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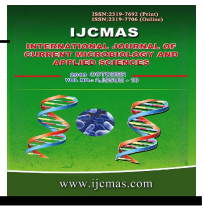

Original Research Article

Phytochemical screening , *Invitro* Anti-bacterial and Antioxidant activity of the *Psidium guajava* root bark

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Keywords

Antioxidants; *Psidium guajava*; Free radicals; Antibacterial activity; Cup plate method; Phyto-chemicals.

A B S T R A C T

Aim of the present work was to evaluate potential *in-vitro* antioxidant and antibacterial activity of alcoholic extract of root bark of *Psidium guajava (PG)* of family Myrtaceae. The antioxidant activity of alcoholic extract of *PG* root bark was evaluated by DPPH, Lipid Peroxidation and Nitric oxide Scavenging activity. The concentration of alcoholic extract were (3-800 ug/ml). The *PG* extract showed maximum scavenging activity at a concentration of 800 µg/ml. The percentage inhibition of the scavenging of the DPPH was found to be 75.6% where as Lipid Peroxidation was 82.4% and nitric oxide scavenging activity was found to be 85.6%. The antibacterial activity of the alcoholic extract of *Psidium guajava* root bark was carried out and it was determined by the cup plate method against *Staphylococcus aureus, Escherichia coli, Proteu vulgaries* and *Bacillus subtilis*. Streptomycin was used as a positive control. The zone of inhibition of alcoholic extract of *PG* against various microorganisms was measured and compared with standard control, *PG* showed antibacterial activity at the concentration of 240 mg/ml against all the bacterial strains however, maximum activity with zone of inhibition (3 cm and 2.8 cm) against Staphylococcus aureus and Bacillus subtilis. Escherichia coli and Proteus vulgaries exhibited moderate antibacterial activity. The preliminary phytochemical screening of alcoholic extract of *PG* showed the presence of pharmacological important constituents like tannins, flavonoids, alkaloids, saponin glycosides. Hence on the basis of the results obtained in the phytochemical study, the antioxidant activity of *PG* may be due to flavonoids and antimicrobial activity could be due to tannins. Phytochemical analysis intended to serve as a major resource for information on analytical and instrumental methodology in the plant sciences.

Introduction

To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system, that functions interactively and synergistically to

neutralize free radicals. Thus, antioxidants are capable of stabilizing the deactivating free radicals before they attack cells (Valko *et al*., 2007).

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoans.The discovery of synthetic antimicrobial compounds having so many adverse effects (Rajeswar *et al*., 2005). Hence alternative herbal medicines are selected.
Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants growing in different regions of the world. For centuries, plants have been used throughout the world as drugs and remedies for various diseases since they have greater potential for producing newer drugs of great benefit to mankind (Efferth and Greten, 2012). In this context, Medicinal plants are rightly said to be Tradition of yesterday and drugs of tomorrow .India is one of the largest users of medicinal plants using more than 7000 plant species for cure and has an abundance of plants, used in the traditional treatments of various disease on an empirical basis (Jain 1994; Ali *et al*.,2008).

In modern times the utility of medicinal plants declined with the advent of synthetic drugs. Therefore, more and more people showing their effort for natural treatment, as it is free from dangerous side effects. Thus the proposed investigations may provide support to the folklore use of medicinal plants. Hence efforts are being made to search easily available alternative medicines with low cost and less or free of side effects.

