WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 3, 1167-1174. Research Article ISSN 2277– 7105

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT OF *PSIDIUM GUAJAVA* AGAINST BACTERIAL PATHOGEN

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Article Received on
06 Jan. 2020,
Revised on 27 Jan. 2020,
Accepted on 16 Feb. 2020
DOI: 10.20959/wjpr20203-16892

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ABSTRACT

Psidium guajava L. commonly known as guava is used for the medicinal purposes. Every part of the plant like leaves, bark, fruit and roots is used to treat various diseases. In the present study, Preliminary phytochemical analysis of methanol, ethanol, ethyl acetate, petroleum ether, chloroform and water leaf extract prepared by cold extraction method of *Psidium guajava* L. was carried out by chemical tests and antibacterial activity was determined by agar well diffusion method against *Staph. aureus, Salmonella typhimurium, Salmonella enteritidis, Salmonella paratyphi B, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli*. Alkaloids, carbohydrates, tannins, phytosterols and cardiac glycosides were present in all extracts. However, flavonoids, antraquinine, saponins, aminoacids were absent in all extracts. All the extracts inhibited the growth of bacterial pathogens under investigation. Ethyl acetate extract showed highest antibacterial activity against *Salmonella typhimurium* and *Escherichia coli* followed by aqueous and chloroform against *Escherichia coli* and *Salmonella paratyphi B* respectively. The inhibition zone of gentamycin [reference control] was 32 mm.

KEYWORDS: *Psidium guajava,* cold extraction, agar well diffusion, gentamycin, alkaloids, saponins.

INTRODUCTION

Antibiotics are used to treat the bacterial diseases. But use of antibiotics has many side effects. Another problem throughout the world is antibiotic resistance development. Hence, there is an urgent need to resolve the problems like antibiotics resistance among the

pathogenic microorganisms for effective antimicrobial property having no side effects and can be an alternate as nutraceuticals. Medicinal plants have been identified and used throughout human history. They are used locally in the treatment of various infections cause by fungi, bacteria, virus and other parasites. The phytochemicals found in medicinal plants possesses antibacterial activity. Thus they could be better option to antibiotics. [Rahman et al. 2001].

Psidium guajava L. commonly known as guava belongs to family *Myrtaceae* [Khare, 2007; Ahmed, 2010]. The tree is native of tropical America. Today, the tree is cultivated in nearly all the countries of tropical world belt including India, China, Thailand, Malaysia, Indonesia and Japan [Tepsorn and Reihard, 2009]. Since long time different parts of *Psidium guajava* L. have been used to treat various diseases. The bark of tree has been used for treatment of diarrhoea, malaria and dysentery [Ahmed, 2010]. The leaves are chewed to cure dental diseases [Elekwa *et al*., 2009]. In India, young leaves of Guava are used as remedy against cough[Tepsorn and Reihard, 2009, Ahmed, 2010].

MATERIALS AND METHODS

Plant material: *Psidium guajava* (Linn) leaves were collected from the tree growing in botany garden, D.B.F.Dayanand College of Science, Solapur in the month of January. The leaves were identified in the department of Botany, D.B.F.Dayanand College of Science, Solapur [Maharashtra].

Test Microorganisms: Pure cultures of *Staph. aureus, Salmonella typhimurium, Salmonella enteritidis, Salmonella paratyphi B, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli* were obtained from Government medical college, Solapur, Maharashtra, India.

Preparation of plant extract:[50gm %w/v] Fresh leaves were washed with sterile distilled water three times. 50 gm of washed leaves were then ground with 90ml sterile distilled water in electronic grinder. The mixer was kept aside for 45 minutes for extraction. It was then filtered through whatman filter paper no.1.To the residue again 10 ml of sterile distilled water was added filtered similarly after 15 minutes. The extract was aloowed todry by evaporating solvent in incubator at 37^0C overnight. The extract was stored in sterile petri dish at 4^0 C.The same procedure was repeated by using methanol, ethanol, ethyl acetate, petroleum ether & chloroform as solvents for extraction instead of sterile distilled water.

