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In-vitro and *In-vivo* antioxidant activity of aqueous extract of bark of *Psidium guajava* Linn.

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ABSTRACT

The objectives of this study was to examine the antioxidant activities of aqueous extracts of bark of *Psidium guajava* bellowing to family Myrtaceae, which is originated in Mexico and extends all over the South America, Europe, Africa and Asia. The *Psidium guajava* is used traditionally for the treatment of various ailments like antioxidant, hepatoprotective, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory and antinociceptive activities. For the antioxidant activity of aqueous extract of bark of *Psidium guajava* Linn, *In-vitro* and *In-vivo* methods are used. *In-vitro* assessment of the ability of the extract to scavenge the Hydrogen peroxide (H_2O_2) was determined with reference to the synthetic antioxidant vitamin C and ascorbic acid was used as standard antioxidants. The scavenging activity of plant extract on hydrogen peroxide was found similar as scavenging activity of vitamin C. The *Psidium guajava* showed the strong *In-vitro* antioxidant activity and exhibited over 86.01% inhibition at 12 µg/ml concentration. For *In-vivo* antioxidant activity catalase (CAT) enzyme level was used**.** CAT levels were measured in fresh liver tissue using ELISA. CAT in hepatic tissue compared to control group. CAT enzymes were significantly increased when treated with aqueous extract of bark of *Psidium guajava*. In human beings, the highest levels of CAT are found in the liver, kidney and erythrocytes, where it is believed to account for the majority of H_2O_2 decomposition. These results show that the *Psidium guajava* could be considered as a natural antioxidant source.

Keywords: Antioxidant activity, *Psidium guajava* Linn, catalase activity, Hydrogen peroxide, aqueous extract.

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INTRODUCTION

The *Psidium guajava* Linn is one of the most important crops belonging to genus *Psidium* and the Myrtaceae family [1]. *Psidium guajava* is used as a traditional medicine in certain culture. *Psidium guajava* is originated in the tropical South America [2] and grows wild in Bangladesh, India[3], Thailand, Brazil, Florida, West Indies, California and also in several other countries. The important guava-growing states in India are Uttar Pradesh, Bihar, Maharashtra, Assam, West Bengal and Andhra Pradesh [4]. Guava is rich in antioxidants compounds and contains a high level of ascorbic acid ranging from 174.2 to 396.7 mg/100 g fresh fruit [5].

Chemical Constituents

Nutritional value of guavas are often included among super fruits, being rich in dietary fiber, vitamins A and C, folic acid; and the dietary minerals, potassium, copper and manganese. Having a generally broad, low calorie profile of essential nutrients, a single common guava fruit contains about four times the amount of vitamin C as an orange. The food value and contents of guava fruit is listed in the (Table 1) [6, 7] However, nutrient content varies across guava species. The barks are known to possess the longer amounts of vitamins and minerals and have such high levels of polyphenolic antioxidant [8.] *Psidium guajava* is also reported as anticancer, anti-inflammatory, hepatoprotective, anti-plasmodial, anti-diarrhea, anti-diabetic and antihypertensive activities like α-pinene and β-pinene, ellagic acid (Figure 1) [9-12]. Guava has antioxidant properties attributed to polyphenols like Guavin B (**Figure 2**) found in its barks [13].

Table 1: Common nutritional value of *Psidium guajava*.

Sr. No.	Nutritional value	per 100 g
1.	Energy	285 kJ (68 kcal)
2.	Carbohydrates	14.32 g
3.	Sugars	8.92 g
4.	Dietary fiber	5.4 g
5.	Fat	0.95 g
6.	Protein	2.55 g
7.	Vitamins	36-50 mg
8.	Calcium	17.8-30 mg

