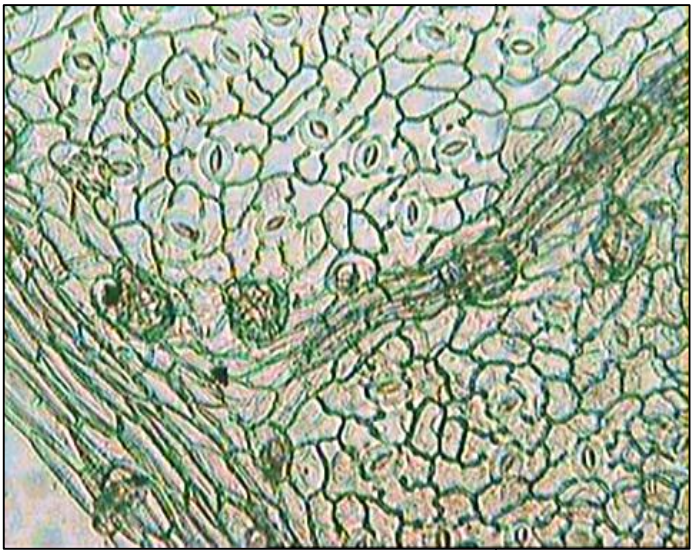

Fig. 4: Epidermis of *Prunus persica* leaf

Microbial load: The procedure of microbial load determination described in Indian Pharmacopoeia. The leaves powder of *P. persica* were evaluated for determination of microbial load by following two methods namely spread plate method and pour plate method.

Table 4: Microbial load: Leaves of *P. persica*

By Spread Plate Method (aerobic)	2524×10^3 Microbes/g of crude drug*
By Pour Plate Method (anaerobic)	234×10^3 Microbes/g crude drug*

*n=3, (Mean value)

Preliminary phytochemical screening: The successive extraction was carried out using petroleum ether, ethyl acetate and ethanol as solvents, the extract was dried using rotary evaporator and percentage yield was determined (Table 5).

Table 5: Percentage yield on successive extraction in soxhlet apparatus

Extracts	Yield (% w/w)
Petroleum ether	3.59%
Ethyl acetate	7.25%
Ethanol	16.62%

Preliminary phytochemical screening of all the three extracts of *p. persica* leaves was performed to have an idea of the phytochemical groups present in the plant part (**Table 6**).

HPTLC Profile: The present study was first to report the HPTLC fingerprint of ethanol and ethyl acetate extracts of *P. persica* leaves showing maximum number of components 16 and 15 respectively at 400nm. From the HPTLC studies, it has been found that ethanol and ethyl acetate extracts contain a mixture of compounds. The study revealed that *P. persica* showed best results in solvent systems Chloroform: Methanol (9:1) for ethanol extract and Hexane: Chloroform: Methanol (7:2:1) for ethyl acetate extract respectively. After scanning and visualizing the plates in absorbance mode at both 254 nm &366 nm and visible light range (400-600nm after spraying with anisaldehyde sulphuric acid reagent) best results were shown at 400nm. The HPTLC images shown in **Fig. 8** indicate that all sample constituents were clearly separated without any tailing and diffuseness. The results from HPTLC finger print scanned at wavelength 400 nm for ethyl acetate extract of *P. persica* leaf revealed the presence of fifteen polyvalent phytoconstituents. The R_f values ranged from 0.06 to 0.99. It is also clear from the chromatogram that out of 15 components, the component with R_f values 0.95 & 0.82 were found to be more predominant as the percentage area is more with 28.85% and 12.91% respectively.

Table 6: Phytochemical screening of various extracts of leaves of *P. persica*

S. No.	Phytoconstituents	Pet. ether Extract	Ethyl acetate Extract	Ethanol Extract
1	Alkaloids	-	-	+
2	Glycosides	-	+	+
3	Tannins and Phenols	-	+	+
4	Flavonoids	-	+	+
5	Steroids/ Triterpenoids	-/-	-/-	+/-
6	Proteins and Amino acids	-	+	+
7	Carbohydrates	-	+	+
8	Fats and fixed oils	+	+	-

(+) = Present, (-) =Absent

The HPTLC finger print scanned at wavelength 400 nm for ethanol extract of *P. persica* leaf showed sixteen polyvalent phytoconstituents and corresponding ascending order of Rf values ranged from 0.02 to 0.98 in which highest concentration of the phytoconstituents was found to be 30.26% and its corresponding R_f value was found to be 0.98 and was recorded. TLC plate showed different color phytoconstituents of *P. persica* ethanol extract by presence of one greenish, three purple, two yellow, one pink and three merged yellowish orange bands showing the presence of terpenoids, steroids and saponins after spraying with anisaldehyde sulphuric acid reagent. Thus, the developed chromatogram is specific with selected solvent system, R_f value and serve the better tool for standardization of the extract. HPTLC fingerprint of a plant species helps in the proper identification and quality control of a particular plant species and also provides basic information regarding isolation, purification, characterization and identification of marker chemical compounds of the species [15].

