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Original Article

PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTI MICROBIAL ACTIVITY OF WHITE & PINK *PSIDIUM GUAJAVA L*INNAEUS

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ABSTRACT

Objective: The objective of the present study was to evaluate phytochemicals, antioxidant and anti microbial activity of white and pink (Guava) *Psidium guajava L*innaeus.

Methods: Healthy and disease free fresh leaves and fruits of white and pink *Psidium guajava* Linnaeus were collected and extracted using ethanol and aqueous solvents and their contents were analysed for biological efficacy.

Results: The phytochemical analysis of extracts revealed the presence of phenols, glycosides, flavonoids and steroids. The leaf extracts of *P. guajava* determined by FRP (Ferric reducing power assay) method showed potent antioxidant activity. The antibacterial activity was analysed against five clinically significant organisms by disc diffusion method and leaf extracts of *P. guajava* Linn, showed maximum zone of inhibition against *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae.* The most resistant bacteria were found to be *Escherchia coli* against *P. guajava* fruit extract that showed no activity.

Conclusion: This study reveals that guava leaf extracts exhibits better phytochemical, antioxidant and antibacterial activity than fruit extracts.

Keywords: *Psidium guajava Linn*, Phytochemicals, FRP, Disc diffusion.

INTRODUCTION

Medicinal plants are the chemical goldmines, generally known for its acceptability by human and animal system. The world Health Organization estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs [1] as they have antioxidant, antibacterial, antifungal and antiviral activity [2, 3] The various indigenous systems such as Siddha, Ayurveda and Unani use several plants species to treat different ailments [4].

Guava, belongs to the Family Myrtaceae, has its origin in the tropical South America and grows widely in Bangladesh, India, Thailand, Brazil, Florida, West Indies, California and also in several other countries [5]. The guava leaf extract is used for the treatment of various types of gastrointestinal disturbances such as vomiting, diarrhoea, inhibition of the peristaltic reflex, gastroenteritis, spasmolytic activity, dysentery, abdominal distention, flatulence and gastric pain [6]. It is widely used as antispasmodic, antidiarrhoeal, antidepressant, antiinflammatory, anticough and sedative agent.

MATERIALS AND METHODS

Healthy and disease free fresh leaves and fruits of white and pink *Psidium guajava* Linn were collected from local market of Avadi. The leaves and fruits were washed with distilled water, dried (in shade for a week), sliced, covered with aluminium foil and kept at 4 °C for 24 h. Then it was subjected to centrifugation at 8000 rpm for 10 min. The resultant supernatant was filtered using Whatmann No. 1 filter paper. The crude extract was subsequently oven dried at a temperature of 45 °C to form a powdery residue. The powdered residue was dissolved in ethanol and aqueous solvents and used for further studies.

Phytochemical screening

The phytochemical screening of leaves and fruits of white and pink *P. guajava Linn* were carried out as described by Nweze *et al.*, 2004, Senthilkumar and Reetha 2009 [7, 8]. The samples were screened for carbohydrates, alkaloids, flavonoids, phytosterols and sterols, anthocyanin and betacyanin, phenols, tannins, saponin, glycosides and proteins.

Test microorganisms

The test microorganisms used for antimicrobial analysis were clinical isolates of *Bacillus cereus, Streptococcus pneumoniae, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa*. The bacterial strains were maintained on Nutrient Agar (NA) in the Department of Microbiology, Annai violet college of Arts and Science, Chennai, India.

Antibacterial activity

Antibacterial activities of leaves and fruits of white and pink *P. guajava Linn* was carried out by the disc diffusion method using the Kirby-Bauer technique [9, 10]. All the bacterial strains were maintained on Nutrient Agar (NA). Pure culture was inoculated into MHA (Muller Hinton agar) plate and sub cultured at 37 °C for 24 h. Inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/l saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of 1.5×10^8 cfu/ml. Standardized inoculum was transferred and spread evenly on a MHA (Muller Hinton agar) plate to yield a lawn culture. Sterile Whatmann No. 1 filter paper discs (~5 mm diameter), impregnated with plant extracts (10 µg/disc) was placed on the inoculated MHA plates and allowed to diffuse for half an hour at 4 °C and incubated at 37ºC for 24 h. Disc containing 5% DMSO (Dimethyl Sulphoxide) served as negative control and Tetracycline (36 µg), served as positive control. The plates were observed for the presence of inhibition of bacterial growth that was indicated by the clear zone around the disc. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity [11, 12]. All experiments were carried out in triplicates under strict aseptic conditions. The activities are expressed as resistant, intermediate and sensitive if the zone of inhibition was less than 7 mm, 8-10 mm and more than 11 mm respectively.