Preliminary Phytochemical Analysis of Extract [Raamann N.**2006]**

Preliminary phytochemical analysis was done to find out the active chemical principle of the plant.

Detection tests of plant extracts:

Detection of Alkaloids: 50 mg of Solvent free extract was mixed with few ml of dilute HCL and filtered. The filtrate was used for various tests as follows.

1. Mayer's test -To a small aliquot of filtrate in a test tube, a drop of Mayer's reagent was added. Development of white or creamy precipitate indicated the positive test.
2. Wagner's test - To a small aliquot of filtrate in a test tube, a few drops of Wagner's reagent were added. Development of reddish-brown precipitate indicated the positive test.
3. Hager's test - To a small aliquot of filtrate in a test tube one ml of Hager's reagent was added. Development of yellow precipitate indicated the positive test. Detection of Carbohydrates:

Detection of carbohydrates: Benedict's test - To a 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated for 2 min in a boiling water bath. A characteristic colored filtrate indicated the presence of sugar.

Detection of Amino acids and proteins: 100 mg extract was dissolved in 10 ml distilled water and filtered through Whatman no.1 filter paper. The filtrate was used to test presence of proteins and amino acids.

Biuret test - One drop of 2% copper sulphate solution was added to 2 ml of filtrate. To this, 1ml of ethanol was added followed by addition of excess of potassium hydroxide pellets. Development of pink color in the ethanol layer indicated presence of proteins.

Ninhydrin Test- Two drops of ninhydrin solution were added to 2 ml of aqueous filtrate. Development of purple color indicated presence of amino acids.

Detection of Saponins: Foam test - 50 mg of extract was dissolved in 20 ml of distilled water. The suspension was shaken in a graduated cylinder for 15 min. Development of two cm layer of foam indicated the presence of Saponins.

Detection of Tannins: Ferric chloride test – 50 mg of extract was dissolved in 5 ml of distilled water and then a few drops of 5% Ferric chloride were added. Development of dark green color indicated the presence of tannins.

Detection of flavonoids: Magnesium and hydrochloric acid reduction test - 50 mg of the extract was dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid were added drop wise. Development of pink to crimson colour indicated presence of flavonoids.

Detection of anthraquinones: 50 mg of extract was dissolved in distilled water. 1ml dilute ammonia solution was added to 2 ml of extract and shaken vigorously. Development of pink color in ammonia layer indicated presence of anthraquinones.

Detection of Cardiac glycosides: Killer kiliani test - 50 mg of the extract was dissolved in distilled water and filtered. Then 1ml of glacial acetic acid and a drop of Ferric chloride and a drop of concentrated sulfuric acid were added to 2 ml of filtrate. Development of green blue color to upper layer and reddish-brown color at the junction of two layers indicated the presence of cardiac glycosides.

Detection of fixed oils and fat: Spot test- A small aliquot of extract was pressed between two filter papers. Development of oil stain on the paper indicated the presence of fixed oils.

Antibacterial activity of Plant Extract [Cruickshank et.al., 1975]

Antibacterial activity of Plant Extract was determined by agar diffusion method. For this, fresh [overnight] isolated colony of test organism was suspended in sterile saline to get turbidity of 0.5 McFarland standards. o.1 ml. of this suspension was spread aseptically on sterile Muller Hinton agar medium [Hi media]. Then the wells [6 mm diameter] were bored by sterile cork borer. 0.2 ml. of each extract was added to the wells. It was allowed to diffuse by keeping in freeze for 20 minutes.0.2 ml of each solvent was added in another well to test its antibacterial activity. The zone of inhibition if obtained with only solvent was then subtracted from the zone of inhibition obtained with plant extract in that solvent. Antibiotic gentamycin [10 mcg/ml][Hi Media] disc was used as standard positive control. After diffusion of extracts the plates were incubated at 37 ^{0}c for 24 hours. Zones of inhibition were then measured in mm. For each extract three replicates were maintained.

