Medicinal Value of *Psidium guajava* Linn Leaves: Phytochemical Analysis and Folk Use in Dysentery Treatment

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KEYWORDS
Psidium guajava Linn, Juice of leaves, Phytochemical Screening, HPTLC Fingerprinting, Physical constants, dysentery application.

Abstract

Present work is dealing with the study of *Psidium guajava* Linn plant's Leaves (*PGL).* The juice of the plant is applicable to medicinal use for the purpose of getting rid of the dysentery (more specifically in animals). In this context, this research for investigation of the leaves was carried by using high performance thin layer chromatography (HPTLC) technique to obtain their fingerprint of the phytochemicals present in it. Present study is reported the eleven (11) peaks in the screening of phytochemicals for the *PGL* collected first time from the Konkan region (western ghat's part) of Maharashtra located near 17.98°N, 73.47°E in the map of India. Study further extended confirmed favorable outcomes over the use in treating the animal dysentery by the survey of local folk and by the antibacterial examination of *PGL* leaves.

Introduction:

From ancient times to modern times, a long period of course, humans have been being aware about few hundreds of medicinal plants. Among those plants, many plant's species still exist and used their parts as traditional medicine (like Ayurveda in India) by people in their routine life. Many species of plant belong to plant kingdom, contain the substance of medicinal value, left un-discovered; however, some among those are constantly being screened for their possible pharmacological value (Ali et.al 1999).

The chemicals derived from such substances of plants are recognized as Phytochemicals, which is nothing but the secondary metabolic compounds (alkaloids, flavonoids, glycosides, phenolic acids, saponins, sesquiterpenes, and triterpenoids) in plants (Chaudhary et al.,1992) in Such bioactive compound analyzed by conducting the Phytochemical Screening Assay procedure.(Olajide et al.,1999), Using modern controlled techniques, their standardization by ensuring quality (in medicine) have been being done; even W.H.O. already initiated this (Quality control 1989).High-performance thin layer chromatography (HPTLC) based method (chromatogram) is a good alternative among other modern methods to estimate the active constituents with better resolution and with reasonable accuracy in a shorter time and (Dhan et al.,1989).(Sethi et.,al1996).

Psidium guajava Linn plant belong to the family Myrtaceae is known as Guava a low growing tree of height usually 6 to 25 ft, but some varieties reach to 40 ft as per environmental conditions (Willis et al.,1985) Already some works are reported in literature for PGL.

PGL finding bioactive compounds including phytochemicals (Rashmi Tambeet.al 2014 Seemakurthi et al.,2023) There are about seventy-two different phenolic compounds (bioactive compound) in *PGL* s already reported in high-performance liquid chromatography study (Díaz-de-Cerio et al.,2016) seventeen types of triterpenoids, thirty types of flavonoids, and nineteen types of sesquiterpenoids in *PGL* s are reported (Jiang et al., 2016); five quercetin glycosides are reported in *PGL* s; two new benzophenone galloyl glycosides (guavinosides A and B) and one quercetin galloyl glycoside (guavinoside C) are also reported 9Matsuzeki et al 20100; The diphenylmethane Shu et.al (Rashmi Tambe, 2014) sesquiterpenoid-diphenylmethane meroterpenoids (generally known as psiguadials A and B) (Sho et.al 2012) and psiguanins A–D (1–4) (Sho. et al.. 210) are also reported for *PGL* s in literature. These covers treating chronic diseases, such as diabetes, and neurodegenerative and cardiovascular diseases, cancer etc. (Rasuli et.al 2017) local traditional medication *PGL* 's juice is preferred to feed to dysentery animal patients to cure. There is a scope lie into to search phytochemicals in *PGL* for treating dysentery. Juice was made from leaves; some pictorial appearance of plant and leaves of *Psidium guajava* Linn are shown in Fig.1.Such work has yet to be reported for the western ghat's of Konkan region of Maharashtra state Hence there is scope for *Psidium guajava Linn.*to conduct study inspecting the finger printing the fingerprints of phytochemical present in it for trees observed in this region .

