



# Phytochemical screening, antimicrobial, antioxidant and cytotoxic potential of different extracts of *Psidium guajava* leaves

Aarti Raj<sup>1</sup> · Vikas Menon<sup>1</sup> · Nitin Sharma<sup>1</sup>

Received: 25 February 2020 / Revised: 16 July 2020 / Accepted: 21 July 2020 / Published online: 2 September 2020  
© Society for Plant Research 2020

## Abstract

*Psidium guajava* (Myrtaceae) is an important food crop and medicinal plant supporting its traditional uses. The aim of the present study was to evaluate phytochemical, antimicrobial, antioxidant and cytotoxic activity of various solvent extracts of leaves of *P. guajava*. Phytochemical analysis revealed the presence of terpenoids, tannins, steroids, flavonoids, saponins, alkaloids and highest amount of phytochemicals was reported with methanol, ethanol and acetone extract. Total phenolic and flavonoid content was found to be highest in acetone extract ( $256.32 \pm 4.56$  mg/g GAE) and n-butanol extract ( $198.64 \pm 1.23$  mg/g RE) respectively. The antimicrobial assay revealed that acetone extract ( $13.5 \pm 2.12$  mm) exhibit maximum zone of inhibition against *P. aeruginosa*; while acetone ( $14 \pm 0.71$  mm) and ethanol ( $14.5 \pm 2.12$  mm) showed maximum zone of inhibition against *S. aureus*. Chloroform extract ( $10.2 \pm 1.13$  mm) showed maximum inhibition to the growth of *C. albicans*. Guava leaves also possess antioxidant activity which was evaluated in terms of % DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity and was found highest in acetone extract (41%). Cytotoxic activity was evaluated in terms of stimulation index using bovine lymphocytes and highest stimulation index was found with methanol extract ( $1.432 \pm 0.193$ ). This study revealed that acetone extract of guava leaf comprises effective antimicrobial, antioxidant and cytotoxicity and can be used for therapeutic applications.

**Keywords** *Psidium guajava* · Phytochemicals · Solvent · Antimicrobial · Antioxidant · Cytotoxicity

## Introduction

Medicinal plants are known for their acceptability by human and animal system. According to a report from World Health Organization, about 80% of the population of developing countries depend on traditional plant-based medicines, for their primary health care needs (Hassan 2009) due to their antioxidant, antibacterial, antifungal and antiviral activity (Barbour et al. 2004; Yasunaka et al. 2005). Indigenous systems such as Siddha, Ayurveda and Unani depend upon several plants species to treat different ailments (Rabe and Staden 1997).

The present study has been carried out on *Psidium guajava* which is a fruit tree with medicinal importance and is a native of South America. It is commonly known as guava,

lemon guava, mubera, mupeera etc. Every part of this tree, whether its fruit, leaves, bark or roots, have been used for treating stomach-ache and diarrhoea in many countries. Ethanolic extracts of stem have a high anti-diabetic activity (Mukhtar et al. 2006; Rai et al. 2007). Guava contains a large number of antioxidants and phytochemicals including essential oils, polysaccharides, minerals, vitamins, enzymes, and triterpenoid acid alkaloids, steroids, glycosides, tannins, flavonoids and saponins (Smith and Siwatibau 1975). Guava contains a higher content of vitamin C and vitamin A. Guava is also a very good source of the pectin which is an important dietary fiber. It has high content of flavonoids (Das 2011), fructose sugar (Khan and Ahmad 1985) and carotenoids (Naseer 2018). Its leaves, pulp and seeds have been used for treatment of gastrointestinal and respiratory disorders (Barbalho et al. 2012). The leaf extract was found to possess anticestodal (Tangpu and Yadav 2006), analgesic, anti-inflammatory properties (Ojewole 2006), antimicrobial (Nair and Chanda 2007), hepatoprotective (Roy et al. 2006) and antioxidant activities (Chen and Yen 2007). In addition,

✉ Vikas Menon  
cct.vikas@cgc.edu.in

<sup>1</sup> Department of Biotechnology, Chandigarh Group of Colleges, Landran, Mohali, Punjab, India

the leaf extract is used in many pharmaceutical preparations as a cough sedative.

Guava leaf extract contains flavonoids, mainly quercetin derivatives, which are hydrolyzed in the body to give the aglycone quercetin which is responsible for the spasmolytic activity of the leaves (Lozoya et al. 1994). Quercetin has several pharmacologic actions; it inhibits the intestinal movement, reduces capillary permeability in the abdominal cavity (Zhang et al. 2003) and possesses dose-dependent antioxidant properties (Nakamura et al. 2000), anti-inflammatory activity (Middleton et al. 1985; Amella et al. 1985), antiviral and antitumor activities (Kaneuchi et al. 2003). It also inhibits the aldose reductase enzyme (Chaundry et al. 1983). Several flavonoids have been reported from guava leaf such as quercetin, avicularin, guaijaverin, isoquercetin, hyperin, quercitrin, quercetin 3-O-gentiobioside, quercetin 4'-glucuronide (Lozoya et al. 1994; Kandil et al. 1997; Arima and Danno 2002). Keeping in mind the importance of *P. guajava*, the present study was conducted to analyze the phytochemical analysis, antimicrobial activity, antioxidant activity and cytotoxicity activity of different extract of *P. guajava* leaves.

## Materials and methods

### Plant Sample and chemicals

Fresh leaves of *P. guajava* were collected from different regions of Ambala District of Haryana, India. The solvents such as methanol, ethanol, chloroform, n-butanol, n-hexane, ethyl acetate, petroleum ether, acetone used were obtained from Loba Chemie Pvt. Ltd., Mumbai India. Antibiotics such as Gentamycin and Fluconazole, DPPH and MTT were obtained from Sigma-Aldrich Co. LLC, Mumbai.

