Original Research Paper

Volume - 14 | Issue - 09 | September - 2024 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Biological Science

PRELIMINARY PHYTOCHEMISTRY, FLUORESCENT ANALYSIS AND HPTLC PROFILE OF PSIDIUM GUAJAVA (LINN.) LEAVES EXTRACT

Rupali P. Shirsat

Department Of Botany, Shri Dr. R. G. Rathod College Of Arts And Commerce, Murtizapur Dist- Akola (ms) 444107

ABSTRACT Psidium guajava (L) is a common fruit plant in the Myrtaceae family. The fruits of this plant are being eaten for their nutritious value and taste. Here, the present attempt is to study the preliminary phytochemistry of the leaves of this plant, its fluorescent analysis, and HPTLC profiling of ethanolic extract. It was observed that the plant is rich in major phytochemicals and the crude powder showed different coloration when treated with available laboratory reagents and chemicals indicating presence of a variety of phytochemicals. Further, the HPTLC profile indicates the availability of alkaloids, saponins, flavonoids, quercetin, vanillic acid, and parvoside- I. This suggests that the leaves extract contains different medicinally important phytochemicals; thus, the sample could be used as a future candidate for the formulation of related drug.

KEYWORDS : Psidium guajava (L), Myrtaceae, Phytochemicals, Fluorescence analysis, HPTLC.

INTRODUCTION

Psidium guajava (L.), commonly known as Guava is a member of the family Myrtaceae. The plant is native to Central and Southern America. It is a common fruit plant in central and northern India. Traditionally its fruits are eaten by the people for their nutritious value and taste. Several workers reported guava as a functional food rich in multifaceted nutrition[1-3]. It was reported earlier that the fruit is rich in vitamin A, vitamin C, dilatory fibers, and antioxidants [1]. However, very few scientists have worked on the phytochemistry of its leaves and their possible role in medicine. Here is an attempt to investigate the fundamental chemical compounds of leaves of guava plants with respect to its preliminary phytochemistry, fluorescent analysis, and HPTLC profile.

MATERIAL AND METHODS

The plant material of guava was collected from Dr. Panjabrao Deshmukh Krishi Vidyapith Horticulture Department, Akola (MS) India. The plant was identified as per the flora of Marathwada[4] , photographed and leaves were collected for further phytochemical analysis. The leaves were shade-dried for 7-8 days and later ground to fine powder to obtain crude drug which was used for further experimentation.

Preliminary Phytochemical Analysis: All the phytochemical tests for the major phytochemical compounds were performed as per the established protocols[5-6].

Fluorescent Analysis: The fluorescent analysis was done employing the methods elaborated by Jagtap and Koche[7].

HPTLC analysis: For the HPTLC analysis, the crude powdered sample was sent to Qualichem Laboratory, Nagpur (MS) India, and the chromatogram was interpreted in the light of recent researches.

OBSERVATION AND RESULTS

Psidium guajava L. belongs to the Mrytaceae family, commonly known as Guava; it is a shrub or small tree up to 10 m tall. Smooth, green leaves are elliptic with short petioles (7-15 cm long, 3-5 cm wide). The leaf margin is entire, has smooth edges, and not toothed. The upper leaf surface is smooth, while the lower surface is slightly down. Guava is commonly a tropical fruit cultivated in many regions. The stems are covered in a smooth, light reddish brown, hairy skin line epidermis. As per the literature of traditional medicine, guava is rich in vitamin C and strengthens the immune system, it can reduce constipation and stomach-aches and reduce body inflammation. The leaves are used to regulate blood sugar levels and cure acne, eczema and respiratory issues.

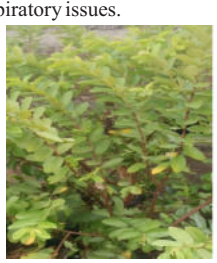

Fig. 1: Plant Psidium guajava L. in its habitat

Preliminary Phytochemical Analysis:

The preliminary phytochemical analysis of P. guajava leaves is presented in table-1. The qualitative tests were caried out for 09 major phytochemicals including alkaloids, phenolics, flavonoids, steroids, quinones, glycosides, saponins, and tannins. It was noted that most phytoconstituents are present in ethanolic extracts. The extract of distilled water showed the presence of terpenoids, steroids, phenolics and tannins. The qualitative tests of ethanolic extract for alkaloids, flavonoids, terpenoids, quinones, steroids, phenolics, glycosides, and Tannins. In the chloroform extract, only terpenoids, flavonoids, and phenols and tannin (Table-1). Thus, it indicates that among the selected solvents, ethanol is the most suitable solvent to obtain the most number of phytochemicals from the crude plant leaves powder. The experimental results are given in Table 1.