Thus, the present work is dealing with the study of *Psidium guajava* Linn plant's Leaves. Juice of the plant is applicable to medicinal use for the purpose to get rid of the dysentery. Therefore, in this research the investigation of the leaves was performed by HPTLC method and Antimicrobial activity.(Vieira et al.,2001)

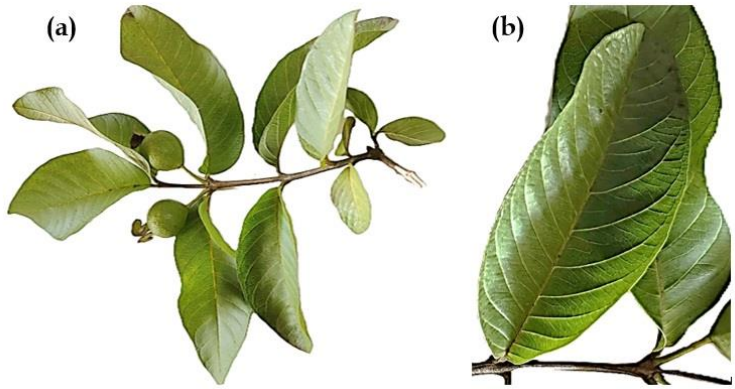


Fig. 1. Leaves of *Psidium guajava* Linn., sampled at 17.98 latitude and 73.48 longitude in western ghat Maharashtra, shows: **(a)** left-stem photograph of stem shows leaves and fruits; **(b)** photograph shows surface morphology of a leaf. (Photograph is captured by author BGR at Poladpur, Konkan region Maharashtra from the same region for the use of the collection, study and sampling in this research)

1 Materials and Methods

1.1. Materials

Extracted powder from the leaves of *Psidium guajava* Linn is a prime material in this research which was obtained first (details given in next sub-section 2.2). Analytical Reagents (AR) grade ethanol, methanol, toluene, ethyl acetate and formic acid from Merck with a minimum assay of 99.9% purity were used. HPTLC experiment conducted on the CAMAG Lino mat 5 machine setup (Camag, Muttenz, Switzerland) situated at Nanded Science College, Nanded. The application parameters used in 'Linomat 5' are: N_2 inert gas for spray, Methanol dosage as a sample solvent, 150 nl/s of speed and 0.2 ul predose volume. TLC aluminum sheets of silica gel G60 F254 of 200 µm thickness plate- 05 x10cm (Merck, Mumbai) paper was used as a stationary phase. Preliminary phytochemical analysis of leaf extracts and study of antimicrobial activity of *PGL* extract was done as per method described by Wagner (1998), Harborne (1988) and Eike Reich (2006) (Hernandz et al.,@000)

1.2. Collection of leaves and Extraction method

Leaves of *Psidium guajava* Linn were collected from the Poladpur, district Raigad, Konkan region of Maharashtra (a part of western ghat's) situated in Raigad district approximately at latitude 17.98 and longitude 73.48 in the world map of India. A good quality of leaves was bifurcated and sampled for the extraction of powder and juice from those. One of those single stems and a leaf are shown in Fig. 1. *PGL* is known as "Peru" in local language; its collected Linn brought to the laboratory for further analysis. The *PG's*. The leaves were first rinsed with running tap water several times in order to reduce surface items and debris. They were then spread for drying in the shade. After that the plant material was made to dry, then it was grinded with a mixer grinder to form fine powder. An extraction was done on a 20 g aliquot of powdered leaves using methanol, in a Soxhlet extractor (Borosil) for a period of 24 hours. Finally, the extraction process involved the removal of methanol through evaporation on a hot plate upon which the extraction solution was deposited on a watch glass. The dry extract was solved in methanol, 5 ml of the solution and filtered usnong Whatman filter paper. The filtered extract was used in the next phytochemical analysis and HPTLC profiling process as well.