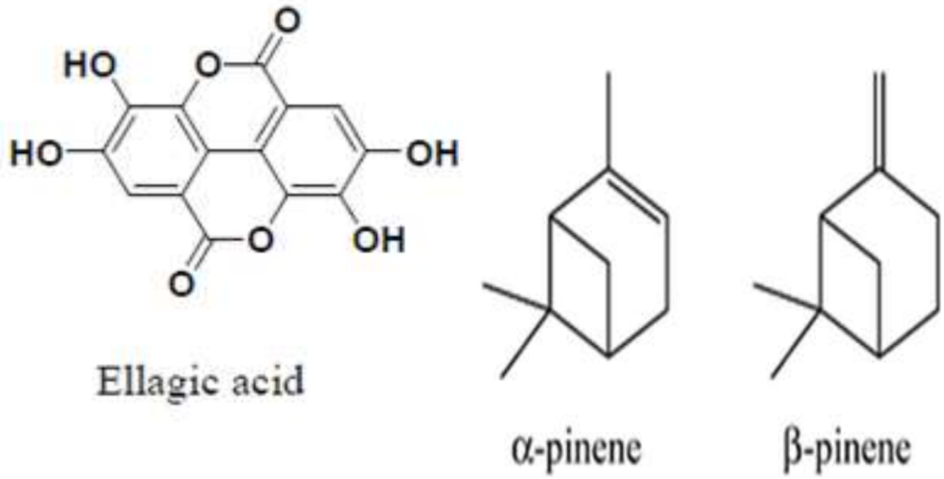
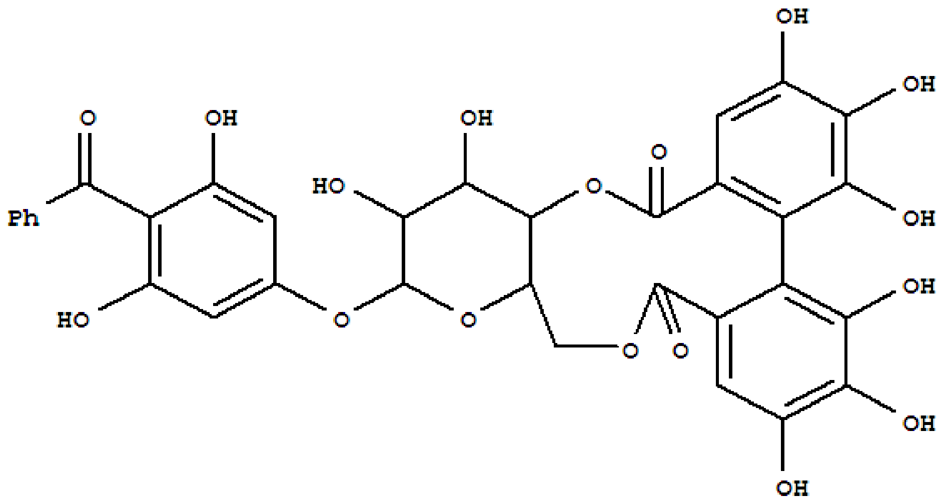


Figure 1: Chemical structure of α-pinene and β-pinene, Ellagic acid.



Guavin B

Figure 2: Chemical structure of Guavin B as antioxidant activity.

Many human diseases are caused by oxidative stress that results from imbalance between the formation and neutralization of pro-oxidants [14]. Oxidative stress initiated by free radicals, such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide and peroxy nitrite, play a vital role in damaging various cellular macromolecules. These include DNA molecules, proteins along with lipid peroxidation [15]. The involvement of active oxygen and free radicals in the pathogenesis of certain human diseases, including cancer, aging and atherosclerosis is increasingly being recognized [16]. However, human cells have an array of protecting mechanisms to prevent the production of free radicals and oxidative damage [17].

These mechanisms include both enzymatic and non-enzymatic antioxidants such as superoxide dismutase, catalase, glutathione reductase, ascorbic acid and tocopherol [18.] Free radicals, especially the oxygen radical, superoxide, when formed could lead to the formation of other radicals. In fact, the toxicity of $O_2^{\cdot}$ in living organisms is due to its conversion into $OH^{\cdot}$ and reactive radical –metal complexes. Superoxide and hydrogen peroxide are converted into $OH^{\cdot}$ and other reactive radical complexes through the iron-catalyzed Haber-Weiss reaction or the superoxide driven Fenton Reaction [19- 21].

Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention. There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery.

Various methods are used to investigate the antioxidant property by both *in-vivo* and *in-vitro* methods. For *In-vitro* study Hydrogen Peroxide (H_2O_2) Scavenging Activity was performed and for *In-vivo* study catalase activity was used.

MATERIALS AND METHOD

Plant material

The bark of *Psidium guajava* was collected in November 2015 from a local area of UTTHAN, Jhalwa, Allahabad, Uttar Pradesh, India. The Plant specimen is authenticated by "BOTANICAL SURVEY OF INDIA, ALLAHABAD". Reference No. - 98642.