Psidium guajava

This plant was selected for the present study based on phytoconstituents like tannins, saponins and flavanoids in stem, leaves and roots. There is no work done on root bark of *Pisidium guajava Pisidium guajava* (*PG)* (Myrtaceae) commonly known as the poor man's apple of the tropics has a long history of traditional use much of which is being

validated by scientific research. It is a dicotyledonous shrub, a small tree about 33ft (10) in hight, with spreading branches, the guava is easy to recognize because of its smooth, thin, copper-colored bark that flakes off, showing the greenish layer beneath and also because of attractive "bony" aspect of its trunk, flowers are fragrant with white, The fruit extending a strong, sweet, musky odour when ripe, Guava rich in tannins, phenols, flavanoids, essential oils, lectins, vitamin, fatty acids etc. The therapeutic activity of guava is attributed to the presence of flavanoids. The tannins demonstrated anti-microbial activity as well as flavanoids demonstrated anti-oxidant activity (Morton, 1987). The stem bark contains 12-30% of tannins, poly phenols, resin and crystals of calcium oxalate (Nadkarni and Nadkarni, 1999), amritoside (Conway, 2001). The roots, stem bark and leaves of the plant rich in tannins (Quisumbing, 1978), leukocyanidins, sterols and gallic acid (Lwu, 1993), quarcetin (Wyk *et al*,

1997). *Guava* fruit is higher in vitamin C than citrus fruits (Okwu and Ekeke, 2003). *Psidium guajava* is well known traditional medicinal plant and is used in various indigenous systems of medicine. The fruits are often included among super fruits, being rich in dietary fibre, vitamins A,C, folic acid and dietary minerals such as potassium, copper and manganese (Hassimotto *et al*., 2005). It is used in many diseases such as anti-inflammatory, diabetes, hypertension, carries wounds, analgesic and antipyretic effects (Gutierrez *et al*., 2008). The root bark of guava dried and roasted slightly and powdered, a pinch of this powder taken in buttermilk checks diarrhoea, an especially useful remedy in infants and children (Ali *et al*., 1996).

In the Philippines the decoction made from the cortex of the bark and roots were used for washing ulcers and wounds (Quisumbing, 1978). While in Panama, Bolivia and Venezuela, the bark is used in the treatment of dysentery and skin aliments (Conway, 2001). In Kinshasa and Congo the bark is used as anti amoebic (Geidam *et al*., 2007) and also used to expel the placenta after the child birth and used to treat skin infection, vaginal hemorrhage, wounds, fever, dehydration and respiratory disturbances, stomachache, tooth aches and constipation (Gutierrez *et al*., 2008).

P. guajava leaves reported to have antioxidant activity (Masuda *et al*., 1999), antibacterial activity (Rogerio, 2005) and kidney problems (Ticzon, 1997) antiulcer activity (Swarnamoni , 2009). Fruit extract in combination of bark, leaf and root aqueous extract showed anti cancer activity (Sato *et al*., 2010). Aqueous extract of *P guajava* leaves possesses anti diarrhoeal activity (Xavier *et al*., 2002) and hypotensive activity (Ojewole, 2006), Flower extract of *PG* also used as a poultice for conjunctivitis (Ayensu, 1978).

Materials and Methods

Collection and Preparation of plant extract

Psidium guajava roots was collected from Sri Padmavathi Mahila University campus, Tirupati in the month of August and authenticated by Dr. Madhava Chetty, Department of Botany, SVU, Tirupati, roots were washed thoroughly under running tap water and bark was separated from root and were shade dried and powdered.

The root bark powder of *PG* (25 gm) was macerated in alcohol for 24 hrs in a round bottom flask and it was subjected to reflux for 3 hrs, cooled and filtered through whatmann filter paper no.1. Repeated the process for 3 times in the same solvent until plant material become color less. The collected filtrate was subjected to solvent evaporation by using Rotary flash evaporator, the residue (extract) was collected and stored in dessicator until use. The alcoholic extract of *PG* was brown in colour and percentage yield was 10.2%.

Growth and maintenance of test organisms for Anti microbial studies

Bacterial cultures of Escherichia coli, Proteus vulgaries,Bacillus Subtilis, Staphylococcus aureus were obtained from culture collection centre; Dept of microbiology; Sri Padmavathi Mahila Vishwavidhyalayam (SPMVV), Tirupati, were used as antimicrobial test organisms. The bacteria was maintained on nutrient broth at 37 ^{0}C.

