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Phytochemical screening and HPTLC fingerprinting of leaf extracts of *Psidium guajava* Linn.

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ABSTRACT
Objective: To establish physical constants and fingerprint profile of ***Psidium guavaja Linn*** using high performance thin layer chromatography (HPTLC) technique. **Methods:** Preliminary phytochemical screening was done, physical constants were evaluated 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:** Preliminary phytochemical screening of the extracts showed the presence of alkaloids, triterpenes, tannins, saponins, glycosides, phenolic compounds and flavonoids. The proximate analysis showed satisfactory result with respect to foreign matter, moisture content, ash value and extractive values. HPTLC finger printing of methanol extract of leaf powder revealed presence of three polyvalent phytoconstituents with their R_f value 0.95, 1.11, 1.41 at 220nm. Component number 3 at R_f 1.41 showed maximum concentration and presence of total five components with their R_f value 0.18, 0.91, 1.21, 1.42, 1.52 at 450nm.Component number 4 at R_f 1.41 showed maximum concentration. Aqueous extract of leaf powder showed total six components with their R_f value 0.29, 0.74, 0.85, 0.96, 1.31 at 220nm.Component number 4 at R_f 0.96 showed maximum concentration. **Conclusions:** It can be concluded that HPTLC fingerprint analysis of leaf powder extract of *Psidium guavaja* can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

Keywords: *Psidium guavaja*, Phytochemical Screening, Physical constants, HPTLC Fingerprinting.

1. Introduction
India is one of the 12-mega biodiversity centre having over 45,000 plant species. Its diversity is unmatched due to the presence of 16 different agro climatic zones, 10 vegetative zones and 15 biotic provinces. India also has equivalent to 3/4th of its land exclusive economic zone in the ocean harbouring a large variety of flora and fauna, many of them with therapeutic properties. About 1500 plants with medicinal uses are mentioned in ancient text and around 800 plants have been used in traditional medicine. But more attention has been focused mainly on their active constituents [1]. The plant kingdom still hold many species of plant containing substance of medicinal value which have yet to be discovered and the large no. of plant are constantly being screened for their possible pharmacological value in addition to already exploited plants [2].
Many medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system, (Ayurveda) and proposed for their interesting multilevel activities. Amongst the medicinal plants used in Ayurvedic preparations for their therapeutic action, some have been thoroughly investigated and some of are still to be explored [3].
Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture and an important tool in bioactive compound analyses [4].
Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards [5].

HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time [6]. High-performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. HPTLC also facilitates repeated detection of chromatogram with same or different parameters [7].

Guava (*Psidum guajava L.*) belongs to the Family Myrtaceae [8]. It is a low growing tree of 6 to 25 ft (1.8 to 7.5 m) high, but in some varieties may reach 40 ft (12 m) under favourable conditions. *P. guajava* is an American fruit which is well known throughout the tropics, and in some countries it has become naturalized on waste lands. Guava tree has long history of medicinal uses that are still employed till date. The infusion decoction made from the leaves and/or bark of the tree has been used for treatment of diarrhea, malaria and dysentery [9]. It has been used in the treatment of sore throats, vomiting and menstrual complications [10]. Tender leaves are chewed for bleeding gum and bad breath. It is said to prevent hangover if chewed before drinking. Indians also use it as a douche for vaginal discharge, and to tighten vaginal walls after childbirth [11]. A decoction of the bark or leaves is used topically to treat wound and skin sores. These therapeutic uses of guava are expected to be due to the presence of some components [12].

2. Materials and Methods

2.1 Plant material

The plant specimens for the proposed study were collected from Garden of Allana College of Pharmacy, Pune, Maharashtra, India and authenticated by Dr. P.G. Diwakar, Joint Director, Botanical survey of India, Pune, Maharashtra, India.