Preparation of Extract

The bark was dried under the shade, powdered with a mechanical grinder and passed through a 40 mesh sieve. The successive solvent cold extraction method used to obtain various extracts including Petroleum ether, chloroform, ethyl acetate, ethanol and distilled water extracts. The solvents were removed from the extracts under reduced pressure by using a rotary vacuum evaporator. The percentage of yield of extract was obtained and is dissolved in their respective solvents for performed antioxidant activity.

Animals

Healthy, adult male Albino Wistar rats (100-200gm) were procured from the Central Drug Research Institute, Lucknow used for study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light; and 12 h dark cycle; 23 ± 2^{o} C, 50± 5%, relative humidity), they were fed with standard rat pellet diet. The Institutional Animal Ethics Committee approved the study. IAEC Number SIP- IAEC/001/09/16.

***In vitro* antioxidant activity**

Hydrogen peroxide scavenging activity

Scavenging activity of hydrogen peroxide by the plant extract was estimated using the method of *Ruch et al* [22]. Plant extract (4 ml) prepared in distilled water at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the plant extract without H_2O_2. The amount of hydrogen peroxide radical inhibited by the extract was calculated using the following equation:

$$H_2O_2 \text{ radical scavenging activity} = \{(Abs_{control} - Abs_{sample}) / (Abs_{control})\} \times 100$$

Where; $Abs_{control}$ is the absorbance of H_2O_2 radical + phosphate buffer;

Abs_{sample} is the absorbance of H_2O_2 radical + sample extract or standard.

***In vivo* antioxidant activity**

Determination of catalase activity

The activity of catalase was assayed following the method described by *Pari and Latha* [23]. The percentage inhibition was evaluated following decrease in absorbance at 620 nm. The liver was homogenized in 0.01 M phosphate buffer (pH 7.0) and centrifuge at 5000 rpm. The reaction mixture consisted of 0.4 ml of hydrogen peroxide (0.2 M), 1 ml of 0.01 M phosphate buffer (pH 7.0) and 0.1 ml of liver homogenate (10% w/v). The reaction of the mixture was stopped by adding 2 ml of dichromate-acetic acid reagent (5% $K_2Cr_2O_7$ prepared in glacial acetic acid). The changes in the absorbance was measured at 620 nm and recorded. Percentage inhibition was calculated using the equation:

$$\% \text{ catalase inhibition} = [(\text{normal activity} - \text{inhibited activity}) / (\text{normal activity})] \times 100\%.$$

Statistical Analysis

The data was represented as mean ± SEM. Results was analyzed by one way ANOVA followed by Dunnett's multiple comparison tests using Graph pad instat 3.0 software. Results were expressed as the mean ± S.E.M. for statistical analysis of the data group means, were compared by one-way analysis of variance (ANOVA) followed by Tukey's post-test for multiple comparisons. $p < 0.001$ was considered to be statistically significant.

RESULTS AND DISCUSSION

***In-vitro* antioxidant activity**

Antioxidant activity of aqueous extract of bark of *Psidium guajava* according to the H_2O_2 radical scavenging activity

In this study, Table 2 shows the antioxidant activity of the aqueous extract of bark of *Psidium guajava* evaluated through *In-vitro* test i.e. H_2O_2 radicals scavenging activity (Figure 3). Ascorbic acid was used as standard antioxidants in the scavenging activity of aqueous extract of bark of *Psidium guajava* on hydrogen peroxide Scavenging activity (Figure 4). The antioxidant activity of aqueous extract of bark of *Psidium guajava* was compared to Vitamin C (ascorbic acid) (Figure 5).

Table 2: Hydrogen peroxide radical scavenging activity of aqueous extract of *Psidium guajava* bark.

Sample	Concentration (µg/ml)	% Inhibition	IC_{50}	r^2
	2	55.50 ± 0.0008		
	4	65.59 ± 0.001		
Aqueous extract	6	72.27 ± 0.002	1.940	0.974
	8	81.10 ± 0.001		
	10	89.69 ± 0.001		
Vitamin C			1.900	0.991

Each value represents the Mean, SD, ± SEM of six animals (n=6). r^2– regression coefficient.

