

Antibacterial and Free Radical Scavenging Activities of stem bark of *Psidium guajava* Linn

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Abstract

Psidium guajava has been widely used in traditional systems of medicine for a variety of diseases. In the present study, stem bark of *Psidium guajava* was evaluated for its antimicrobial and antioxidant activities. The agar disk diffusion method was used to study the antibacterial activity of ethanolic extract of stem bark of *Psidium guajava* (EPG) against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. Free radical scavenging assays such as 2, 2-diphenyl-1-picryl hydrazyl (DPPH) and nitric oxide radical scavenging assays were performed. The EPG showed antibacterial activity against all three bacterial stains. It was observed that the EPG effectively scavenged free radicals in a dose dependent manner and antioxidant activity was compared to standard antioxidant such as ascorbic acid.

Key words:

Antibacterial, Antioxidant, *Psidium guajava*

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Introduction:

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. India has rich medicinal plants flora of more than 7500 species. Of these, 4635 species are used commercially on large scale. Over 50% of all modern

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clinical drugs are natural product origin and natural products play important roles in drug development in the pharmaceutical industry [1].

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The pharmacological industries have produced a number of new antibiotics; resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents. Therefore, actions must be taken to reduce this problem, such as to minimize the use of antibiotics, develop research of resistance, among microorganism and to continue studies to develop new drugs, either synthetic or natural to control pathogenic microorganism. Antioxidants are one such substance which has the capability to neutralize free radicals or their actions. Recently, there has been growing interest in natural antioxidants of plant origin because they have greater application in the food industry for increasing the stability and shelf life of food products. Moreover, they also find use as nutraceuticals and phyto-pharmaceuticals as they have significant impact on the status of human health and disease prevention [2].

The plant *Psidium guajava* is a shrub or small tree up to 8 m tall, with smooth, peeling bark. Leaves short-petioled, elliptic ovate to elliptic-oblong, often rounded at base, lathery, pubescent on lower surface, to 15 cm long and 6m wide; lateral nerves 10-18 pairs, prominent on under surface. Flower large, white and fragrant, to 2 cm long. Fruit(berry) 3-10 cm across, green to light yellow when ripe, or red in some varieties, pulpy; fresh creamy white to yellow or red, depending on variety. Numerous varieties have been developed in india and elsewhere in the tropics. Stem irregularly fluted when old. Bark quite smooth, pale pinkish brown or buff white gray patches, exfoliating in very thin woody plates. The plant traditionally used as astringent, haemostatic, constipating and antiemetic as well as used to treat

haemorrhages, diarrhea and dysentery especially in children [3].

The plant has been reported various pharmacological activities including hepatoprotective [4], cytotoxicity [5] and anti-diabetic activity [6]. Therefore, it is worthwhile to use modern science and technology tools for verifying therapeutic potential of *Psidium guajava* Stem bark as antimicrobial and antioxidant agent as per international standards.

Materials and Methods:

Collection and identification of the plant material:

The stem bark of *Psidium guajava* was collected from the Morena, Madhya Pradesh in the month of November 2010. The plant was authenticated at Department of Botany, Safia college of Science, Bhopal, Madhya Pradesh and the Voucher specimen (234/BOT/SAFIA/11) has been preserved at Department of Pharmacology, Technocrats Institute of Technology-Pharmacy for future reference.

Preparation of extract:

The collected, cleaned powder of stem bark of *Psidium guajava* was used for the extraction process. The powder of stem bark of *Psidium guajava* (500 g) material was evenly packed in the soxhlet apparatus and extracted with ethanol by hot continuous extraction process for about 26 h and concentrated by vacuum distillation to reduce the volume 1/10. The concentrated extract was placed in desiccator to remove the excessive moisture [7].

Preliminary phytochemical screening:

The preliminary phytochemical screening of ethanolic extract of stem bark of *Psidium guajava* (EPG) was subjected to determine the presence of various phytoconstituents [8].

Antibacterial activity:

Microbial strains:

The strains were Gram-positive (*Bacillus subtilis* MTCC 1134) and Gram-negative (*Escherichia coli* MTCC 3261 and *Pseudomonas aeruginosa* MTCC 647) obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

Disc diffusion method:

In vitro antimicrobial activity was screened by disc diffusion method [9]. The Mueller Hinton Agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petri-plates. The plates were allowed to solidify for 5 min and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. The different concentrations of EPG were loaded on 5 mm sterile individual discs. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 h. Negative control was prepared using respective solvent. The standard drug Gentamycin (10 µg/disc) was used as positive control. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Antioxidant activity:

DPPH scavenging activity:

The antioxidant activity of EPG and standard were as assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical activity [10]. The diluted working solutions of EPG were prepared in methanol. Ascorbic acid was used as standard in 1-100 µg/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank and % inhibition was calculated.

Nitric oxide scavenging activity:

Nitric oxide radical scavenging activity was determined according to the method reported by [11]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of EPG at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated.

Calculation of percentage inhibition:

The percentage inhibition of radical production by the test sample was calculated using the formula:

$$\text{Inhibitory ratio} = \frac{A-B}{A} \times 100$$

Where, A is the absorbance of control;

B is the absorbance with addition of test sample

Linear regression analysis was used to calculate IC₅₀ values.

Results and Discussion:

The qualitative chemical investigation of EPG was carried out to check the presence of various phytoconstituents and it was revealed that the presence of flavonoids, phenolic compounds, tannins, terpenoids and sponins (Table-I).