Table 1: Preliminary phytochemical test of Psidium guajava L.

Sr. no.	Test	Distilled water	Ethanol	Chloroform
1	Alkaloid	-	+	-
2	Flavonoid	-	+	+
3	Terpenoids	+	+	+
4	Quinones	-	+	-
5	Steroids	+	+	-
6	Phenol	+	+	+
7	Glycosides	-	+	-
8	Saponin	-	-	-
9	Tannin	+	+	+

Note: The results are average of triplicate analysis.

Fluorescent analysis:

Fluorescent analysis of the leaf powder of P. guajava was done as per the method of Jagtap and Koche (2023). The main objective behind this is to give some important tests to authenticate powdered drug material of said plant and to identify the adulteration if any. During this study, the powder was tested as it is and in combination with different chemicals and reagents present in the laboratory. The powder as such appear green under sunlight but in UV light the color changes to dark green. With concentrated HCL, the powder extract showed a dark brown color under normal sunlight, however, under UV light it turns light green. But in 50% HCL, it appears light green that turn into yellow under UV light. With concentrated H_2SO_4, the powder extract appears reddish brown and turns blackish brown but in the case of 50% H_2SO_4, it was yellow in normal sunlight and turned brown in UV light. With NaOH, the powder appeared orange in normal sunlight and turns yellow under UV- light. With ferric chloride solution, the powder showed a black appearance in sunlight that turned dark green while with 50% nitric acid, the powder showed a yellow color in sunlight that turned brownish green. The experimental proof is given in photoplate-2.

Table 2. Fluorescent analysis of P. guajava leaf powder

Sr. no.	Test (Powder)	Sunlight	UV- Light

1	Powder such as	Green	Dark green
2	Powder + HCL	Dark brown	Light green
3	Powder + 50% HCL	Light green	Yellow
4	Powder + H2SO4	Redish brown	Blackish brown
5	Powder + 50% H2SO4	Yellow	Brown
6	Powder + NaOH solution	Orange	Yellow
7	Powder + Ferric Chloride 5%	Black	Dark green
8	Powder + Nitric acid 50%	Yellow	Blackish green

HPTLC profiling:

The HPTLC analysis facility was availed from Qualichem Laboratories Pvt. Ltd. Nagpur. The analysis of done using a glass tank chamber (10 x 10 cm) with a solvent system Toluene: Ethyl Acetate: Formic acid (5:4:0.2). The solvent front position 70.00 mm, dryer used- Oven with temperature 60oC and time 5 minutes. The detector used was CAMAG TLC Scanner "Scanner_171005" S/N 171005 (2.01.02) with a scanning speed of 20mm/S and data resolution of 100mm/step. The analysis was done on two wavelengths 254 nm and 366 nm.

HPTLC analysis of P. guajava leaf extract on 254 nm:

At 254 nm, the HPTLC chromatogram showed 15 different peaks (Fig. 2) and visualized the same number of bands on the TLC visualizer (Fig. 2). The details of different peaks are presented in Table-3, including peak number, start rf, maximum rf, the height of peak and peak area, etc. This information is necessary to identify the respective compounds giving that peak or band.