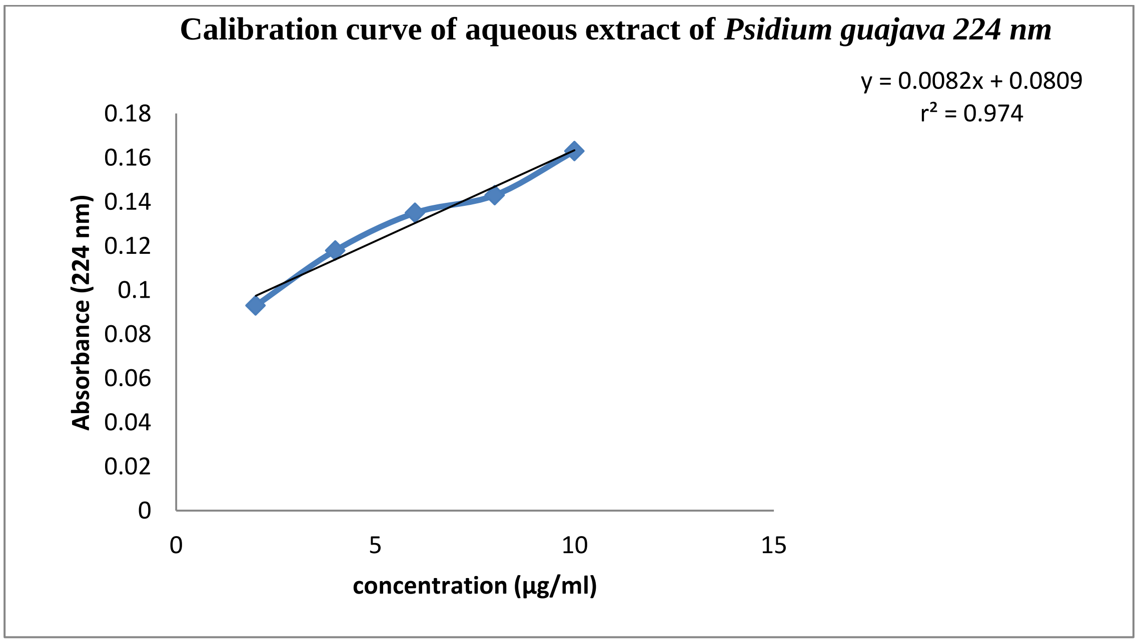


Figure 3: Hydrogen peroxide radical scavenging activity aqueous extract of bark *Psidium guajava.*