Recently much attention has been directed towards plant extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants play a vital role in covering the basic health needs in developing countries and the plants

may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world population. There are about 45,000 plant species in India with capacity to produce a large number of organic chemicals concentrated hotspot in the region of Eastern Himalayas, of high structural diversity [12]. In the present work EPG showed higher activity to the test bacteria such as *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The antibacterial activity of *Bacillus subtilis* and *Pseudomonas aeruginosa* showed more zone of inhibition, compared to *Escherichia coli*. The EPG showed significant activity to the test bacteria such as *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* when compared to standard drug Gentamycin and results were reported in Table-II.

The antioxidant activity of plants is mainly contributed by the active compounds present in them and may contribute to the improvement in quality of life by delaying onset and reducing the risk of degenerative diseases associated with aging. Nitric oxide (NO) is a potent pleiotropic mediator of physiological process such as smooth muscle relaxant, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities. Although nitric oxide and superoxide radicals are involved in host defense, over production of these two radicals contributes to the pathogenesis of some inflammatory diseases. Moreover in the pathological conditions, nitric oxide reacts with superoxide anion and form potentially cytotoxic molecules,

peroxynitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspect of inflammation and tissue damage seen in inflammatory diseases [13]. EPG significantly inhibited nitric oxide in a dose dependent manner (Table-III) with the IC₅₀ being 281.57 µg /ml. DPPH assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration. When DPPH radicals encounter a proton donating substance such as an antioxidant, it would be scavenged and the absorbance is reduced. Thus, the DPPH radicals were widely used to investigate the scavenging activity of some natural compounds [13]. In the present study, the EPG was investigated in comparison with the known antioxidant ascorbic acid. The IC₅₀ values for DPPH radical with ascorbic acid and EPG was found to be 91.85 and 229.17 µg/ml, respectively as shown in Table-IV.

Conclusion:

The present study suggested that EPG have great potential as antimicrobial agent against enteric bacterial pathogens and they can be used as alternative medicine in the treatment of enteric bacterial. The results obtained in the present study indicate that EPG exhibits free radical scavenging activity. Both antimicrobial and antioxidant activity may be due to strong occurrence of polyphenolic compounds and terpenoids. These findings provide scientific evidence to support traditional uses and indicate a promising potential for the development of antimicrobial and antioxidant drug from *Psidium guajava* plant.

Table 1: Preliminary phytochemical screening of EPG

Name of the tests	EPG
Alkaloids	-
Glycosides	-
Steroids	-
Flavonoids	+
Tannins & Phenolic Compounds	+
Terpenoids	+
Carbohydrates	-
Fixed oils & Fats	-
Proteins & Free amino acids	-
Gums & Mucilage	-
Saponins	+

+ = Positive; - = Negative

Table-II: Anti bacterial activity of EPG by disk diffusion method

Test samples (µg/ml)	Zone of inhibition (mm)		
	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
EPG 150	12	10	08
EPG 250	16	14	14
EPG 350	14	12	09
Gentamycin 20	21	17	22

Table-III: Nitric oxide radical scavenging effect of EPG

Concentration (µg/ml)	Percentage of inhibition	
	Ascorbic acid	EPG
10	24.85	16.05
50	36.47	20.97
100	47.04	27.31
150	60.42	31.54
200	78.73	37.88
250	-	49.85
300	-	51.61
IC₅₀ (µg/ml)	104.08	281.57

Table IV: DPPH radical scavenging effect of EPG

Concentration (µg/ml)	Percentage of inhibition	
	Ascorbic acid	EPG
10	26.14	6.78
50	40.18	17.12
100	59.12	26.88
150	72.58	33.36
200	86.86	44.12
250	-	58.12
300	-	60.48
IC₅₀ (µg/ml)	91.85	229.17

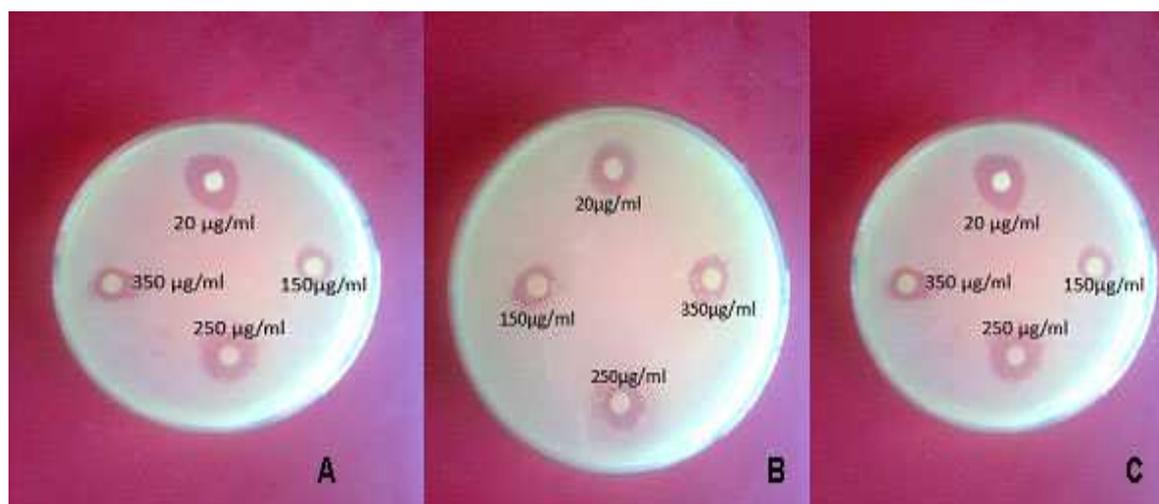


Figure no: 1 Antibacterial activity of EPG (A= *B. subtilis*; B= *E. coli*; C= *P. aeruginosa*)

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