### Media and microbial strains

Two Bacterial strains *Staphylococcus aureus* (MTCC096) and *Pseudomonas aeruginosa* (MTCC741) and a fungal strain i.e. *Candida albicans* (MTCC227) were used to analyze the inhibitory effect of various solvent extract of leaves of *P. guajava*. Both bacterial and fungal strains were procured from Institute of Microbial Technology, Chandigarh and maintained on Muller-Hinton agar (MHA) and Potato Dextrose (PDA) agar slants at 37 °C and 30 °C respectively. The antibacterial and antifungal assays were performed on Muller-Hinton agar (MHA) and Potato Dextrose (PDA) agar medium using agar well diffusion method (Perez et al. 1990).

### Preparation of leaves extracts using different solvents

The collected leaves (1 kg) were thoroughly washed with running tap water followed by rinsing in distilled water. The leaves were completely dried for two weeks in shady area, and grounded to powder using an electric grinder. Extracts of different solvents (Methanol, ethanol, chloroform, n-butanol, n-hexane, ethyl acetate, petroleum ether, acetone) were prepared using a cold maceration method, whereas decoction method was used to prepare aqueous extract and filtrate was allowed to dry and stored at 4 °C till use (Kumar et al. 2016).

### Qualitative analysis of phytochemicals in various solvent extracts

The various solvent extracts were subjected to preliminary phytochemical analysis for the presence or absence of different bioactive components such as phenolics, flavonoids, tannins, saponins, alkaloids, glycosides, and carbohydrate using standard methods (Khandelwal 2008; Guleria et al. 2016).

### Determination of total phenolic and flavonoid content in various in various solvent extracts

The total phenolic content (TPC) was determined using Folin–ciocalteu reagent method (Singleton et al. 1999; Kumar et al. 2018, 2020); whereas total flavonoid content (TFC) of different solvent extracts of leaves of *T. foliolosum* were quantified by using aluminum chloride (AlCl<sub>3</sub>) method (Zhishen et al. 1999; Kumar et al. 2018, 2020). Gallic acid and Rutin was used as standard for calculation of TPC and TFC respectively.

### Agar well diffusion method for antimicrobial activity

Antimicrobial activity in various solvent extracts of leaves of *P. guajava* was evaluated in terms of zone of inhibition using agar well diffusion method (Perez et al. 1990). Gentamycin (for antibacterial activity) and Fluconazole (for antifungal activity) were used as a positive control, and Dimethyl sulfoxide (DMSO) was used as a negative control. In agar well diffusion method, the bacterial/fungal culture of 0.5 McFarland Standard ( $\sim 2 \times 10^8$  colony forming units (CFU/mL)) was uniformly spread on the surface of MHA/ PDA plates using sterile cotton swabs. The wells were punched with the cork borer (6 mm) in the agar. Approximately 50  $\mu$ L of the prepared extract (50 mg/mL in DMSO) of *P. guajava* were added into the wells, allowed to stand at room temperature

for about 1 h and incubated at 37 °C for bacteria and 30 °C for fungal growth. After 24 h (bacteria)/48 h (fungus) of incubation, the zone of inhibition was measured using a HiAntibiotic Zone scale-C (Himedia Biosciences, Mumbai (India)).

### DPPH radical scavenging activity of solvent extracts of *P. guajava*

DPPH radical scavenging activity of the extract was measured by the method described by Barros et al. (2007) with modifications. For performing DPPH assay, 10 test tubes were covered with aluminium foil to mark as 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10. In each test tube, 200 µL of each extract (1 mg/mL), 800 µL of 0.1 M Tris–HCl buffer (pH-7.4) and 1 mL of DPPH solution (0.004%) was added, mixed thoroughly and incubated in dark for about 30 min. After incubation, the absorbance was taken at 517 nm. For blank, 1.2 mL of ethanol and 800 µL of Tris–HCl was used. For control, 1 mL of DPPH solution, 200 µL of ethanol and 800 µL of 0.1 M Tris–HCl was taken. Ascorbic acid (10 mg/10 mL) was used as standard antioxidant and standard curve was prepared using various dilutions of ascorbic acid ( $10^{-1}$  to  $10^{-8}$ ). To 200 µL of ascorbic acid sample from each dilution, 800 µL of Tris–HCL buffer and 1 mL of DPPH solution was added and incubated in dark for 30 min and the absorbance was taken at 517 nm.

Percentage scavenging activity was calculated using following formula-

$$\% \text{ DPPH radical scavenging activity} = \{A_{(\text{control})} - A_{(\text{sample})}\} / A_{(\text{control})} \times 100$$

### In vitro blood lymphocyte proliferation response by MTT assay

Lymphocytes from bovine blood were extracted using Histopaque 1077 and suspended in PMI1640 media supplemented with 10% fetal calf serum (FCS), 10 mM HEPES buffer, 2 mM l-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. Lymphocyte suspension was diluted to  $5 \times 10^6$  live cells/mL using culture media (RPMI 1640). To 100 µL of diluted cells, add 50 µL of each extracts and mitogen (concanavalin A, 25 µg/mL in RPMI), and then final volume was adjusted to 200 µL using RPMI media. The reaction mixture was incubated at 37 °C in a humidified CO<sub>2</sub> incubator (95% air and 5% CO<sub>2</sub>) for 48 h. After incubation, supernatant was discarded without disturbing the adhered cells and then 25 µL of MTT (5 µg/mL of PBS) solution to each well and again allowed to react for 4 h in a humidified CO<sub>2</sub> incubator. Then 100 µL of DMSO was added to each mixture in order to dissolve crystals formed.