1.3. Preparation of Sample for HPTLC:

Preparation of sample solution: In a 10 ml volumetric flask, 10mg of dried extract of *Psidium guajava* leaves is mixed with 10 ml dimethyl formaldehyde. Using Whatman filter paper, the sample solution was filtered. The 1ml amount of this solution was inserted to10 ml dimethyl formaldehyde filled 10ml volumetric flask by using the pipetted by replicating with the work seen in literature (Tiwari et al.2012) (Om etal .2014).

1.4. HPTLC experimental method

The TLC plates used previously were washed with analytical grade methanol and then dried at 60°C. The development of the plates was carried out using toluene, ethyl acetate, and formic acid (5:4:0.3) as mobile phase, placed in the Camag HPTLC twin-trough chamber of 10 x 10 cm. The chamber was first flooded with filter paper for 15 minutes and the plates were allowed to ethylene diamine tetra-acetic (EDTA) for 10 minutes. Further development was carried out to 85.0 mm of the solvent front before the plate was allowed to dry using a stream of air.

The 'Lino mat 5' with syringe filled by pure sample, developer chamber, Visualizer, and scanner were plugged in and connected to workstation pc, made ready with Win Cat automation tool/software for experiment. Extracted samples of leaf were placed into the system of vial or syringe placed into the 'Lino mat 5' and run for printing the band (bar-line) of sample. This conducted experiment pre-used the following: 100 µl Syringe size, 20.0 mm of Application position Y and Band length is 6.0 mm. The dimension of slit is 5.00 x 0.45 mm, and the scanning speed of light (from Micro Optimize optical system) was 20 mm/s with data resolution of 100 µm/step.

Then sample loaded TLC was placed in development chamber with a mobile phase absorption to form the separation of constituents in the sample due to their travel along with mobile phase. After half an hour, it was placed out and dried. The bands were examined by Visualizer with 254 nm and 366 nm wavelength for confirmation of bands separation and presence of standards. Further, these separated bands were quantified by HPTLC densitometric scanning using 'Camag TLC Scanner 4' in the absorption mode (multi wavelength Scanning) by placing developed TLC into it using the D2 (Deuterium) & W (Tungsten) lamps with the second order optical filter and with the automatic detector mode at high voltage 263 V. To obtain data in digital format. Scanning was done from 200 nm to 700 nm five wavelengths to observe a good absorption for selection for the study. Measurements were finally taken on 254 nm wavelength for analysis.

The Win CATS software (version 1.4.8) (as a Planar Chromatography Manager) is employed in the HPTLC experiment and used to print band to obtain the data, performed analysis of data related works and to draw results to make final report. For this, the data filtering was done by Savitsky-Golay 7, and the lowest slope baseline correction is used with the minimum peak threshold of slope 5. The minimum height, minimum area and maximum height of the peak threshold properties were set to 10 AU and 50 and 990 AU respectively for this. With automatic display scaling setting, the Track start and end positions were set.

1.5. Method of Survey and antimicrobial study related to Dysentery

Survey has been conducted at different places with some questions regarding the treatment and cure of domestic animals from their dysentery along with the emphasis on *PGL* leaves. Antibacterial study has been conducted in Biocyte institute of Research and Development (Bird Lab) Sangli Maharashtra. Details of both investigations are provided in the result section.

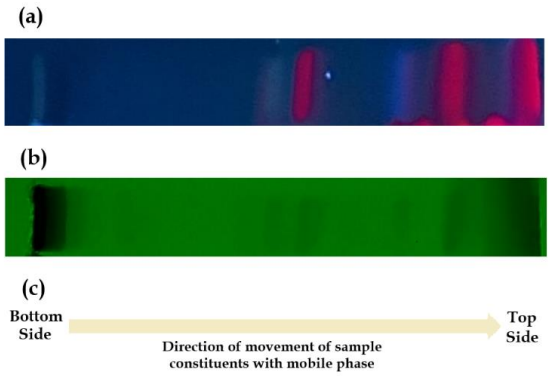


Fig. 2. The HPTLC fingerprint scanning profile of the extract of the leaves of *guajava* Linn. leaves at wavelength (a) 366 nm and (b) 254 nm. Both the figures illustrating the bands appearance due

to the constituents in the samples moved with mobile phase which is shown in (c); (c) showed that those were moved from the 'Bottom side' (shown in left hand side) to the 'Top Side' (shown in the right-hand side) of the figures (a) and (b).