Thus, the present study provides sufficient information about phytoconstituents present in the ethyl acetate and ethanol extracts of *P. persica* and in the identification, standardization and quality control of this medicinal plant.

Table 7: Data pertaining to HPTLC fingerprint of Ethyl acetate extract of *P. persica* leaf at 400 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area%
1	0.02	2.4 AU	0.05	130.6 AU	1.21	0.06	122.3 AU	107.50 AU	1.14
2	0.07	10.5 AU	0.09	136.3 AU	0.75	0.11	134.5 AU	175.2 AU	1.80
3	0.11	0.30 AU	0.18	18.8 AU	0.37	0.20	13.7 AU	41.2 AU	0.39
4	0.23	14.5 AU	0.29	140.5 AU	3.73	0.31	135.7 AU	389.3 AU	3.86
5	0.21	26.4 AU	0.41	152.3 AU	1.82	0.44	143.4 AU	692.2 AU	7.05
6	0.34	4.6 AU	0.49	137.9 AU	2.62	0.53	134.5 AU	401.3 AU	3.37
7	0.38	35.2 AU	0.51	148. AU	2.14	0.56	144.3 AU	322.6 AU	2.84
8	0.45	75.4 AU	0.57	170.9 AU	6.21	0.61	162.3 AU	482.3 AU	5.60
9	0.54	23.4 AU	0.65	174.7 AU	5.27	0.65	169.4 AU	388.2 AU	4.87
10	0.68	85.3 AU	0.70	165.8 AU	5.55	0.70	163.1AU	748.3 AU	6.18
11	0.71	0.42 AU	0.74	233.4 AU	9.16	0.75	229.5 AU	850.4 AU	8.00
12	0.78	22.5 AU	0.80	292.4 AU	11.95	0.82	289.8 AU	1345.7 AU	12.91
13	0.82	12.6 AU	0.84	298.8 AU	7.88	0.86	293.4 AU	913.4 AU	8.36
14	0.89	33.1 AU	0.92	535.6 AU	26.28	0.95	524.9 AU	3033.2 AU	28.85
15	0.93	76.6 AU	0.97	277.4 AU	3.89	0.99	270.3 AU	553.33 AU	4.79

Table 8: Data pertaining to HPTLC fingerprint of Ethanol extract of *P. persica* leaf at 400 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area%
1	0.01	1.8 AU	0.02	111.2 AU	1.28	0.02	109.2 AU	24.8 AU	0.31
2	0.03	15.6 AU	0.04	138.3 AU	1.70	0.05	136.4 AU	15.4 AU	0.73
3	0.04	1.33 AU	0.06	108.5AU	0.41	0.07	102.7 AU	32.8 AU	0.37
4	0.06	11.5 AU	0.09	191.5 AU	0.25	0.11	189.7 AU	310.2AU	2.81
5	0.12	23.4 AU	0.14	106.3 AU	0.29	0.14	104.9 AU	58.3 AU	0.54
6	0.16	4.4 AU	0.19	104.8 AU	2.38	0.22	103.8 AU	95.65AU	1.22
7	0.22	25.2 AU	0.28	381.2 AU	11.53	0.30	377.5 AU	783.4AU	8.14
8	0.40	31.3 AU	0.43	310.3 AU	17.65	0.44	30.6 AU	1022.1AU	13.60
9	0.48	63.3 AU	0.52	145.9 AU	2.84	0.52	141.6 AU	230.7AU	2.42
10	0.54	55.7 AU	0.55	168.4 AU	3.23	0.56	172.5 AU	405.2AU	3.46
11	0.61	1.4 AU	0.65	209.4 AU	9.37	0.65	205.6 AU	755.5AU	8.49
12	0.64	2.8 AU	0.67	170.4 AU	4.31	0.69	167.2AU	289.5AU	3.52
13	0.66	20.6 AU	0.70	211.5 AU	7.28	0.74	210.6 AU	584.6 AU	6.05
14	0.76	38.4 AU	0.78	252.6 AU	8.48	0.80	242.6 AU	922.4AU	9.83
15	0.81	66.6 AU	0.82	275.8 AU	7.93	0.84	268.8 AU	850.8AU	8.26
16	0.90	28.4 AU	0.96	605.4 AU	28.65	0.98	600.2 AU	3247.5AU	30.26