The amount of MTT formazan produced during the incubation was measured by an ELISA reader at a test wavelength of 550 nm and a reference wavelength of 630 nm. The results were based on the optical density at the wavelength of 550 nm (OD<sub>550</sub>) and expressed as a stimulation index (SI), which was calculated as follows: (Soper et al. 1978; Mosmann 1983; Norian et al. 2015):

Stimulation index (SI) = OD<sub>550</sub> of stimulated cells - OD<sub>550</sub> of blank / OD<sub>550</sub> of unstimulated cells.

## Results and discussions

### Phytochemical analysis

All the extracts were tested for the presence of different phytochemicals and the results was summarized in Table 1. All the extracts also varied in number of phyto-compounds present and comparison of number of phyto-compounds showed higher number of phytocompounds were present in extracts of methanol, ethanol, acetone and Aqueous extract prepared through decoction method. Least number of bioactive compounds were reported from n-hexane and ethyl acetate extract (Fig. 1). Several studies have reported the presence of alkaloids, flavonoids, glycosides, polyphenols, reducing compounds, saponins and tannins in the different solvent extract of *P. guajava* leaves (Uboh et al. 2010; Biswas et al. 2013; Gayathri and Kiruba 2014).

### Quantitative analysis of phytochemicals in various extracts of *P. guajava* leaves

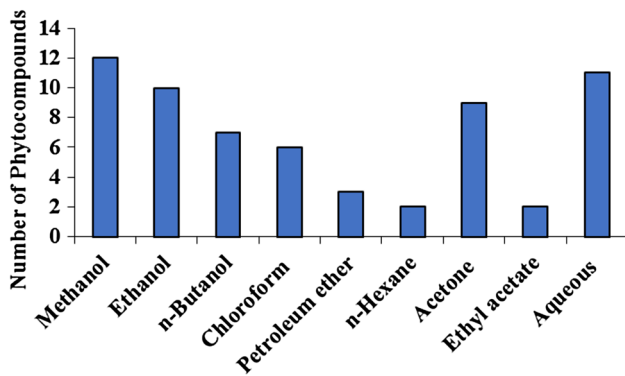
The amount of total phenolic (TPC) and flavonoid content (TFC) was calculated using spectrophotometric methods and the results were showed in Fig. 2. Among all extracts, acetone extract of *P. guajava* leaves showed higher amount of TPC ( $256.32 \pm 4.56$  mg/g GAE) followed by n-butanol extract ( $210.32 \pm 4.3$  mg/g GAE), while TFC was higher in n-butanol extract ( $198.64 \pm 1.23$  mg/g RE) followed by acetone extract ( $165.43 \pm 2.44$  mg/g RE). Amount of TPC and TFC was also quantified by various researchers (Nantitanon et al. 2010; Ashraf et al. 2016; Camarena-Tello et al. 2018) in different solvents. However, there may be variation in TPC and TPC in solvents of different polarities, which can be explained due to difference in the chemical nature of solvents, variation in agro-climatic conditions, maturity at harvest, difference in extraction method and polarity of extracting solvent (Bae et al. 2012).

**Table 1** Qualitative screening of phytochemicals in various extracts of *P. guajava*

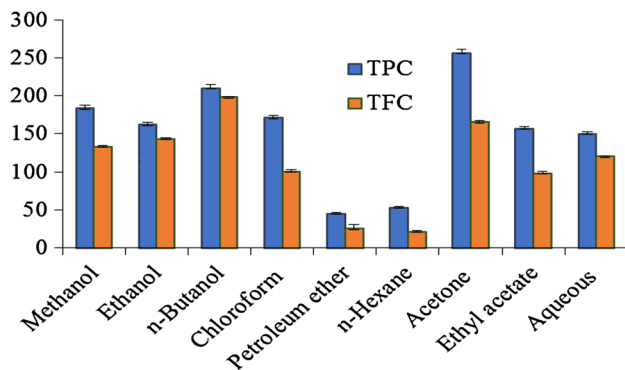
S. No	Phytochemicals	M	E	But	C	PE	Hex	Ac	EA	Aq
1	Alkaloids	+	+	-	-	-	-	-	-	-
2	Flavanoids	+	+	-	-	-	-	+	+	+
3	Steroids	+	+	-	+	-	-	+	-	+
4	Terpenoids	+	+	+	+	-	-	+	-	+
5	Quinones	+	-	-	-	-	-	+	-	+
6	Phenols	+	+	+	-	-	-	+	+	+
7	Starch	+	+	+	+	+	+	+	-	+
8	Anthocyanine	+	-	-	-	-	-	-	-	-
9	Protein	-	-	-	-	-	-	-	-	-
10	Carbohydrate	+	+	+	+	+	+	-	+	+
11	Cellulose	+	+	+	+	+	-	+	+	+
12	Glycoside	+	+	+	-	-	-	+	-	+
13	Saponins	-	-	-	+	-	-	-	-	+
14	Tannins	+	+	+	-	-	-	+	-	+

M methanol, E ethanol, But n-Butanol, C chloroform, PE petroleum ether) Hex n-Hexane, Ac acetone, EA ethyl acetate, Aq aqueous

(+) indicate presence while (-) indicate absence of phytochemicals



**Fig. 1** Comparison of number of phytochemicals present in various extracts of *P. guajava* leaves



**Fig. 2** Comparison of total phenolic content (TPC) and flavonoid content (TFC) in various extracts of *P. guajava* leaves. TPC was expressed as mg/g Gallic acid equivalents; while TFC was expressed as mg/g Rutin equivalents. Values are expressed as mean ± S.D. of two readings

Díaz-de-Cerio et al. (2015) quantified different category of phenolic compounds in Guava leaves infusions and ultrasound aqueous extract using HPLC–ESI–MS method. Four different classes of phenolic compound such as Gallic and ellagic acid derivatives, flavonols, flavanones, and flavan-3-ols and some benzophenones were quantified.