2 Results and Discussion

2.1. Preliminary outcome from Visualizer

Preliminary experimental outcome contains primary experimental data of HPTLC, after developing film, in the form of Fingerprint for the wavelength 366 nm and 254 nm as shown in Fig. 2(a) Fig. 2(b). The more bands are observable in Fig. 2(a) in the Visualizer; it indicated that, 'at 366 nm wavelength, good amount of absorption of light taken placed by the separated constituent (due to travel with mobile phase or retarded) in sample. However, at 254 nm, due to the wavelength in ultraviolet light, the bands might not have been able to be observed very efficiently in the Visualizer, as shown in Fig. 2(b). It needed the observation in Scanner with wavelength near ~254 nm. However, with necked eyes observation, it seems some bands visible, which inferred the absorption of light have been taken placed by the separated constituent (due to travel with mobile phase or retarded) in sample, might be less efficiently (subjected to the limitations of scanned camera of the Visualizer).

2.2. HPTLC Scanner Investigation

HPTLC outcome details are provided in Fig. 3 and Table 1. The graph plotted in the Fig. 3 is illustrating that the Retardation factor (Rf) of the constituents in sample in X-axis and the corresponding adsorption in A.U. Fig. 3 depicted total eleven peaks which means 11 constituents present in sample were separate and move with mobile phase. in Y-axis. The peak has numbered in accordance with the order of increase in Retardation factor in the X-axis direction. The retardation factor for the peaks from 1 to 11 are found 0.01(1), 0.14(2), 0.18(3), 0.34(4), 0.38(5), 0.43(6), 0.5(7), 0.52(8), 0.58(9), 0.67(10) and 0.80(11) (the round bracket contains the corresponding peak number) as tabulated in Table 1.

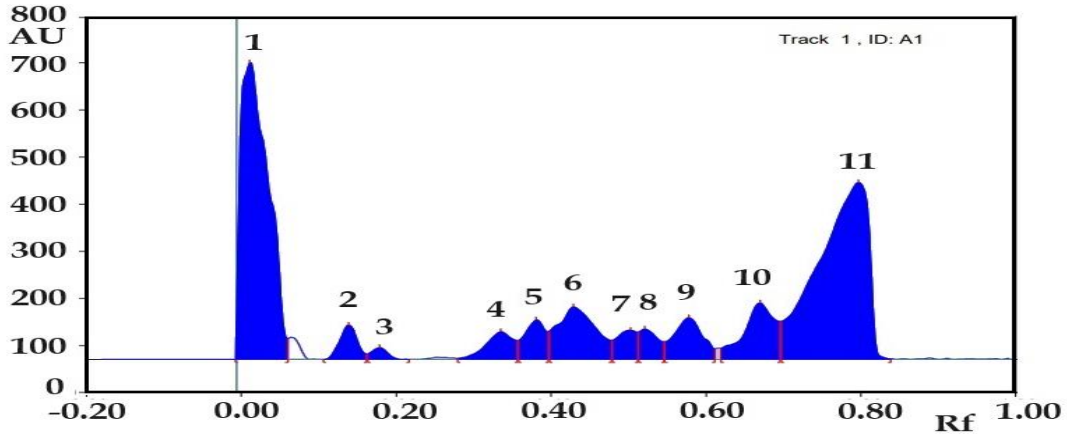


Fig. 3. Spectra of HPTLC Chromatogram: HPTLC of methanolic extract of *Psidium guajava* (Resolution at 220 nm; vol-20µl, mobile phase- Mobile phase methanol. -n-hexane-ethyl acetate (7:3)

The HPTLC analysis has obtained high-resolution data and showed different peaks of the PGL leaf extract. It was runs along with the standard and perceived to be validated the presence of

phytochemical compounds from the chromatogram after their derivatization. The result from HPTLC fingerprint scan of PGL at wavelength 254 nm has shown the presence of polyvalent phytoconstituents and its corresponding Rf value in ascending order are found from -0.1.to 0.8, in which the highest concentration of the phytoconstituents was found to be34.45%at0.80Rf. This is recorded in Table 2. The graphical representation showed eleven different peaks of polyvalent phytoconstituents.