Chemicals

Chemicals and solvents were used in this study was analytical grade.

In vitro antioxidant methods

DPPH radical scavenging activity

DPPH radical scavenging activity was measured by the spectrophotometric method. To an ethanolic solution of DPPH (200 μM*)*, 2 ml of test compounds dissolved in ethanol were added at different concentrations (3-800 μg/ml). An equal amount of ethanol was added to the control. After 20 min the decrease in absorbance of test mixtures (due to quenching of DPPH free radicals) was read at 517nm.using calorimeter and the percentage inhibition was calculated (Vani *et al*., 1997).

Assay for NO scavenging activity

Sodium nitroprusside (5 mM) in phosphate buffer pH 7.7 was incubated with 3, 6, 12, 25, 50, 100, 200, 400 and 800 μg/ml concentrations of drug dissolved in a suitable solvent (alcohol) and tubes were incubated at 25°C for 120 minutes. At intervals, 0.5ml of incubation solution was removed and diluted with 0.5 ml of Griess reagent. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent N-napthyl ethylenediamine was measured at 546 nm in (Marcocci *et al*., 1994).

Lipid peroxidation:

Preparation of rat brain homogenate (Youdim, 1990).

The extent of lipid peroxidation in rat brain homogenate was measured *in vitro* in terms of formation of thiobarbituric acid reactive substance (TBARS). Different concentrations of the extract (3-800 μg/ml) were made up with ethanol the ethanolic extract was expressed in terms of dry weight (mg/ml) in ethanol. These samples were individually added to the brain homogenate (0.5 ml). This mixture was incubated with 0.15 *M* KC1 (100 μl). Lipid peroxidation was initiated by adding 100 μl of 15 mM $FeSO_4$ solution. The reaction mixture was incubated at 37 °C for 30 min. An equal volume of TBA:TCA (1;1.1ml) was added to the above solution followed by the addition of 1ml BHT. This final mixture was heated on a water bath for 20 min at 80 °C and cooled, centrifuged and absorbance read at 532 nm using a spectrophotometer (Shimadzu 160 IPC) (Yoshikawa *et al*., 1983). The percentage inhibition of lipid peroxidation was calculated by comparing the results of the test with those of controls not treated with the extract as per the formula:

Formula: $\%\text{Inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$

(above all the tests were in triplicate)

Assay of antibiotic (Streptomycin) by the agar well-diffusion method (Heilman, 1945).

Agar-well diffusion method

To evaluate the antimicrobial activity of plant extract by using microorganisms like Escherichia coli, Proteus vulgaries, Bacillus subtilis, Staphylococcus aureus, Agar well diffusion method was used. In this method, the plant extract was dissolved in appropriate solvent. Agar plates were prepared and inoculated with the microbial suspension and then agar was punched to form well of 7 mm diameter. Different plant test extract (mg//ml) was suspended in the wells. A control well was loaded with streptomycin (1mg/ml). The plates were then incubated

at 37°C for 24 to 48 hrs. After incubation, all plates were analyzed for the appearance of inhibition zone around the extract loaded well and the clear zone of growth inhibition was measured using the serial dilution method of diameter (cm) (Okeke *et al*., 2001).

Preliminary phytochemical analysis

Phytochemical analysis was carried out according to standard protocol(kokate *et al*.,2010)

Result and Discussion

Antimicrobial activity of alcoholic extract of root bark of *Psidium guajava* was evaluated by well agar diffusion method. *Psidium guajava* at concentrations of 10, 20, 40, 80, 160, 240 mg/ml was selected. The antimicrobial activity was maximum at a concentration of 240 mg/ml against Staphylococcus aureus and Bacillus subtilis as compared to Streptomycin (1mg/ml) and the activity was moderate against Proteus vulgaris and E.coli when compare to Streptomycin. *Psidium guajava* alcoholic extract showed minimum activity at 10, 20, 40, 80, 160 mg/ml concentration against all organisms compared to Streptomycin (Table.1; Fig. 1).