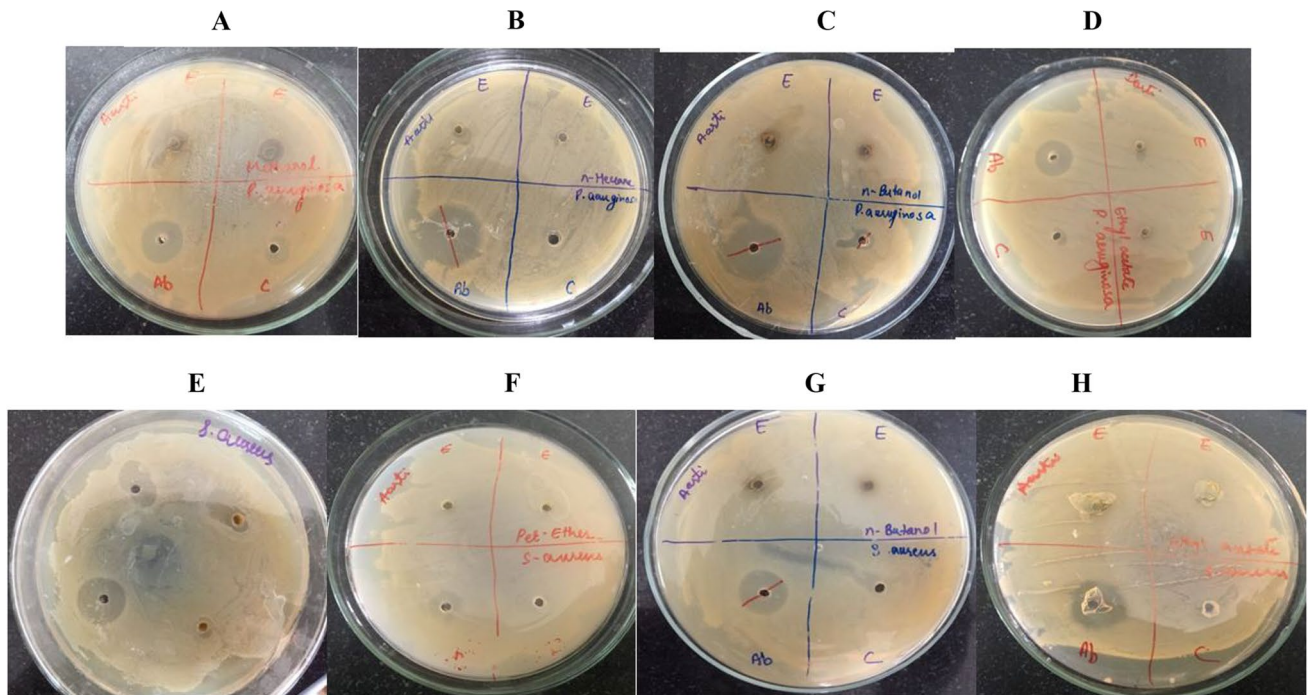
**Antimicrobial activity of various extracts of *P. guajava***

Antimicrobial activity of various extracts of *P. guajava* was evaluated against bacterial and fungal strains and expressed in terms of diameter of zone of inhibition. The data of zone of inhibition was given in Table 2. Acetone (13.5 ± 2.12 mm), ethanol (13 ± 1.41 mm) and methanol (12.5 ± 3.53 mm) extract showed maximum zone of inhibition against *P. aeruginosa*; while acetone (14 ± 0.71 mm) and ethanol (14.5 ± 2.12 mm) showed maximum zone of inhibition against *S. aureus* (Fig. 3). Chloroform (10.2 ± 1.13 mm) and ethanol extract (8.75 ± 0.50 mm) showed maximum inhibition to the growth of *C. albicans* (Fig. 4). The higher antibacterial activity of guava against gram positive bacteria and moderate against the gram-negative bacterial strains was also reported in previous study (Nair and Chanda 2007). Essential oil from guava also showed activity against *Salmonella* and *S. aureus* (Gonçalves et al. 2008). Aqueous and methanol extract of the guava leaves reported to inhibit the growth of bacteria by producing a remarkable zone of inhibition (Puntawong et al. 2012). Antifungal activity of leaves of *P. guajava* showed in the present study was in accordance with the

**Table 2** Diameter of Zone of Inhibition against tested bacterial and fungal strains

Extracts	Diameter of Zone of Inhibition (mm)						
	Control (C)	<i>Pseudomonas aeruginosa</i>		<i>Staphylococcus aureus</i>		<i>Candida albicans</i>	
		Antibiotic (Ab)	Extract <sup>a</sup>	Antibiotic (Ab)	Extract <sup>a</sup>	Antibiotic (Ab)	Extract <sup>a</sup>
Ethanol	0	24	13 ± 1.41	18	14.5 ± 2.12	13	8.75 ± 0.50
n-Hexane	0	22	2.5 ± 0.53	20	0	11	7 ± 1.41
Acetone	0	22	13.5 ± 2.12	20	14 ± 0.71	12	5.3 ± 1.41
n-Butanol	0	15	0	20	6.5 ± 2.12	9	7.5 ± 0.71
Methanol	0	18	12.5 ± 3.53	12	0	15	5.75 ± 1.21
Petroleum ether	0	20	7.5 ± 0.71	12	0	11	0
Ethyl acetate	0	13	0	15	0	12	1.5 ± 0.53
Chloroform	0	12	3 ± 1.41	20	10 ± 0.71	14	10.2 ± 1.13
Aqueous	0	18	6.5 ± 0.71	16	10 ± 0.71	11	0