RESULT AND DISCUSSION

Table 1: Preliminary Phytochemical Analysis of leaf extract of *Psidium guajava* L.

Sr no.	Phytochemicals	Name of test	Aq	Meth	Eth	EA	PE	Chl
1	Alkaloids	Mayer test	+	+	+	+	+	+
		Wagner test	+	+	+	+	+	+
		Hager test	+	+	+	+	+	+
2	Carbohydrates	Benedict test	+	+	+	+	+	+
3	Saponins	Foam test	-	-	-	-	-	-
4	Proteins	Biuret test	-	-	-	-	-	-
5	Amino acids	Ninhydrin test	-	-	-	-	-	-
6	Anthraquinones	-	-	-	-	-	-	-
7	Tannins	Ferric chloride test	+	+	+	+	+	+
8	Flavonoids	Magnesium & HCL reduction test	-	-		-	-	-
9	phytosterols	Liebermann Burchard test	+	+	+	+	+	+
10	Cardiac glycosides	Killer Kiliani test	+	+	+	+	+	+
11	Fixed oils and fats	Spot test	--	--	--	--	--	--

[Meth= methanol, EA= Ethyl acetate, PE= Petroleum ether, Chl= chloroform, aq=aqueous]

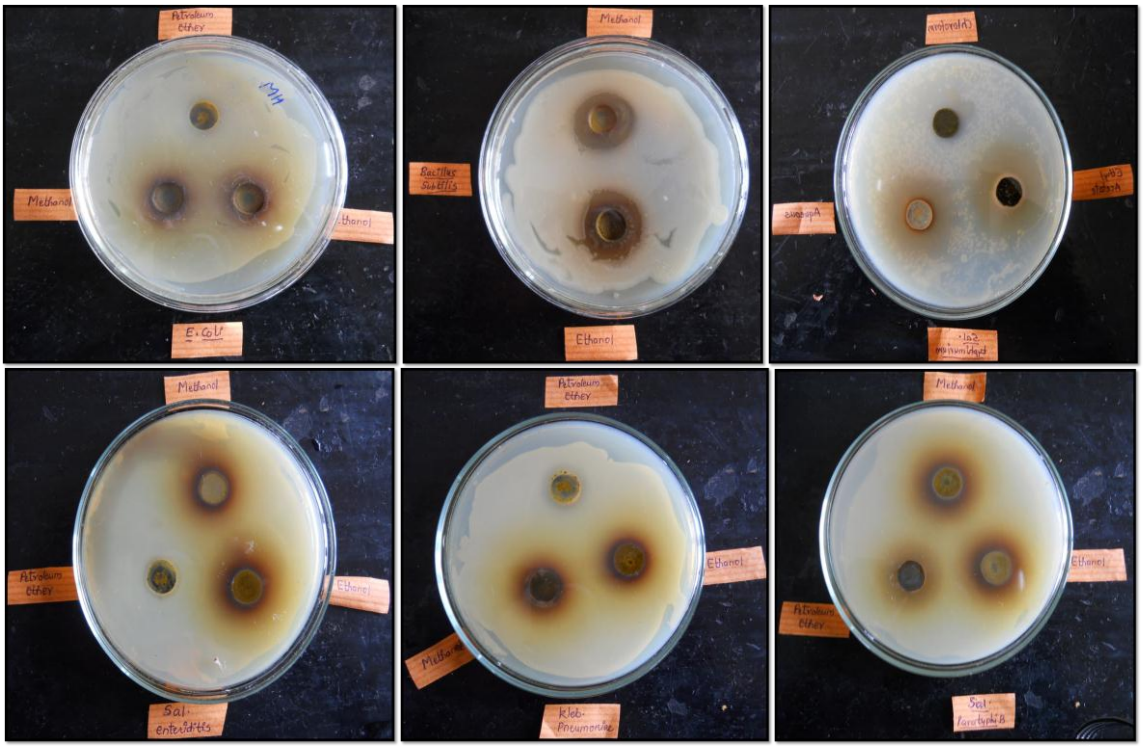
Table 1 shows the preliminary phytochemical analysis of extracts of *Psidium guajava* L. leaves. Alkaloids, carbohydrates, tannins, and cardiac glycosides were present in all extracts however saponins, proteins, amino acids, anthraquinones, fixed oils and fats and flavonoids were absent in the extracts of *Psidium guajava* L. leaves. Preliminary phytochemical analysis of methanolic extracts leaf of *Psidium guajava* L. revealed the presence of antimicrobial compounds such as flavonoids, steroids and tannins [Dhiman *et al*., 2011].