Table 1. Details of HCTL Profile :

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	0.0 AU	**0.01 Rf**	630.1 AU	37.25 %	0.06 Rf	43.8 AU	**17066.9 AU**	**32.42 %**
2	0.11 Rf	0.1 AU	**0.14 Rf**	72.7 AU	4.30 %	0.16 Rf	11.3 AU	1234.3 AU	2.34 %
3	0.16Rf	11.5 AU	**0.18 Rf**	24.8 AU	1.46 %	0.20 Rf	0.1 AU	385.6 AU	0.73 %
4	0.28 Rf	1.9 AU	**0.34 Rf**	58.5 AU	3.46 %	0.36 Rf	39.7 AU	1562.5 AU	2.97 %
5	0.36 Rf	40.2 AU	**0.38 Rf**	83.9 AU	4.96 %	0.40 Rf	58.5 AU	1689.1 AU	3.21 %
6	0.40 Rf	59.7 AU	**0.43 Rf**	111.4 AU	6.58 %	0.48 Rf	40.4 AU	**4113.2 AU**	**7.81 %**
7	0.48Rf	40.8 AU	**0.50 Rf**	62.1 AU	3.67 %	0.51 RI	57.7 AU	1206.0 AU	2.29 %
8	0.51 Rf	58.0 AU	**0.52 Rf**	64.2 AU	3.80 %	0.55 Rf	37.5 AU	1167.2 AU	2.22 %
9	0.55 Rf	37.5 AU	**0.58 Rf**	88.4 AU	5.22 %	0.61 Rf	22.5 AU	2472.7 AU	4.70 %
10	O.62 Rf	24.2 AU	**0.67 Rf**	119.8 AU	7.08 %	0.70 Rf	80.5 AU	3606.7 AU	6.85 %
11	0.70 Rf	80.8 AU	**0.80 Rf**	375.7 AU	22.21 %	0.84 RI	1.3 AU	**18133.7 AU**	**34.45 %**

In term of area under curve of peak, the Rf value of phytoconstituents (at maximum position in peak) from the highest area concentration to the lowest area concentration were observed as 0.80, 0.01, 0.43, 0.67, 0.58, 0.38, 0.34, 0.14, 0.50, 0.52 and 0.18 (shown in round bracket) for peak number as 11 (34.5), 1 (32.42), 6 (7.82), 10 (6.85), 9 (4.70), 5 (3.21), 4 (2.97), 2 (2.34), 7 (2.29), 8 (2.22) and 3 (0.73) respectively. First four phytoconstituents have covered 81.53% of total area which shows constituent of the phytochemical corresponding to peaks 11, 1, 6 and 10 have major contribution quantitively.

Similarly in term of Maximum height, the Rf value of phytoconstituents (at maximum position of peak) from the highest Maximum height to lowest Maximum height is observed as 0.01, 0.80, 0.67, 0.43, 0.58, 0.38, 0.14, 0.52, 0.50, 0.34 and 0.18 for the peak numbers 1 (37.25), 11 (22.21), 10 (7.08), 6 (6.58), 9 (5.22), 5 (4.96), 2 (4.30), 8 (3.80), 7 (3.67), 4 (3.46), and 3 (1.46) respectively. First four have covered 73.12% of total, which shows constituent of the phytochemical corresponding to peaks 1, 11, 10 and 6 have qualitatively major contribution. Thus, phytochemical with retardation factor 0.01, 0.80, 0.67 and 0.43 Rf are understood as main bioactive chemicals which abundantly found in the *PGL* leaves.

Khandelwal KR (2005) is opinion about such relative results from the HPTLC seems coincident with the authors opinion; according to him, the HPTLC method is simple, rapid, accurate, reproducible, selective and economical for quality and quantitative determination of plant material (Atuti et al.2018), (Wilson et al.,2017).