<sup>a</sup>Values are mean ± S.D. of two readings



**Fig. 3** Agar well diffusion method of antibacterial activity of various extracts of *P. guajava* against *P. aeruginosa* (a–d), where plate A-methanolic extract; plate B-n-hexane extract; plate C-n-Butanol

extract; plate D-Ethyl acetate extract and against *Staphylococcus aureus* (e–h) where plate A-Methanolic extract; plate B-Pet ether extract; plate C-n-Butanol extract; plate D-Ethyl acetate extract

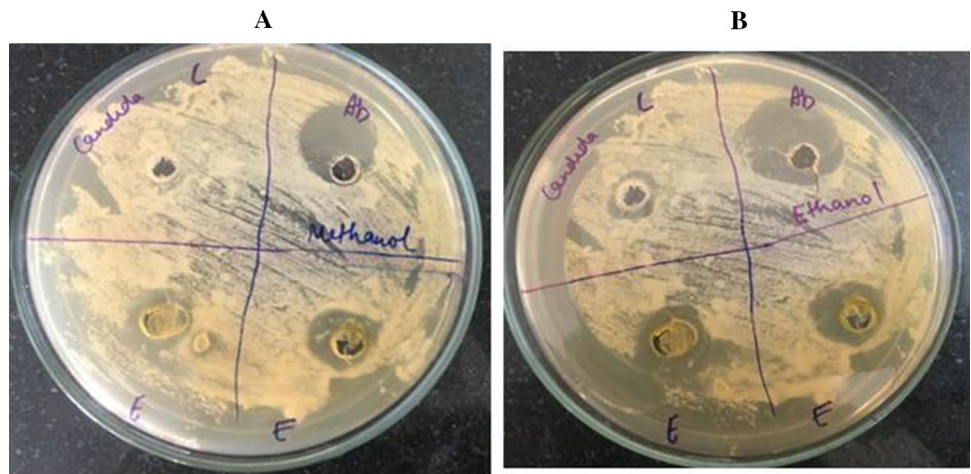
reports of Morais-Braga et al. (2017) and Shinde et al. (2018).

### Antioxidant activity in terms of DPPH radical scavenging

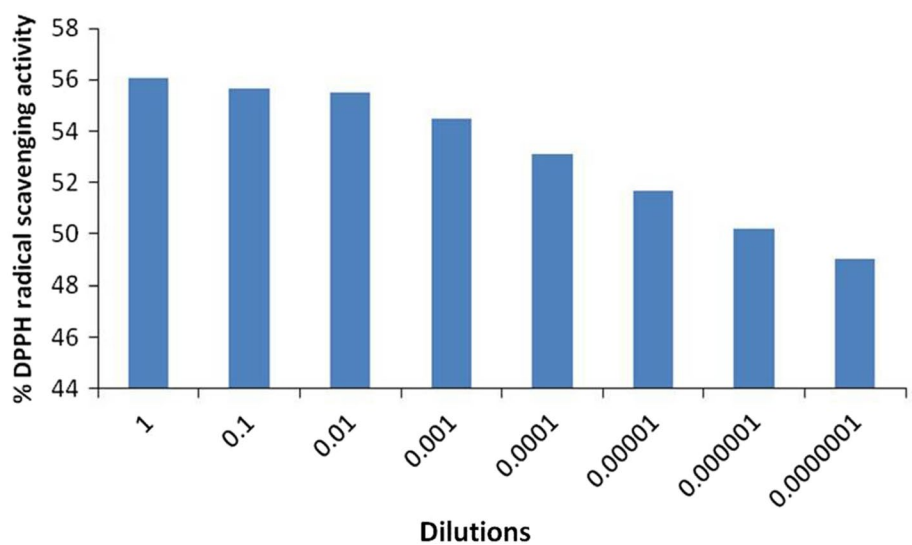
Antioxidant activity followed concentration-dependence, as radical scavenging activity of ascorbic acid increase with increase in concentration (Fig. 5). Comparison of % DPPH

radical scavenging activity of various solvent extracts of *P. guajava* was done at same concentration (100 µg/mL) and found that acetone extract (41%) showed highest radical scavenging followed by chloroform (40%) and n-butanol extract (35.9%). Pet ether and n-hexane extract showed least radical scavenging activity (Table 3). Ascorbic acid an important antioxidant compound is present in leaves in excess (He and Venant 2004; Thaipong et al. 2005). The other phytochemicals which are present in guava are

**Fig. 4** Agar well diffusion method of antifungal activity of various extracts of *P. guajava* against *C. albicans*. Plate A-Methanolic extract; Plate B-Ethanollic extract