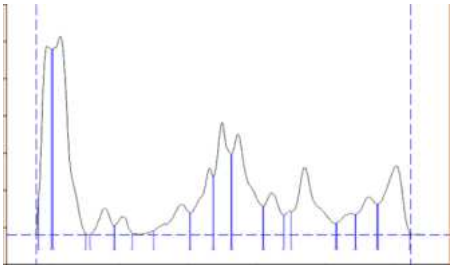

Fig. 2. HPTLC chromatogram of P. guajava leaf extract at 254 nm

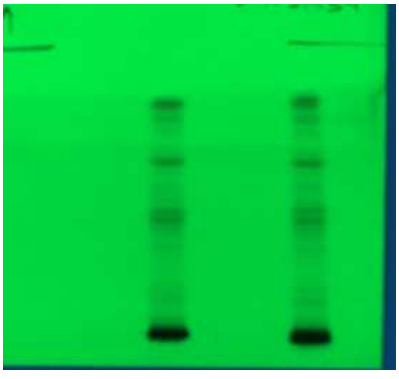

Fig. 3. HPTLC Bands for P. guajava leaves extract at 254 nm

Table-3: Details of HPTLC chromatogram of P. guajava leaf extract at 254 nm.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Compound
1	-0.04	8.0	-0.00	605.7	13.52	0.01	590.9	10424.6	9.28	Un-identified
2	0.01	592.7	0.01	603.3	13.47	0.02	586.9	5371.5	4.78	Un-identified
3	0.02	587.1	0.03	620.9	13.86	0.12	47.3	16150.3	14.37	Un-identified
4	0.12	47.5	0.16	113.9	2.54	0.19	64.7	3776.8	3.36	**Saponin**
5	0.20	65.7	0.22	83.7	1.87	0.25	28.0	2143.4	1.91	Un-identified
6	0.29	36.3	0.39	119.4	2.67	0.41	96.2	5707.4	5.08	Un-identified
7	0.41	96.8	0.46	229.1	5.12	0.47	211.3	6286.4	5.59	Un-identified
8	0.47	213.4	0.50	372.5	8.32	0.52	309.3	8638.3	7.69	Un-identified
9	0.52	310.7	0.53	350.6	7.83	0.56	180.7	7324.9	6.52	**Pervoside-I**
10	0.57	179.3	0.58	183.2	4.09	0.60	128.5	3556.6	3.17	Ferulic acid
11	0.60	128.6	0.63	168.1	3.75	0.67	83.6	5641.0	5.02	**Alkaloid**
12	0.67	83.9	0.69	110.6	2.47	0.70	106.5	1887.3	1.68	Un-identified
13	0.70	106.5	0.74	260.8	5.82	0.83	76.4	11506.7	10.24	Vanillic acid
14	0.85	97.2	0.93	213.0	4.76	0.94	198.4	9550.9	8.50	Un-identified
15	0.95	198.6	0.99	443.7	9.91	1.04	0.5	14400.3	12.82	**Quercetin**

From the chromatogram of leaves extract of P. guava, which showed 15 peaks at 254 nm, 04 peaks were identified. These were peak numbers 4, 9, 11 and 15 having maximum Rf values of 0.16, 0.53, 0.63, and 0.99. These compounds identified were saponin, pervoside -I, Alkaloid, and Quercetin. Other peaks were unidentified.

HPTLC analysis of P. guajava leaf extract on 366 nm:

At 366 nm, the HPTLC chromatogram showed 13 different peaks (Fig. 4) and visualized the same number of bands on the TLC visualizer (Fig. 5). The details of different peaks are presented in table-4, including peak number, start Rf, maximum Rf, the height of peak and peak area, etc. This information is necessary to identify the respective compounds giving that peak or band.

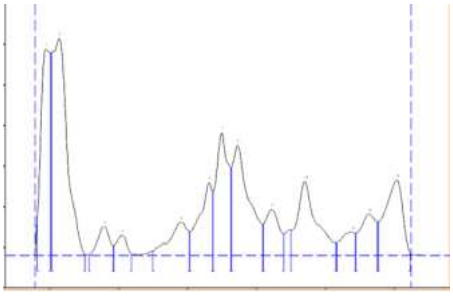

Fig. 4. HPTLC chromatogram of P. guajava leaf extract at 366 nm

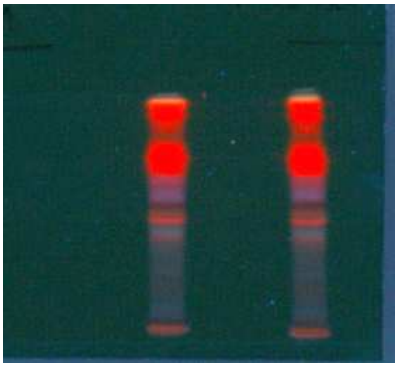

Fig. 5. HPTLC band visualization of P. guajava leaf extract at 366

Table 4: Details of HPTLC chromatogram of P. guajava leaf extract at 366 nm.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	98.0	-0.01	507.4	19.25	0.00	498.9	9792.9	12.83	Un-identified
2	0.00	498.4	0.03	534.2	20.27	0.10	0.4	16414.1	21.50	Un-identified
3	0.11	0.4	0.16	70.7	2.68	0.18	23.5	1705.5	2.23	**Saponin**
4	0.19	24.0	0.21	49.3	1.87	0.24	2.6	1040.8	1.36	Un-identified