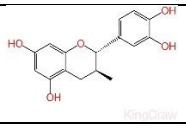
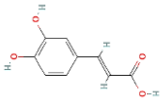
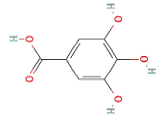
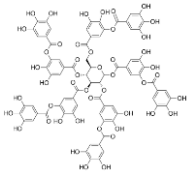
Mobile phase is hydrophobic and surface coating of thin layer is a hydrophilic nature. Due to this, the phytocompound having more retardation factor or separation is highly non-polar in nature and stabilized at hydrophobic. Similarly, the phytocompound having less retardation factor

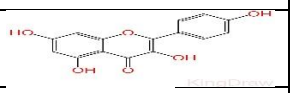
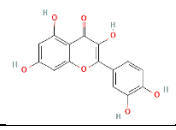
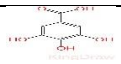
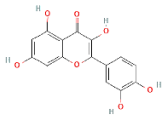
or separation is highly polar in nature and stabilized as hydrophilic. Near zero is highly polar and hydrophilic i.e., found for the Rf number 0.01 for first peak and thereafter little less 0.14 for second peak. Best non-polar and highest stabilized hydrophobic constituent is found at Rf = 0.80 for eleventh peak.

2.3. Discussion on present Phytochemical

The name of all eleven (11) constituents can be found from their corresponding Rf value. It needed to be done these Rf's comparison with the standard of their pure solvent form. Form these work the name of constituent are found as given in the Table 2 with its formula and chemical structure .

Table 2: Details of the HPTLC detected Phytochemicals.

Peak No.	Rf Max Pos.	From	To	Name of Constituent / Citation	Name of Constituent And Chemical formula	2 D Chemical structure
1	**0.01 Rf**	-0.01 Rf	0.06 Rf	belupanone	Catechin 0.11	
2	0.14 Rf	0.11 Rf	0.16 Rf	lupeol	Catechin 0.11 Gallic acid 0.21	
3	0.18 Rf	0.16Rf	0.20 Rf		Gallic acid 0.21	
4	0.34Rf	0.28 Rf	0.36 Rf	catechin	Caffeic acid (Rf=48/49 (3,4-Dihydroxycinnamic acid) 180.16 g/mol IUPAC: (*E*)-3-(3,4-dihydroxyphenyl) prop-2-enoic acid	$C_9H_8O_4$ 
5	0.38 Rf	0.36 Rf	0.40 Rf		Gallic acid IUPAC name: 3,4,5-trihydroxybenzoic acid170.12 g/mol Quercetin	$C_7H_6O_5$ 
6	0.43Rf	0.40 Rf	0.48 Rf	Epicatechin	Tannins / Quercetin Tannins is a type of polyphenol. $C_{76}H_{52}O_{46}$ Tannic acid IUPAC name: 1,2,3,4,6-penta-*O*-{3,4-dihydroxy-5-[(3,4,5-trihydroxybenzoyl)	

					oxy] benzoyl}-D-glucopyranose 1701.19 g/mol	
7	0.50Rf	0.48Rf	0.51 RI		Kaempferol	
8	0.52 Rf	0.51 Rf	0.55 Rf		Quercetin-3-o-sulphate	
9	0.58 Rf	0.55 Rf	0.61 Rf			Unknown
10	0.67Rf	O.62 Rf	0.70 Rf		Gallic acid	
11	0.80 Rf	0.70 Rf	0.84 RI		Quercetin IUPAC name: 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one 302.23 g/mol.	$C_{15}H_{10}O_7$ 

The analysis was repeated three times to study the possibility of interference from the other compounds of the extract estimation analysis. The superimposable peaks observed at the same positions (Rf) of corresponding constituent of present Phytochemicals and thus all three have confirmed the presence of the Phytochemical in the samples which have mentioned in Table 2. Quercetin has estimated the largest concentration and quantity. Other chemicals from the area and height can be assigned to small amount of constituent of PG Linn. extract.