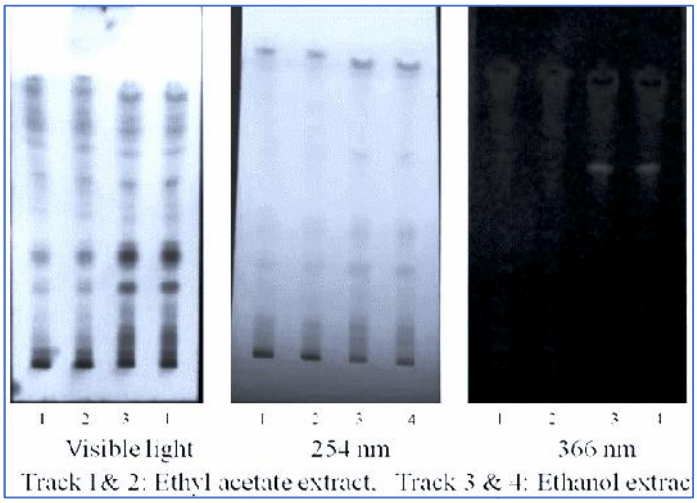

Fig. 5: HPTLC profile of leaf extracts of *P. persica*

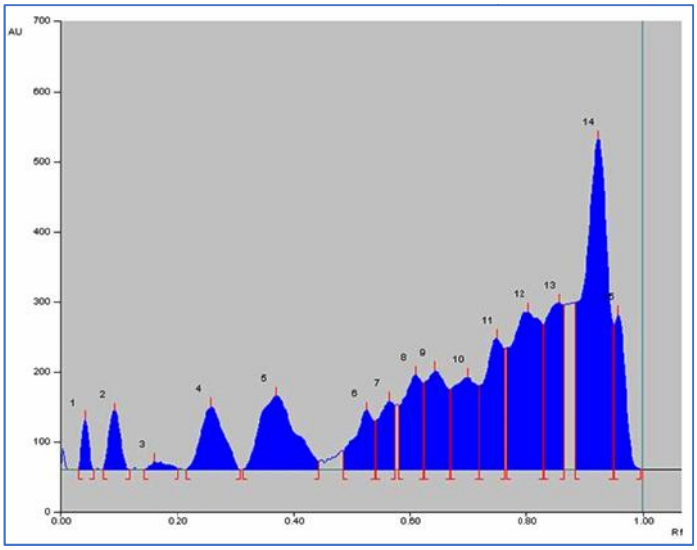

Fig. 7: Chromatogram of ethyl acetate extract of *Prunus persica* leaf at 400 nm

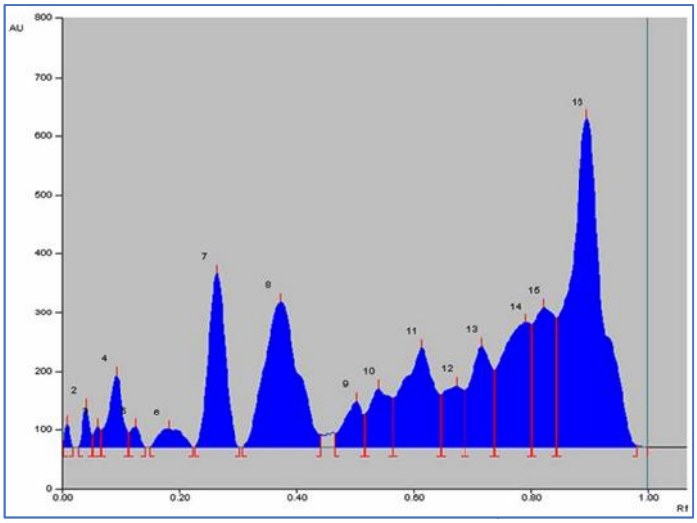

Fig. 8: Chromatogram of ethanol extract of *Prunus persica* leaf at 400 nm

CONCLUSION

Peach is an important common plant, the fruit of which is integral part of diet, being consumed as a seasonal nutritive fruit. Physico-chemical evaluation & Chemical screening of different solvents extracts showing the presence of alkaloids, glycosides, Flavonoids, carbohydrates, fixed oils, steroids, tannins & phenols, amino acids & proteins, all these findings included with HPTLC profile will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the plant, which is gaining relevance in plant drug research, the identification and preparation of monograph of plant. In developing countries like ours one must fully explore this important medicinal plant which might provide us some important "leads/hits" in near future.

DISCLAIMER

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