5	0.30	10.0	0.38	81.7	3.10	0.40	58.2	3093.1	4.05	Un-identified
6	0.41	58.3	0.46	179.3	6.80	0.47	156.4	4787.1	6.27	Un-identified
7	0.47	157.0	0.50	302.1	11.46	0.53	215.7	7775.4	10.18	Un-identified
8	0.53	216.8	0.55	271.2	10.29	0.62	76.6	9425.7	12.35	**Pervoside - I**
9	0.62	76.7	0.64	112.4	4.26	0.68	53.1	3303.5	4.33	**Alkaloid**
10	0.70	62.8	0.74	181.9	6.90	0.83	30.6	7276.5	9.53	**Vanillic acid**
11	0.83	31.0	0.88	57.5	2.18	0.89	54.7	1687.7	2.21	**Flavonoid**
12	0.89	54.8	0.93	102.0	3.87	0.95	82.8	3301.1	4.32	Un-identified
13	0.95	82.8	1.01	185.7	7.05	1.05	3.6	6741.1	8.83	Un-identified

From the chromatogram of leaves extract of P. guava, which showed 13 peaks at 366 nm, 05 peaks were identified. These were peak numbers 3, 8,9, 10 and 11 having maximum Rf values 0.16, 0.55, 0.64, 0.74, and 0.88. These compounds were identified as saponin, pervoside -I, Alkaloid, vanillic acid and flavonoid.

DISCUSSION AND CONCLUSION

The preliminary phytochemistry of the leaves of P. guajava indicates that the plant is rich in phytochemical composition. It showed presence of various phytochemicals. The extract of distilled water showed the presence of terpenoids, steroids, phenolics and tannins. The qualitative tests of ethanolic extract for terpenoids, quinones, steroids, phenolics, glycosides, and Tannins. In the chloroform extract, only terpenoids, flavonoids, and phenols (Table-1). Thus it indicates that among the selected solvents, ethanol is the most suitable solvent to obtain the most number of phytochemicals from the crude plant powder.

Further, the fluorescent analysis showed that- The powder as such appears green under sunlight but in UV light the color changes to dark green. With concentrated HCL, the powder extract showed a dark brown color under normal sunlight, however, under UV light it turns light green. But in 50% HCL, it appears light green that turn into yellow under UV light. With concentrated H2SO4, the powder extract appears reddish brown and turns blackish brown but in case of 50% H2SO4, it was yellow in normal sunlight and turns brown in UV light. With NaOH, the powder appeared orange in normal sunlight and turns yellow under UV- light. With ferric chloride solution, the powder showed a black appearance in sunlight that turned dark green while with 50% nitric acid, the powder showed a yellow color in sunlight that turned brownish green.

From the HPTLC chromatogram of leaf extract of P. guava, which showed 15 peaks at 254 nm, 04 peaks were identified. These were peak numbers 4, 9, 11 and 15 having maximum Rf values 0.16, 0.53, 0.63 and 0.99. These compounds were identified as saponin, pervoside -I, Alkaloid and Quercetin.

From the HPTLC chromatogram of leaves extract of P. guava, which showed 13 peaks at 366 nm, 05 peaks were identified. These were peak numbers 3, 8,9, 10 and 11 having maximum Rf values 0.16, 0.55, 0.64, 0.74, and 0.88. These compounds were identified as saponin, pervoside -I, Alkaloid, vanillic acid and flavonoid.

Our results are in analogy with several other scientists. Some of these are discussed below. he phytochemical screening of aqueous, methanol, ethyl acetate and petroleum ether extracts of P. guajava and revealed the presence of alkaloids, tannins, and steroids by positive reaction with the respective test reagent. The aqueous and methanol extracts contained the most phytoconstituents, according to phytochemical screening. The effectiveness of Psidium guajava L. It has been proven to be successful in treating conditions including diarrhoea, dysentery, gastroenteritis, hypertension, diabetes, caries, pain alleviation, cough, mouth, ulcer, liver damage inflammation, and other widespread illnesses. Guava possesses antibacterial, antidiabetic, antiviral, antioxidant, and anti-inflammatory activities. Due to these biological activities, it can be quite helpful for the prevention and treatment of diseases.