Antioxidant activity

Antioxidant potential of leaves and fruits of white and pink *P. guajava* Linn was determined by Ferric reducing power assay (FRP). All the assays were carried out in triplicate.

Ferric reducing power assay (FRP)

In this assay, Fe3+/ferricyanide complex is reduced to the ferrous form by antioxidants. The Fe2+formed is monitored by measuring the formation of Perl's Prussian blue at 700 nm. The reducing power of leaf and fruit extracts was quantified by the method described previously with minor modification [13]. *P. guajava* Linn leaf and fruit extracts and BHT (Butylated Hydroxy Toluene) as standard (2.5, 5.0, 7.5, 10.0) mg in 1 ml ethanol (50%) were mixed with 2.5 ml phosphate buffer (2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). These mixtures were incubated at 50 °C for 20 min. 2.5 ml trichloroacetic acid (10%) were added and the mixture was centrifuged at 3000 rpm for 10 min.

The upper layer of the solution (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml ferric chloride (0.1%). Control was prepared without any extract or BHT (Butylated Hydroxy Toluene). The absorbance of the blue color mixture was measured spectrophotometrically at 700 nm. Increased absorbance of the mixture indicated increased reducing power.

Total phenolic content (TPC)

Total phenolic content of the extracts were determined by the Folin-Ciocalteau method with slight modification. Triplicates of sample extracts, which consisted of 300 µl were transferred into a test tube and 10 % 1.5 ml of Folin-Ciocalteau's reagent (v/v) together with 1.2 ml of sodium carbonate was added.

The test tubes were wrapped in aluminum foil to prevent light exposure and left to stand for 30 minutes before measuring the absorbance at 765 nm. Distilled water was used as a blank. Total phenolic content, was expressed as mg of GAE (gallic acid equivalents) per 100g of material (mg GAE/100g).

Statistical analysis

The experimental data were expressed as mean±SD. The level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Phytochemical screening

The preliminary phytochemical screening of the leaf and fruit extracts of P. guajava Linn showed varied degrees of presence of secondary metabolites. It revealed strong presence of chemical substances such as tannins, phenols, glycosides and flavonoids in the ethanol leaf extracts. Moderate presence of sterols, carbohydrates and alkaloids was shown in the ethanol leaf extracts. Presence of alkaloids were shown only in the ethanol leaf extract of P. guajava Linn. Saponins and proteins were absent in all the extracts of P. guajava Linn. The aqueous extracts also showed almost similar results for the presence of secondary metabolites but the intensity of color was less, which may be attributed to the fact that some chemicals may not be properly soluble in aqueous solvents [14].

The ethanol leaf extracts of P. guajava Linn showed better presence of secondary metabolites compared to all other extracts (table 1). These results are in accordance with study done by Vikrant et al., 2012 [15]. The pink and white guava extracts showed almost similar results therefore not mentioned separately.

Table 1: Phytochemical screening of leaf and fruit extract of *Psidium guajava Linn*.

S. No	Secondary metabolites	Ethanol extract		Aqueous extract	
		Fruit Extract	Leaf Extract	Fruit Extract	Leaf Extract
1.	Carbohydrate	+	++	+	++
2.	Tannins	++	+++	++	++
3.	Saponins	_	_	_	_
4.	Flavonoids	++	+++	++	++
5.	Alkaloids	-	++	-	-
6.	Anthocyanin	+	+	_	_
7.	Betacyanin	+	+	+	_
8.	Glycosides	+	+++	+	++
9.	Proteins	_	_	_	_
10.	Sterols	++	++	++	+
11.	Phenols	+++	+++	++	++