**Fig. 5** Concentration dependent DPPH radical scavenging activity of ascorbic acid



**Table 3** Comparative % DPPH radical scavenging activity of various extracts of *P. guajava* at same concentration

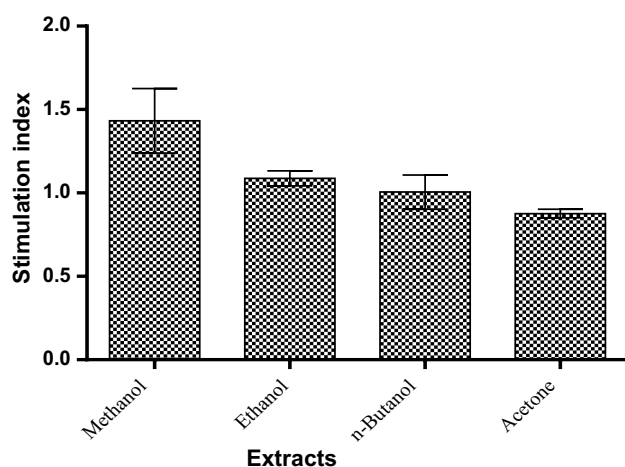
Sr. No	Extracts	% DPPH scavenging
1	Acetone	41
2	n-Butanol	35.9
3	Chloroform	40
4	Ethanol	7.28
5	Petroleum ether	- 12.8
6	Ethyl acetate	2.5
7	Methanol	20.05
8	n-Hexane	0.5
9	Aqueous	4.1

protocatechuic acid, quercetin, ferulic acid, quercetin, gallic acid and caffeic acid and are also important antioxidants. Study from Fernandes et al. (2014), Seo et al. (2014) and Santhoshkumar et al. (2014) also reported the maximum

antioxidant activity of methanol and aqueous extract of guava leaves. Ethanollic extract of guava showed low activity in all antioxidant assays like DPPH and FRAP assay (Vijayakumar et al. 2015). Quercetin isolated from leaves of guava was also reported to possess most active and strong antioxidant activity (Soman et al. 2010; Nantitanon and Okonogi 2012).

**Cytotoxic activity assay**

Stimulation Index of different solvent extracts of *P. guajava* showed that highest stimulation index was found with methanol extract (1.432 ± 0.193) followed by ethanol (1.09 ± 0.045), butanol (1.005 ± 0.103) and acetone extract (0.88 ± 0.026) (Fig. 6). Aqueous leaf extract of *P. guajava* was reported to possess anticancer activity against MCF-7 cell lines using MTT assay (Sukanya et al. 2017). Ashraf et al. (2016) also evaluated in vivo cytotoxic against *Artemia salina* nauplii using brine shrimp lethality assay and



**Fig. 6** Stimulation index of guava leaves in different solvent extracts

in vitro anticancer activity against KBM5 (human chronic myelogenous leukaemia), SCC4 (human tongue squamous carcinoma cells) and U266 (human multiple myeloma cells) of methanol, hexane and chloroform extracts of *P. guajava*. Among all tested extracts, hexane extract showed better cytotoxicity and anticancer effect which may be attributed to presence of tetracosane,  $\alpha$ -copaene,  $\gamma$ -sitosterol, vitamin E and squalene.

## Conclusion

The present study reveals that the plant *P. guajava* is rich in many bioactive compounds which are of medicinal importance. The results also showed that the most effective solvent used for the extraction of phytochemicals are methanol, ethanol and acetone and highest antimicrobial, antioxidant and cytotoxicity activity was reported with acetone extract. As the leaves have been reported for biological importance, therefore, it can be further used to treat many acute and even lethal diseases in future. Also, we know that, there is a rapid emergence of drug resistant organisms which remains ineffective from the present medicines and antibiotics used by us. Therefore, the necessity of continuous searching for new antimicrobial substances and natural products may act as alternative for antibiotic and chemotherapeutic agents. Through phytochemical analysis, the presence of many bioactive compounds such as terpenoids, tannins, steroids, flavonoids, saponins, alkaloids have been observed. However, results indicate that the extraction was best in methanol and ethanol. Therefore, by keeping in mind the benefits and availability of the guava leaves, we should look forward the

isolation of these bioactive compounds for the development of versatile drugs from a versatile tree, *P. guajava*.

**Acknowledgement** The authors acknowledge Chandigarh Group of Colleges, Landran for providing the infrastructural support.

## Compliance with ethical standards

**Conflict of interest** The authors confirm that they have no conflicts of interest with any parties regarding the content of this article.

## References

- Amella M, Bronner C, Briancon F, Haag M, Anton R, Landry Y (1985) Inhibition of mast cell histamine release by flavonoids and bioflavonoids. *Planta Med* 51:16–20
- Arima H, Danno G (2002) Isolation of antimicrobial compounds from Guava (*Psidium guajava* L.) and their structural elucidation. *Biosci Biotechnol Biochem* 66:1727–1730
- Ashraf A, Sarfraz RA, Rashid MA, Mahmood A, Shahid M, Noor N (2016) Chemical composition, antioxidant, antitumor, anticancer and cytotoxic effects of *Psidium guajava* leaf extracts. *Pharmaceut Biol* 54(10):1971–1981
- Bae H, Jayaprakasha GK, Crosby K, Jifon JL, Patil BS (2012) Influence of extraction solvents on antioxidant activity and the content of bioactive compounds in non-pungent peppers. *Plant Foods Hum Nutr* 67:120–128
- Barbalho SM, Farinazzi-Machado FM, de Alvares GR, Brunnati AC, Otoboni AM, Ottoboni B (2012) *Psidium guajava* (Guava): a plant of multipurpose medicinal applications. *Med Aromat Plants* 1(104):2167–2412
- Barbour EK, Al Sharif M, Sagherian VK, Habre AN, Talhouk RS, Talhouk SN (2004) Screening of selected indigenous plants of Lebanon for antimicrobial activity. *J Ethnopharmacol* 93(1):1–7
- Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, Baptista P (2007) Total phenols, ascorbic acid,  $\beta$ -carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chem* 103:413–419
- Biswas B, Rogers K, McLaughlin F, Daniels D, Yadav A (2013) Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two gram-negative and gram-positive bacteria. *Int J Microbiol*. <https://doi.org/10.1155/2013/746165>
- Camarena-Tello JC, Martínez-Flores HE, Garnica-Romo M, Padilla-Ramírez JS, Saavedra-Molina A, Alvarez-Cortes O, Bartolomé-Camacho MC, Rodiles-López JO (2018) Quantification of phenolic compounds and in vitro radical scavenging abilities with leaf extracts from two varieties of *Psidium guajava* L. *Antioxidants* 7(3):34
- Chaundry PS, Cabrera J, Juliana HR, Varma SD (1983) Inhibition of human lens aldose reductase by flavonoids, sulindac and indomethacin. *Biochem Pharmacol* 2:1995–1998
- Chen HY, Yen GC (2007) Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. *Food Chem* 101(2):686–694
- Das AJ (2011) Review on nutritional, medicinal and pharmacological properties of *Centella asiatica* (Indian pennywort). *J Biol Act Prod from Nat* 1(4):216–228
- Díaz-de-Cerio E, Verardo V, Gómez-Caravaca AM, Fernández-Gutiérrez A, Segura-Carretero A (2015) Determination of polar compounds in guava leaves infusions and ultrasound aqueous extract by HPLC-ESI-MS. *J Chem*. <https://doi.org/10.1155/2015/250919>