Saikia et al.,[9] reported that Psidium guajava and Terminalia chebula leaves stand out as remarkable sources of phytochemicals with diverse and potent health benefits. Psidium guajava leaves, with their extensive array of phytochemicals such as flavonoids, polyphenols, and alkaloids, have also emerged as a valuable natural remedy. These leaves showcase an impressive range of health-promoting effects, including antioxidant protection, antimicrobial action, and potential antidiabetic exploration in various health applications, underlining their potential as a complementary approach to modern healthcare. Earlier some other biological reported the phytochemical analysis of P. guajava and their bioactivities [10-13].

From, the study it could be concluded that the plant Psidium guajava has some traditional medicinal values. It is supported by our study that the plant is rich in various bioactive phytochemicals that play a vital role in imparting medicinal and nutritional values to the plants; and further research should be focused on the identification of bioactive compounds as drug conditions for human benefit.

Acknowledgment: The author is grateful to Principal, Shri R. G. Rathod Arts and Science College Murtizapur, Dist- Akola (MS) for providing all necessary facilities during the work.

REFERENCES

[1] Naseer, S., Hussain, S., Naeem, N. et al. (2018). The phytochemistry and medicinal value of Psidium guajava (guava). Clinical Phytoscience, 4, 32 https://doi.org/10.1186/s40816-018-0093-8.
[2] Ugbogu, E. A., Emmanuel, O., Uche, M. E., Dike, E. et al., (2022). The ethnobotanical, phytochemistry and pharmacological activities of Psidium guajava L. Arabian Journal of Chemistry, 15(5), 1-26.
[3] Jeurkar, M. M., Pinpalshende, P. M., Kosalge, S. B. & Thawkar, M. M. (2023). Phytochemistry, Pharmacology and multiferous activity of Psidium guajava. Journal of Emerging Technologies and Innovative Research, 10(02), e96-e115.
[4] Naik, V. N. (1998). Flora of Marathwada, Amrut Prakashan, Aurangabad (MS) India
[5] Harborne, J. B. (1973). Methods of plant analysis. In: Phytochemical methods. Chapman and Hall, London; c1973, p. 49-188.
[6] Gandhi, R. R., Kopare, N. P., Rathod, S. A., Shirsat, R. P. & Koche, D. K. (2022). Physico-chemical, Fluorescent and Phytochemical analysis of Anisochilus carnosus (L.f.) Wall: a Lamiaceae herb from Maharashtra, India. Indian Journal of Applied and Pure Biology, 37(1), 1-12.
[7] Jagtap, T. & Koche, D. (2023) Powder Microscopy, Fluorescence, Qualitative Phytochemistry, and GC- MS analysis of a Neglected Ethnomedicinal Weed - Ruellia brittoniana Leonard, Haya: The Saudi Journal of Life Sciences, 8(5), 71-77.
[8] Lone, Z. A. & Jain, N. K. (2022). Phytochemical Screening of Guava (Psidium guajava L.) International Journals of Innovations in Engineering and Management, 9(6), 7 - 12.
[9] Saikia, M., Konwar, S. & Kalita, B. B. (2023). Exploring phytochemical diversity and antioxidant potential in leaves extracts: A comparative study of Psidium guajava (Guava) and Terminalia chebula (Myrobalan). The Pharma Innovation, 12(12), 1-5
[10] Adamu, A. (2021). Phytochemical screening of guava leaves extract, International Journal of Pure and Applied Science Research. 12(2), 89-95.
[11] Seo, J., Lee, S., Elam, M. L., Johnson, S. A., Kang, J., Arjmandi, B. H. (2014). Study to find the best extraction solvent for use with guava leaves (Psidium guajava L.) for high antioxidant efficacy. Food Science & Nutrition, 2,174–80.
[12] Begum, S., Hassan, S. I., Ali, S. N., Siddiqui, B. S. (2004). Chemical constituents from the leaves of Psidium guajava. Natural Product Research, 18(2),135–40.
[13] Joshi, D. M., Pathak, S. S., Banmare, S., Bhaisare, S. S. (2023). Review of Phytochemicals Present in Psidium guajava Plant and Its Mechanism of Action on Medicinal Activities. Cureus. 15(10), e46364. doi: 10.7759/cureus.46364.