Effect of alcoholic extract of *Psidium gujava* root bark on different antioxidant models

Free radical scavenging activity was carried out in different *invitro* antioxidant models. Several concentrations ranging from (3-800 µg/ml) of *PG* root bark was tested. Results of present study showed that free radicals was scavenged by the test compounds (extract) in a concentration dependent manner up to the given concentration in all the models. The IC_{50} value of DPPH, NO, lipid peroxidation were found to be 300 µg/ml, 280µg/ml, 320 µg/ml respectively (Table. 2, Fig.2,3,4).

Plants are important sources of potentially useful structures for the development of new chemotherapeutic agents (Tona *et al*., 1999). Many reports are available on the antiviral, antibacterial, antifungal, antihelmentic and anti-inflammatory properties of plants (Samy, 2000, Palambo, 2001, Stepnovic, 2003). Some of these observations have helped in identifying the active principles responsible for such activities. In the present study, alcoholic extract of *Psidium guajava* root bark was investigated for their antimicrobial potentiality against 4 clinically important microbial strains.

Among which 2 of them were gram positive and 2 of them were gram negatives was used. The anti microbial activity of *Psidium guajava* was studied by Well diffusion method. Well diffusion method is used extensively to investigate the anti microbial activity of natural substances and plant extracts. *Psidium guajava* showed maximum activity at 240 mg/ml as compared to control used againist all the bacterial strains. In the present investigation, largest inhibition zone diameters were recorded with gram-positive bacteria i.e *S. aureus* and *B. subtilis*, it could be due to presence of tannins and other phytochemicals in root bark of *PG*. Phytochemical screening of alcoholic extract of root bark of *Psidium guajava* contains presence flavonoids, tannins, alkaloids and saponins, these constituents could be responsible for the activity.The zones of inhibition of effective extract was close to the control drug and falls with the

Table.1 Effect of alcoholic extract of *Psidium guajava* root bark on antimicrobial activity

Zones of inhibition (cm)							
	***Psidium guajava* extract (mg/ml)**						**Streptomycin (1 mg/ml)**
Microorganism	**240**	**160**	**80**	**40**	**20**	**10**	
Staphylococcus.aureus	3	2.4	2.7	1.5	1.6	1.8	3.6
Eschericia coli	2.1	2.6	0	1.5	1.5	1.1	3.6
Bacillus subtillis	2.8	2	2.6	2.5	2.6	2	4.4
Proteus vulgaries	2.2	2	2.7	2	1.5	1.5	4.4

Figure.1 Effect of alcoholic extract of *Psidium guajava* root bark on antimicrobial activity