- Fernandes MRV, Azzolini AECS, Martinez MLL, Souza CRF, Oliveira WP (2014) Assessment of antioxidant activity of spray dried extracts of *Psidium guajava* leaves by DPPH and chemiluminescence inhibition in human neutrophils. *Biomed Res Int* 2014:382891 (**Article ID 382891**)
- Gayathri V, Kiruba D (2014) Preliminary phytochemical analysis of leaf powder extracts of *Psidium guajava* L. *Int J Pharmacogn Phytochem Res* 6(2):332–334
- Gonçalves FA, Neto MA, Bezerra JNS, Macrae A, De SOV (2008) Antibacterial activity of guava, *Psidium guajava* Linnaeus, leaf extracts on diarrhea-causing enteric bacteria isolated from seabob shrimp, *Xiphopenaeus kroyeri* (Heller). *Rev Inst Med trop S Paulo* 50(1):11–15
- Guleria S, Dev K, Khosla PK (2016) Comparative analysis of phytochemicals and antioxidant activities of fruit and leaves of *Terminalia chebula* from Himachal Pradesh. *IJBPAS* 5:1195–1206
- Hassan A, Rahman S, Deeba F, Mahmud S (2009) Antimicrobial activity of some plant extracts having hepatoprotective effects. *J Med Plants Res* 3(1):020–23
- He Q, Venant N (2004) Antioxidant power of phytochemicals from *Psidium guajava* leaf. *J Zhejiang Univ Sci A* 5(6):676–683
- Kandil FE, El-Sayed NH, Micheal HN, Ishak MS, Mabry TJ (1997) Flavonoids from *Psidium guajava*. *Asian J Chem* 9:871–872
- Kaneuchi M, Sasaki M, Tanaka Y, Sakuragi N, Fujimoto S, Dahiya R (2003) Quercetin regulates growth of Ishikawa cells through the suppression of EGF and cyclin D1. *Int J Oncol* 22:159–164
- Khan MIH, Ahmad JA (1985) Pharmacognostic study of *Psidium guajava* L. *J Med Plant Res* 23(2):95–103
- Khandelwal K (2008) Practical pharmacognosy. Pragati Books Pvt Ltd, pp 149–156
- Kumar V, Dev K, Sourirajan A, Khosla PK (2016) Comparative antioxidant potential of bark and leaves of *Terminalia arjuna* (Roxb) Wight and Arn from Himachal Pradesh. *Int J Pharm Phytopharmacol Res* 6(1):27–33
- Kumar V, Sharma N, Sourirajan A, Khosla PK, Dev K (2018) Comparative evaluation of antimicrobial and antioxidant potential of ethanolic extract and its fractions of bark and leaves of *Terminalia arjuna* from north-western Himalayas, India. *J Trad Compl Med* 8(1):100–106
- Kumar R, Sharma N, Rolta R, Lal UR, Sourirajan A, Dev K, Kumar V (2020) *Thalictrum foliolosum* DC: an unexplored medicinal herb from north western Himalayas with potential against fungal pathogens and scavenger of reactive oxygen species. *Biocatal Agric Biotechnol* 26:101621
- Lozoya X, Meckes M, Abou-Zaid M, Tortoriello J, Nozzolillo C, Arnason JT (1994) Calcium-antagonist effect of quercetin and its relation with the spasmolytic properties of *Psidium guajava* L. *Arch Med Res* 25:11–15
- Middleton E, Drzewiecki G (1985) Naturalty occurring flavonoids and human basophil histamine release. *Int Arch Allergy Appl Immunol* 77:155–157
- Morais-Braga MF, Carneiro JN, Machado AJ, Sales DL, dos Santos AT, Boligon AA, Athayde ML, Menezes IR, Souza DS, Costa JG, Coutinho HD (2017) Phenolic composition and medicinal usage of *Psidium guajava* Linn.: Antifungal activity or inhibition of virulence? *Saudi J biological Sci* 24(2):302–313
- Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 65(1–2):55–63
- Mukhtar HM, Ansari SH, Bhat ZA, Naved T, Singh P (2006) Antidiabetic activity of an ethanol extract obtained from the stem bark of *Psidium guajava* (Myrtaceae). *Pharmazie* 61:725–727
- Nair R, Chanda S (2007) In-vitro antimicrobial activity of *Psidium guajava* L., Leaf extracts against clinically important pathogenic microbial strains. *Braz J Microbiol* 38:452–458
- Nakamura Y, Ishimitsu S, Tonogai Y (2000) Effects of quercetin and rutin on serum and hepatic lipid concentrations, fecal steroid excretion and serum antioxidant properties. *J Health Sci* 46:229–240
- Nantitanon W, Okonogi S (2012) Comparison of antioxidant activity of compounds isolated from guava leaves and a stability study of the most active compound. *Drug Discov Ther* 6(1):38–43
- Nantitanon W, Yotsawimonwat S, Okonogi S (2010) Factors influencing antioxidant activities and total phenolic content of guava leaf extract. *LWT-Food Sci Technol* 43(7):1095–1103