Table No. 1

S.no	Chemical Test	Aqueous Extract	Chloroform Extract	Methanol Extract	Ethanol Extract
1	**Test for Alkaloids :** a) Dragendorff's test b) Mayer's test c) Hagers's test	 + - -	 + - -	 + - -	 + - -
2	**Test for Tannins :** a) Ferric chloride test b)Lead acetate test c) Potassium Dichromate test d) Dilute HNO_3	 + + + +	 + - - -	 + + + +	 + + + +
3.	**Test for flavonoid** a) Lead acetate test b) Ferric chloride test c) Sodium Hydroxide test d) Shinoda test	 + - + +	 + - + +	 + + + +	 + + + +
4.	**Test for Steroids:** a) Salkowski test b) Liebermann – Burchard Reaction	 + +	 + +	 + +	 +
5.	**Saponification test:** Foam test	 +	 -	 +	 +
6.	**Test for Cardiac Glycosides:** a) Keller-Killiani test b) Legal's test	 - +	 - +	 + +	 + -
7.	**Test for Anthraquinone Glycosides:** Borntrager's test	 -	 -	 +	 +
8.	**Test for Saponin Glycosides:** a) Foam Test b) Heamolytic test	 + +	 - -	 + +	 + +
9.	**Test for Carbohydrates** a) Molisch's test b) Fehlings test c) Benedict test	 + + -	 - + -	 + + +	 + + -
10.	**Test for Proteins:** a) Biuret test b) Millions test	 - +	 - -	 + -	 - -
11.	**Test for amino acids:** Ninhydrin test	-	-	-	-

Note: + indicates presence of phytoconstituents, - Indicates absence of phytoconstituents

2.2 Preparation and Extraction of Plant Material

In the present study the leaf powder of *Psidium guavaja* defatted with petroleum ether and 100 g was packed in a Soxhlet apparatus and extracted successively with chloroform & methanol & ethanol. The extraction was carried out until the extractive becomes colorless. Aqueous extraction was also carried out by decoction method. The extract was filtered through a cotton plug, followed by Whattman filter paper (no.1). The extract was evaporated under reduced pressure using Rotovac evaporator.

2.3 Phytochemical Screening: The phytochemical investigation of the different extracts of *Psidium guavaja* was carried out with standard protocol. The phytochemical tests were carried out with chloroform, methanol, ethanol & water. The results are presented in Table 1.

2.4 Evaluation of physical constants

Foreign Matter, Moisture Content, Total Ash Value, Water Soluble Ash Value, Acid Insoluble Ash Value , Soluble, Extractive Value in Water, Chloroform, Methanol and Ethanol were carried out[13]. The results are presented in Table 2.

Table No 2.

Sr. no.	Evaluation Parameter	Value (%)	St. dev.
1	Foreign Matter	1.09	0.176
2	Moisture Content	10.52	0.15
3	Total Ash Value	7.4	0.264
4	Water Soluble Ash Value	3.633	0.152
5	Acid Insoluble Ash Value	1.063	0.118
6	Water Soluble Extractive Value	7.266	0.115
7	Chloroform Soluble Extractive Value	0.366	0.152
8	Methanol Soluble Extractive Value	9.466	0.098
9	Ethanol Soluble Extractive Value	5.333	0.251

The Methanol soluble extractive value found to be the highest (9.466%) where water soluble (7.266%), Chloroform (0.3%) and Ethanol (5.33%) respectively

2.5 HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of Harborne [14] and Wagner [15] *et al.*

2.5.1 Sample Preparation

Methanolic and aqueous extracts obtained were evaporated under reduced pressure using rotovac evaporator. Each extract residue was re-dissolved in 1ml of chromatographic grade methanol, which was used for sample application on pre-coated silica gel 60F254 aluminum sheets.

2.5.2 Developing Solvent System: A number of solvent systems were tried, for extracts, but the satisfactory resolution was obtained in the solvent n hexane: Ethyl acetate (7:3) & methanol: water (7:3) for methanolic & aqueous extracts respectively.

2.5.3 Sample Application: Application of bands of each extract was carried out (4mm in length and 1 µl in concentration for
Extract) using spray technique. Sample were applied in duplicate on pre-coated silica gel 60F254
Aluminum sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG
HPTLC system, which was programmed through WIN CATS software.