Phytochemical analysis carried out by Singh *et al*., (2012) showed the presence of flavonoids, alkaloids, terpenoids, tannins, saponins and glycosides in methanolic leaf extract of *Psidium guajava* L.

Elekwa *et al*., (2009) investigated phytochemicals in the ethanol, methanol and aqueous extract of stem bark and leaves of *Psidium guajava* L. They have reported the presence of alkaloids (in all the extracts), saponins (in ethanol and methanol), cardenolides with steroided rings (in all the extracts). The presence of these phytochemicals supports the use of this plant in medicine.

Table 2: Antibacterial activity of *Psidium guajava* L.leaf extracts.

Sr.No	Name of test organism	Diameter of zone of inhibition [mm]						
		Aq	Met	Eth	EA	PE	Chl	DMSO
1	*Staph. aureus*	05	20	20	26	15	02	-
2	*Salmonella typhimurium*	30	24	25	32	14	12	-
3	*Salmonella enteritidis*	10	25	28	24	12	02	-
4	*Salmonella paratyphi B*	30	25	22	25	16	26	-
5	*Proteus vulgaris*	18	20	25	20	16	03	-
6	*Pseudomonas aeruginosa*	10	20	20	28	20	28	-
7	*Klebsiella pneumoniae*	11	22	22	22	12	02	-
8	*Escherichia coli*	12	22	22	32	15	30	-



Photograph 1: Antibacterial activity of *psidium guajava* l. Leaf extracts.

Table 2 exhibits the antibacterial activity in all solvent extracts used in the present study. The strong activity was observed with ethyl acetate and chloroform extracts. Maximum activity was revealed in ethyl acetate against *Salmonella typhimurium*[32 mm] *and Escherichia coli*[32] *and Escherichia coli* [30mm] *and Pseudomonas aeruginosa*[28] *in chloroform extract.* However, aqueous extract showed maximum against *Salmonella typhimurium* [30mm] *and Salmonella paratyphi B.*[30mm].

Dhiman *et al*., (2011) investigated in vitro antimicrobial potential of methanol extract of *Psidium guajava* L. leaf and reported that the methanolic extract exhibited antimicrobial activity against *Escherichia coli* with MIC 0.78µg/ml and MBC of 50µ/ml. Further they

noticed appreciable antifungal activity with MIC of 12.5µg/ml. Similar studies of antibacterial activity of methanolic leaves extract of *Psidium guajava* L.by agar well diffusion method against *Staphylococcus aureus* and *Escherichia coli* exhibited significant activity by Ismail Mohammed, 2012. Antimicrobial activity of stem bark and leaves of *Psidium guajava* L by Elekwa *et al*., (2009) by using ethanol, methanol and aqueous extract reported that only aqueous extracts inhibited *Bacillus subtilis* and *Fusarium* species. Antibacterial activity revealed in the present study correlates with investigations by Dhiman *et al*., (2011), Ismail Mohammed, 2012 and Elekwa *et al*., (2009).

CONCLUSION

The present study concludes that *Psidium guajava* L. leaf contains various active antibacterial phytochemicals like alkaloids, saponins, tannins, phytosterols due to wcich it exhibits antibacterial activity against bacteria. Thus this plant can be utilized for drug development.

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