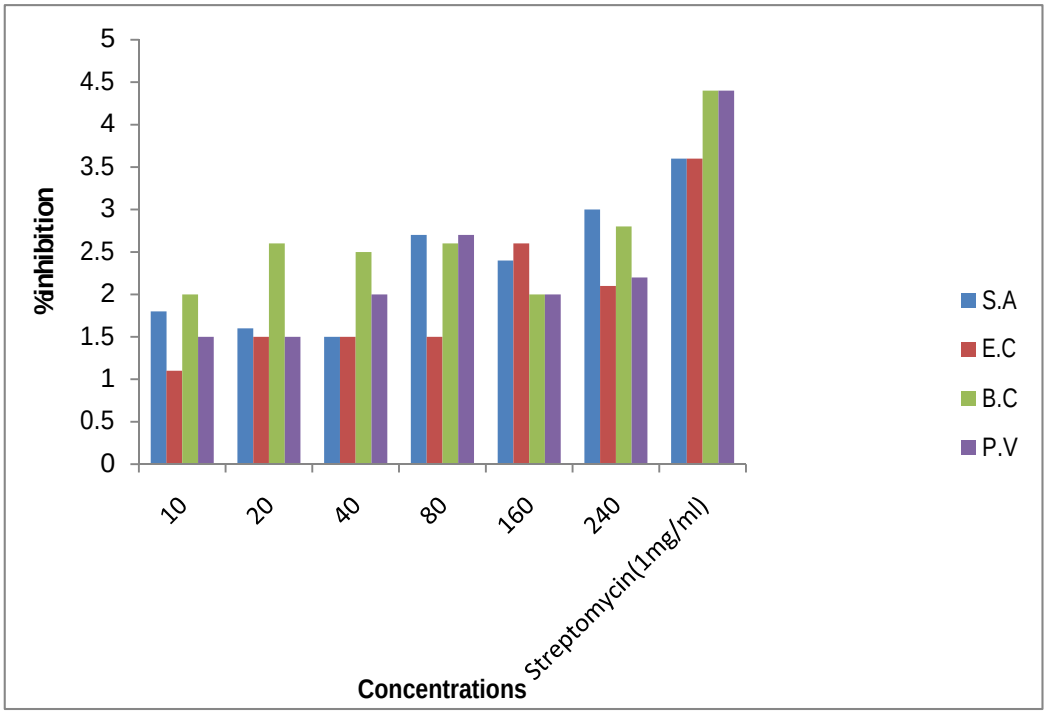

Table.2 Effect of alcoholic extract of *Psidium gujava* root bark on different antioxidant models

S. No	Concentration (µg/ml)	% Inhibition		
		DPPH	NO	Lipid Peroxidation
1	3	4.8	5.6	4.05
2	6	7.8	9.3	9.12
3	12	15.7	15.6	16.2
4	25	45.6	23.7	22.6
5	50	56.93	32.1	33.7
6	100	65.2	51.4	44.5
7	200	66.7	62.2	45.2
8	400	69.3	67.8	52.3
9	800	75.6	85.6	82.4
11	IC_{50}	280 µg/ml	280µg/ml	320 µg/ml

Figure.2 Effect of alcoholic extract of *Psidium guajava* root bark on DPPH 3 method

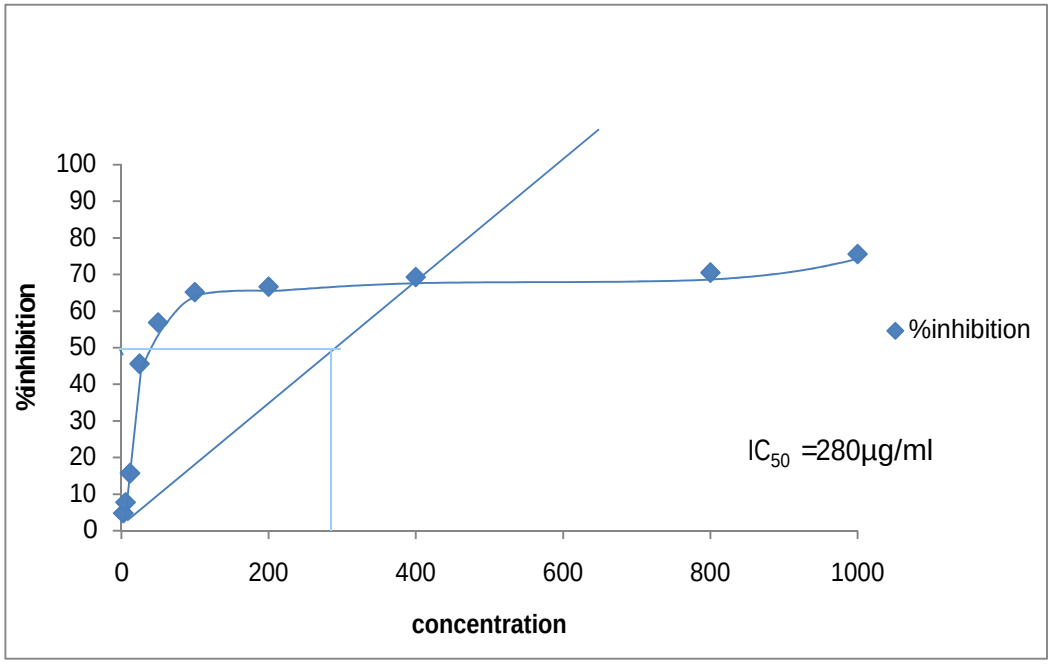

Figure3 Effect of alcoholic extract of *Psidium guajava* root bark on Nitric oxide scavenging activity