- Naseer S, Hussain S, Naeem N, Pervaiz M, Rahman M (2018) The phytochemistry and medicinal value of *Psidium guajava* (guava). *Clin Phytosci* 4(1):1–8
- Norian R, Delirezh N, Azadmehr A (2015) Evaluation of proliferation and cytokines production by mitogen-stimulated bovine peripheral blood mononuclear cells. *Vet Res Forum* 6(4):265
- Ojewole JA (2006) Anti-inflammatory and analgesic effects of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extracts in rats and mice. *Methods Find Exp Clin Pharmacol* 28:441–446
- Perez C, Paul M, Bazerque P (1990) Antibiotic assay by agar-well diffusion method. *Acta Biol Med Exp* 15:113–115
- Puntawong S, Okonogi S, Pringproa K (2012) In vitro antibacterial activity of *Psidium guajava* Linn. Leaf extracts against pathogenic bacteria in pigs. *Chiang Mai Univ J Nat Sci* 11(2):127–134
- Rabe T, Van Staden J (1997) Antibacterial activity of South African plants used for medicinal purposes. *J Ethnopharmacol* 56(1):81–87
- Rai PK, Rai NK, Rai AK, Watal G (2007) Role of LIBS in elemental analysis of *Psidium guajava* responsible for glycemic potential. *Instrum Sci Technol* 35:507–522
- Roy K, Kamath V, Asad M (2006) Hepatoprotective activity of *Psidium guajava* L. leaf extract. *Indian J Exp Biol* 44:305–311
- Santhoshkumar T, Rahuman AA, Jayaseelan C, Rajakumar G, Marimuthu S, Kirthi AV, Valayutham K, Thomas J, Venkatesan J, Kim S (2014) Green synthesis of titanium dioxide nanoparticles using *Psidium guajava* extract and its antibacterial and antioxidant properties. *Asian Pac J Trop Biomed* 7:968–976
- Seo J, Lee S, Elam ML, Johnson SA, Kang J, Arjmandi BH (2014) Study to find the best extraction solvent for use with guava leaves (*Psidium guajava* L.) for high antioxidant efficacy. *Food Sci Nutr* 2:174–180
- Shinde M, Sawant A, Dhekale P, Sharma A (2018) Effect of different extracts of *Psidium guajava* leaves on antibacterial and antifungal activity. *World J Pharmaceut Res* 7(13):523–528
- Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* 299:152–178
- Smith RM, Siwatibau S (1975) Sesquiterpene hydrocarbons of fijiian guavas. *Phytochem* 14(9):2013–2015
- Soman S, Rauf AA, Indira M, Rajamanickam C (2010) Antioxidant and Antiglycative potential of ethyl acetate fraction of *Psidium guajava* leaf extract in Streptozotocin-induced diabetic rats. *Plant Foods Hum Nutr* 65:386–391
- Soper FF, Muscoplat CC, Johnson DW (1978) In vitro stimulation of bovine peripheral blood lymphocytes: analysis of variation of lymphocyte blastogenic response in normal dairy cattle. *Am J Vet Res* 39(6):1039–1042
- Sukanya SR, Sinthu K, Shazhni JA, Priya FS (2017) Qualitative phytochemical screening and assessment of antimicrobial activity of *Psidium guajava* and its cytotoxic studies. *Asian J Appl Sci Technol* 1(9):414–420
- Tangpu TV, Yadav AK (2006) Anticestodal efficacy of *Psidium guajava* against experimental *Hymenolepis diminuta* infection in rats. *Indian J Pharmacol* 38:29–32



- Thaipong K, Boonprakob U, Cisneros-zevallos L, Byrne DH, Pathom N (2005) Hydrophilic and lipophilic antioxidant activities of guava fruits. *Southeast Asian J Trop Med Public Health* 36:254–257
- Uboh FE, Okon IE, Ekong MB (2010) Effect of aqueous extract of *Psidium guajava* leaves on liver enzymes, histological integrity and hematological indices in rats. *Gastroenterol Res* 3(1):32
- Vijayakumar K, Anand AV, Manikandan R (2015) In vitro antioxidant activity of ethanolic extract of *Psidium guajava* leaves. *Int J Res Stud Biosci* 3(5):145–149
- Yasunaka K, Abe F, Nagayama A, Okabe H, Lozada-Pérez L, López-Villafranco E, Muñiz EE, Aguilar A, Reyes-Chilpa R (2005) Antibacterial activity of crude extracts from Mexican medicinal plants and purified coumarins and xanthenes. *J Ethnopharmacol* 97(2):293–299
- Zhang W, Chen B, Wang C, Zhu Q, Mo Z (2003) Mechanism of quercetin as antidiarrheal agent. *Acad J First Med Coll PLA* 23:1029–1031
- Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* 64:555–559

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.