Data is not enough to test statistically. The outcome of survey is purely on the one-to-one communication and interview conducted with survey subjects; listening and noticing and documenting the important points concerned with study. Subjects were resident of Raigad district (the same region from where PGL leaves were collected), age range from 30 to 55 old, mother tongue ‘Marathi’ with dilate mixed with Konkani

Results of the Antibacterial Study

Fig.4 illustrated the first row indicates starting essay of the E. coli bacteria grown at first stage and then after every 6 hours their status in the separate rows; the last row indicated completion of two days status. Antimicrobial screening was performed by the Agar Well plate method at Biocyte Microbiological Testing Center, Sangli.

A loop-full of E. coli was separately grown into 200 ml of sterile molten Antibiotic Assay Medium No. 19 at temperatures which ranged between 40°C - 45°C. The Petri plates were then filled with 15 to 20ml of sterilized agar medium in a measuring cylinder to a level of 3-4mm height. After preparation the plates were spread on an even surface and left to cool to room temperature Before being transferred to the refrigerator to allow the agar to solidify for 15-20 minutes. A very important step was to guarantee that the medium layer would have an equal thickness. An 8-10 mm stainless steel borer was used to punch four to five agar cups on each plate. These plates were titled sample, standard and negative control and were provided with other necessary points of analysis.

To each plate, 100 µl of Solution A containing 1 mg/ml was applied to the agar cup marked Standard. Likewise, 100 µl of Solution B (1: 1mg/ml) was applied on the agar cup on the plate with the compound ID (F3). A 100 µl aliquot of DMSO was added to the agar cup which is labelled N for Negative Control. These plates were allowed to stand for 15-20 minutes at a temperature of between 2-8°C pre incubation diffusion to reduce variation on the time of application. The plates were then incubated for 24-48 hours: respectively, at 30-35°C for bacteria and 20-25°C for yeast and molds. Then the diameters or areas of the inhibition zones were measured and the reading were recorded to the nearest whole number.

During the antibacterial study against *E. coli* it has been found that Sample showed the inhibiting the growth of bacterial strain is positive and good. The result suggested that P. Guajava can need 20 mg/ml quantity of juice to have the similar inhibition result achieved for the t1mg/ml of Streptomycin. This outcome is very suggestive to use P. guajava against E. coli gram negative bacteria and it is cost effective treatment to local folk especially those located at remote or interior areas.

Table 3. Outcome of Antibacterial screening.

Sr.No	Sample	Concentration	Zone of Inhibition (mm) (E.coli)
1	Control	-	-
2	Standard Streptomycin	1mg/ml	36
3	Sample *Psidium guava Linn.*	5mg/ml	10
		10mg/ml	16

Fig. 4. Illustration of the visuals of results in term of photograph for anti-bacterial study of PGL 5mg and 10mg over selected E. Coli bacteria with reference to standard Streptomycin 1mg after 48 hrs of inhibit period.

Conclusions

The present study highlights the antimicrobial potential of *Psidium guajava* (guava) leaf juice against *Escherichia coli*. High-performance thin-layer chromatography (HPTLC) analysis revealed the presence of 11 distinct bioactive compounds in the leaf juice, suggesting a diverse phytochemical profile. Antimicrobial activity testing demonstrated that the leaf juice exhibited

measurable inhibition of *E. coli*, with a zone of inhibition of 10 mm at 5 mg/mL and 16 mm at 10 mg/mL. Although the observed activity is lower compared to the standard streptomycin (36 mm at 1 mg/mL), the results indicate that *P. guajava* leaf juice has potential as a natural antimicrobial agent. Further research is recommended to isolate and identify the active compounds and explore their mechanism of action.

Acknowledgements
Authors (BGR & JMW) are thankful to Science college, Nanded for providing the facility of HPTLC, also thankful to Biocyte institute of Research and Development (Bird Lab) Sangli Maharashtra.

Conflict of Interest
The authors declare no conflict of interest.

Ethical Permission
Ethical permission is not applicable / not required for this research. Neither any kind of medication nor any kind of clinical study were conducted on any animal and human in the present research. No human and animal subjects were injured in the present study.

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