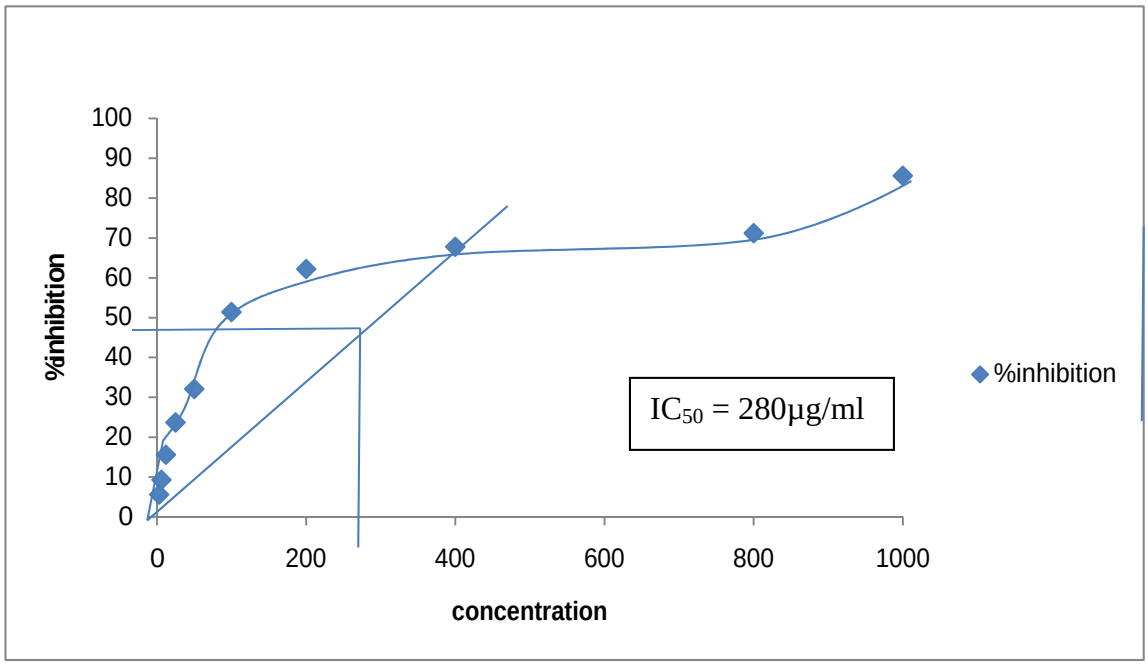

Figure.4 Effect of alcoholic extract of *Psidium guajava* root bark on lipid peroxidation method