+++-Strongly positive++-positive, +-Trace _-Not detected

Table 2: Antibacterial activity of *Psidium guajava* Linn by disc diffusion method (zone of inhibition in mm at 10 µg/disc)

Microorganisms	Zone of Inhibition				
	Ethanol leaf extract (mm)	ethanol fruit extract (mm)	Aqueous leaf extract (mm)	Aqueous fruit extract (mm)	Tetracycline (mm)
Pseudomonas aeruginosa	19	11	10	8	36
Streptococcus pneumonia	11	13	9	8	23
Escherchia coli	10	7	8	5	30
Bacillus cereus	10	11	5	7	20
Klebsiella pneumonia	16	13	12	13	26

(Data is mean±SD of three determinations)

Antibacterial activity

Antibacterial activity of ethanol and aqueous extracts of fruit and leaves of *P. guajava* Linn was analyzed against five clinically significant organisms using disc diffusion method as shown in (table 2). All the extracts tested showed a measurable zone of inhibition. Tetracycline (36 µg) was used as the standard positive control.

The activity of ethanol extracts of *P. guajava* Linn leaves (inhibition zone 10-19 mm) was found to be more pronounced than all other extracts.

Among the test microorganisms used, *Pseudomonas* and *Klebsiella* were found to be the most sensitive against *P. guajava* Linn leaf extract. The most resistant bacteria were found to be *Escherchia coli* against *P. guajava* fruit extracts that showed no activity.

Antioxidant potential

Ferric reducing power assay (FRP)

In the present study, the ability of extracts to reduce iron (III) to iron (II) was determined and was compared with that of standard BHT.

All the extracts showed some degree of electron donating capacity however, ethanol leaf extracts of *P. guajava* Linn showed maximum reduction of iron (III) to iron (II) indicated by higher absorbance at 700 nm, but the capacities were inferior to that of BHT.

The ethanol leaf extracts of *P. guajava* Linn contains higher phenol content and exhibited, higher electron donating capacity. Similar results have been reported in literature [16] (table 3).

Total phenolic content (TPC)

The present study showed that *Psidium* guajava leaves are rich in phenolic constituents which are efficient in donating protons and it accounts for natural antioxidant property (table 4). Therefore, qualitative and quantitative analysis of major individual phenolics in *Psidium guajava* leaves could be useful for explaining the relationships between total antioxidant capacity and total phenolic contents in this plant [17].

Table 3: Antioxidant activity of *Psidium guajava* Linn by Ferric Reducing Power assay method

S. No.	Concentration (µg/ml)	Ethanol extract		Aqueous extract		BHT (nm)
		Leaf (nm)	Fruit (nm)	Leaf (nm)	Fruit (nm)	
1	2.5	0.53±0.01	0.49±0.05	0.40±0.06	0.32±0.21	0.58±0.07
2	5.0	0.56±0.08	0.52±0.09	0.42±0.12	0.36±0.07	0.60±0.05
3	7.5	0.59±0.05	0.58±0.06	0.48±0.08	0.42±0.01	0.64±0.07
4	10.0	0.62±0.03	0.60±0.01	0.50±0.21	0.46±0.11	0.68±0.02

(Data is mean±SD of three determinations)

Table 4: Total phenolic content of *Psidium guajava* Linn

samples	Absorbance (750 nm)	(mg GAE/100g)
Ethanol Leaf extract	0.318±0.04	0.35±0.13
Ethanol Fruit extract	0.263±0.08	0.28±0.25
Aqueous Leaf extract	0.214±0.01	0.20±0.01
Aqueous fruit extract	0.220±0.02	0.23±0.05

(Data is mean±SD of three determinations)

CONCLUSION

This study reveals that *Psidium guajava* Linn leaf exhibits better phytochemical, antioxidant and antibacterial activity than other extracts along with other properties such as antispasmodic, antidiarrhoeal, antidepressant, antiinflammatory, anticough and sedative agent. The guava leaves being natural, abundant and easily available, we can develop many cost effective molecules for various diseases. Further research has to be done to investigate the mechanism of actions with other therapeutic activities.

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CONFLICT OF INTERESTS

Declared None

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