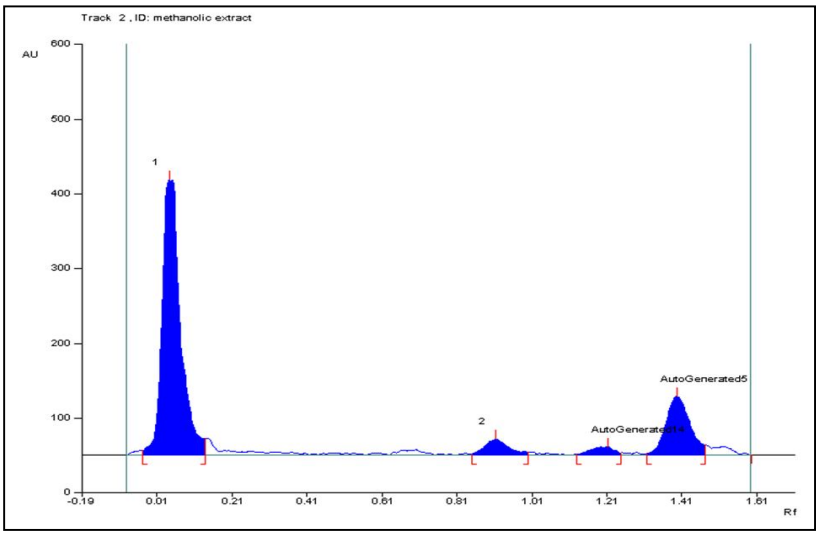

Fig 1: Chromatogram: HPTLC of methanolic extract of *Psidium guavaja* (Resolution at 220 nm; vol-20µl, mobile phase- Mobile phase-n-hexane-ethyl acetate (7:3)

2.6 Development of Chromatogram
After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x 10 cm saturated with solvent n hexane: Ethyl acetate (7:3) & methanol: water (7:3) for methanolic & aqueous extract for 15 minutes. The air-dried plates were viewed in ultraviolet radiation to mid-day light (Figure 1). The chromatograms were scanned by densitometer at 200 nm for methanolic extract & 224 nm for aqueous extract after spraying with anisaldehyde sulphuric acid. The R_f values and finger print data were recorded by WIN CATS software

3. Results and Discussion
3.1 Phytochemical Screening
The phytochemical test on petroleum ether, chloroform, methanol & aqueous extracts of *Psidium guavaja* powder showed the presence of various phytoconstituents like alkaloid, carbohydrate, glycoside, steroid, protein, tannin, terpenoid, flavonoid and phenolic compounds are present (Table no.1)

3.2 Evaluation of physical constants:
The proximate analysis showed satisfactory result with respect to foreign matter, moisture content, Ash value and Extractive values. (Table no.2)

3.3 High Performance Thin Layer Chromatography
3.3.1 HPTLC of methanolic extract: The results from HPTLC finger print scanned at wavelength 220 nm for methanolic extract of *Psidium guavaja* leaf powder. There are three polyvalent phytoconstituents with their R_f value 0.95, 1.11, 1.41, Component number 3 at 1.41at R_f value showed maximum concentration

3.3.2 HPTLC of of MeOH extract of *Psidium guavaja*: The results from HPTLC finger print scanned at wavelength 450 nm for methanolic extract of *Psidium guavaja* leaf powder showed the presence of total five components with their R_f value R_f – 0.18, 0.91, 1.21, 1.42, 1.52 Component number 4 at 1.41at R_f showed maximum concentration.