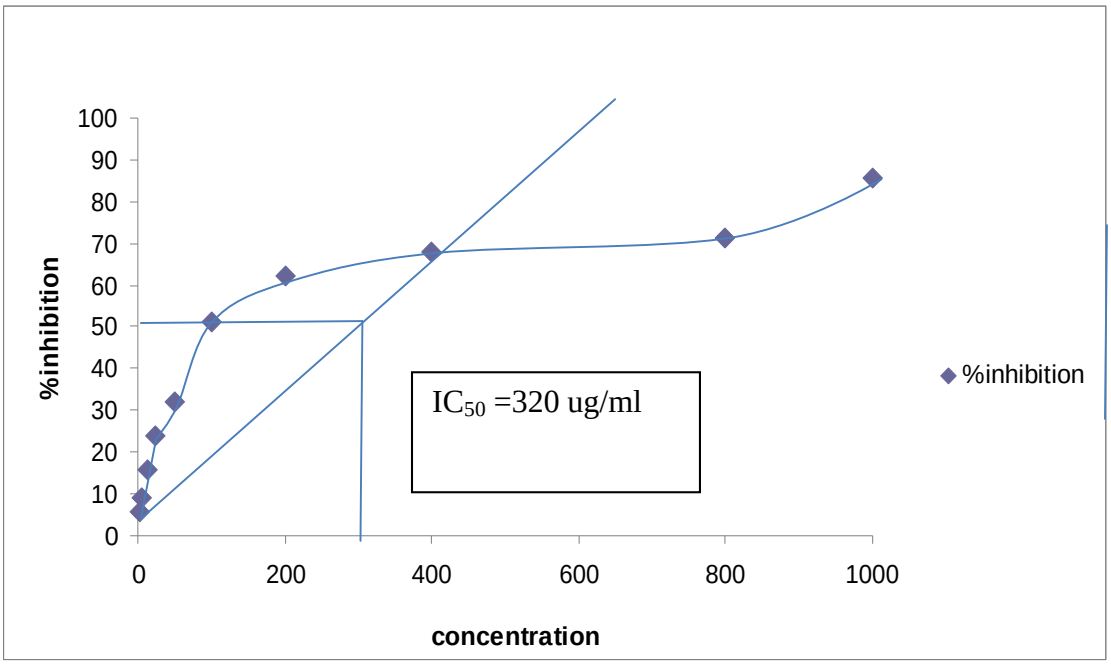

Table.3 Effect of alcoholic extract of *Psidium guajava* root bark on phytochemical analysis

TESTS	RESULTS
Test for tannins	+ve
Test for alkaloids	+ve
Test for flavanoids	+ve
Test for saponin glycosides	+ve
Test for cardiac glycosides	-ve
Test for proteins	-ve
Test for carbohydrates	-ve

+ve = present -ve = absent

Kirby Bains standard for antimicrobial studies (Ogbonnia *et al*., 2008).

Invitro antioxidant activity of alcoholic extract of *PG* was carried out in various antioxidant models. The anti oxidant activity is perhaps related to the H^+ ions donating capability of the extract, which scavenges the peroxyl radical to inhibit or terminate the peroxidation chain. The nitrite produced by the incubation of solution of sodium nitroprusside in standard phosphate buffer at 25° was reduced by alcoholic extract of *PG*. This may be due to the antioxidant principles in the *Psidium guajava root bark*, which compete with oxygen to react with nitric oxide there by inhibiting the generation of nitrite. The alcoholic extract of root bark of *PG* exhibited marked and dose dependent free radical scavenging effect in DPPH radical scavenging assay.

Lipid peroxidation can be prevented either by reducing the formation of free radicals or by supplying the competitive substrate for unsaturated lipids in the membrane or by accelerating the repair mechanisms of damaged cell membrane (Ohkowa *et al*.,1979). The antioxidant activity could be attributed with the flavonoids and alkaloids from extract of *PG* root bark. On the other hand, tannins existing in alcoholic extract of *PG* plant could also play an important role. It has been reported that they have free radical scavenger properties and antioxidant action. Flavonoids existing in the extracts could play an important role and are phenolic compounds widely distributed in the plant kingdom and have several pharmacological properties such as spasmolytic and antidiarrhoeal activities. Flavonoids have been reported to have free radical scavenger properties. Researchers reported that flavonoids are antioxidants found usually in plants, fruits and vegetable and are known to be excellent scavengers of free radicals (Tona *et al* 1999).

The results of the present investigation, we conclude that the alcoholic extract of *Psidium guajava* root bark had significant antibacterial and antioxidant activity. The present investigation provide a support to some uses of the plant in traditional medicine. Further studies are recommends for the differentiating activities against different microorganisms of this extract encourage in identifying and to isolate the novel active components responsible for the antimicrobial and antioxidant activity.

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