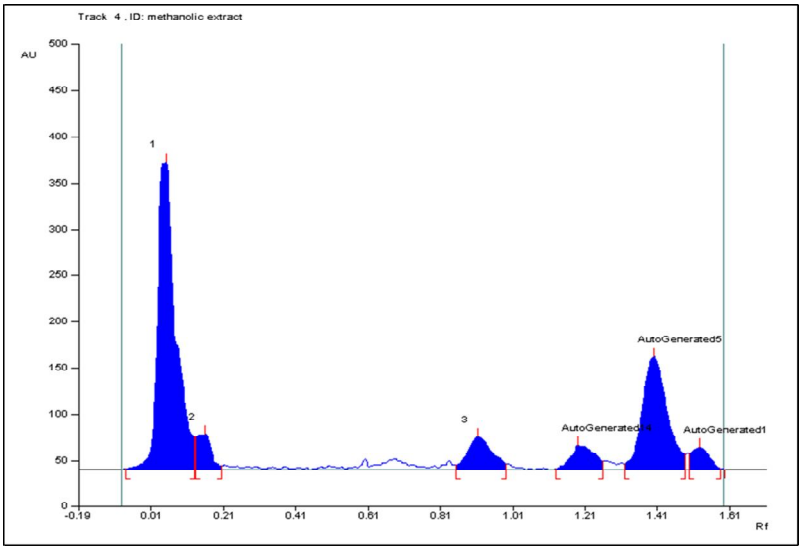

Fig 2: Chromatogram: HPTLC of methanolic extract of *Psidium guavaja* (Resolution at 450 nm; vol-10 µl, Mobile phase-n-hexane: ethyl acetate (7:3)

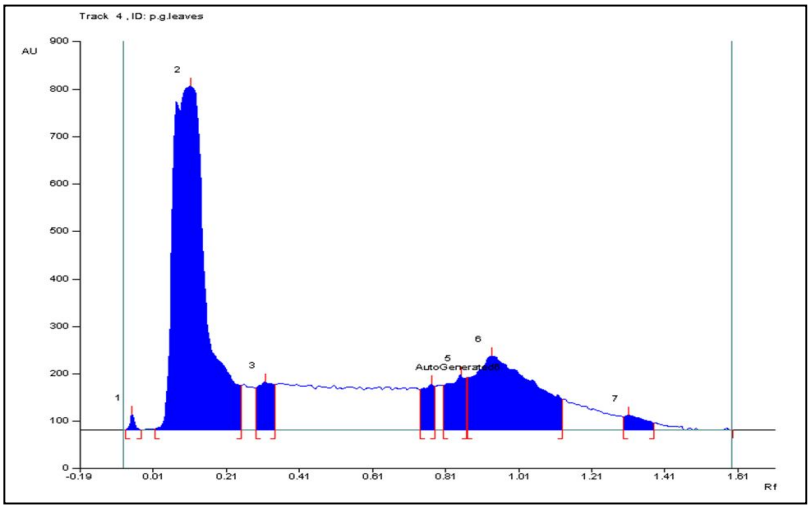

Fig 3: Chromatogram: HPTLC of aqueous extract of *Psidium. guavaja* (Resolution at 220 nm; vol-20 µl, Mobile phase Methanol: water (7:3)

3.3.3 HPTLC of Aqueous extract of *Psidium guavaja*: The results from HPTLC finger print scanned at wavelength 220 nm for aqueous extract of *Psidium guavaja* leaf powder showed the presence of total six components with their R_f value 0.29, 0.74, 0.85, 0.96, 1.31. Component number 4 at 0.96 R_f value showed maximum concentration.

4. Conclusion

Herbal medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the herbal medicine. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants. In the present study preliminary phytochemical screening showed presence of alkaloid, carbohydrate, glycoside, steroid, protein, tannin, terpenoid, flavonoid and phenolic compounds. These methods were also employed to analyze commercial samples to illustrate their application in qualitative (fingerprint) and quantitative determination, demonstrating their feasibility in the quality control of phytoconstituents from mentioned Herbal drugs and formulations. This will help induced to come out uniform standard products, which will restore faith of product and alternative herbal medicine therapy.

Evaluation of all physical constants established shown satisfactory results. Methanolic extractive value was found maximum. HPTLC chromatogram of methanolic and aqueous extract results showed that there are many compounds in *Psidium guavaja*. From the HPTLC studies, it has been found that methanol & aqueous extracts contain not a single compound but a mixture of compounds and so it is established that the pharmacological activity shown by them are due to the cumulative effect of all the compounds in composite.

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