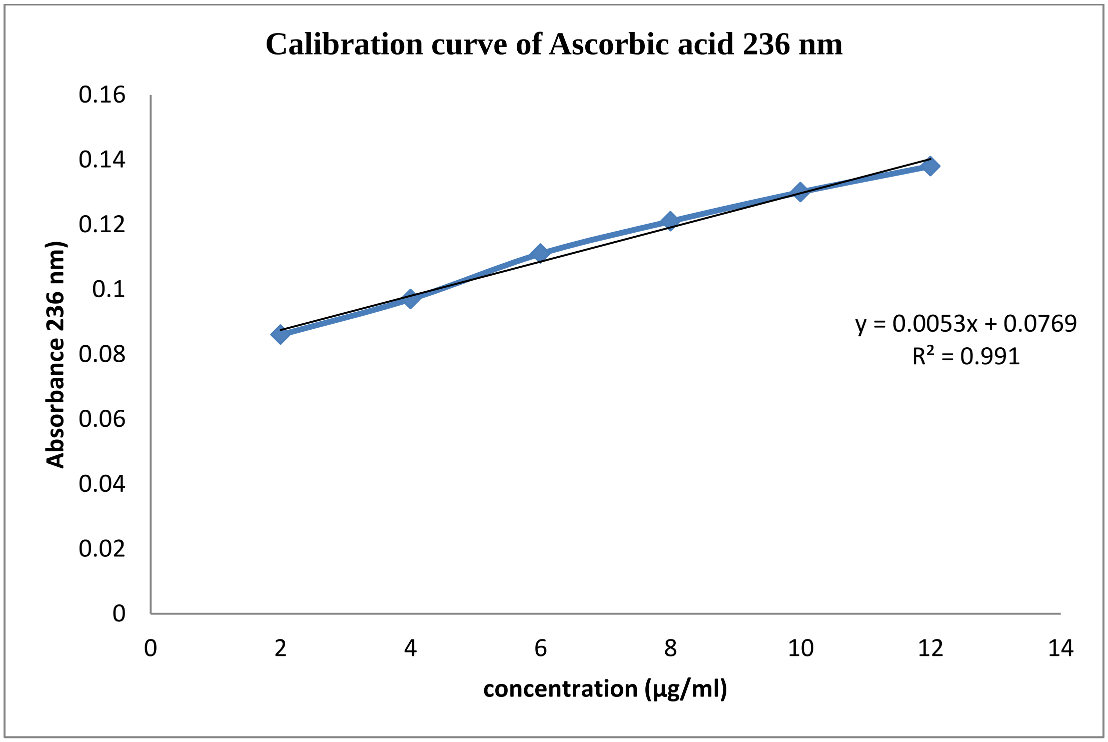


Figure 4: Hydrogen peroxide radical scavenging activity ascorbic acid.

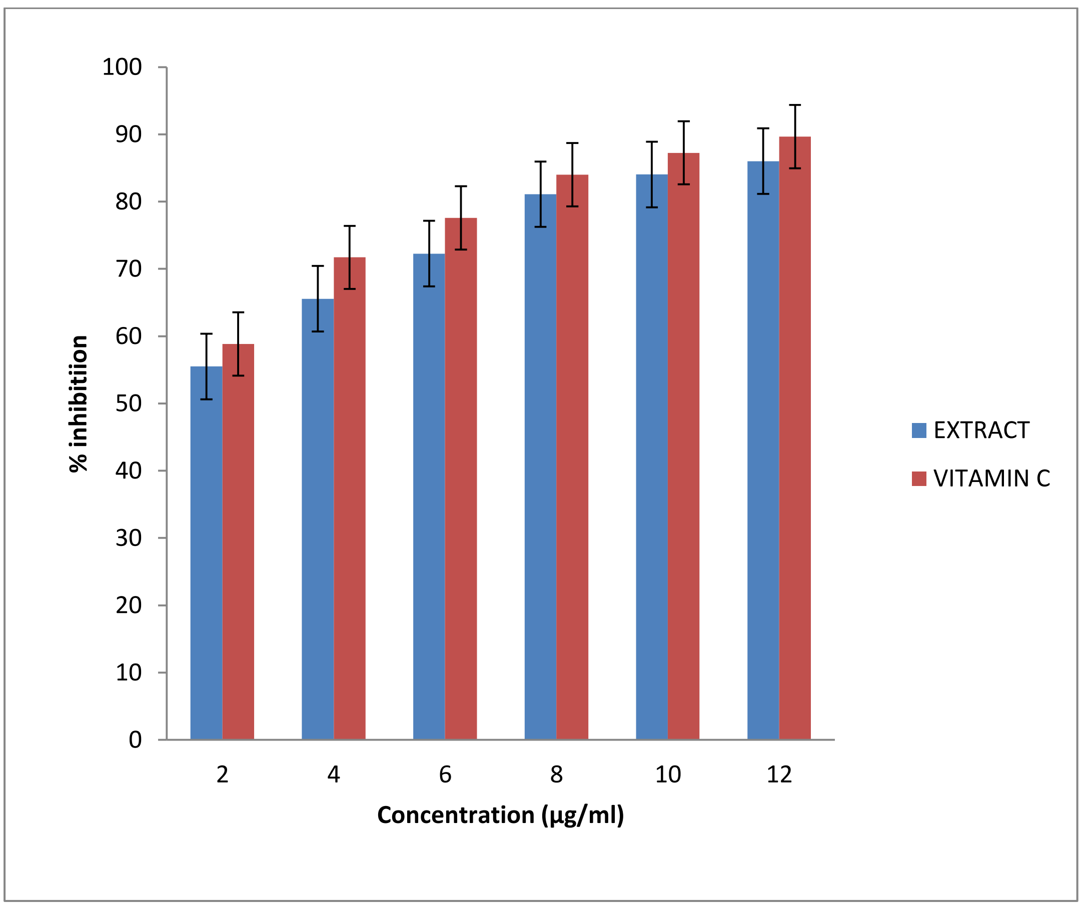


Figure 5: Compare study of aqueous extract of *Psidium guajava* bark with Ascorbic acid.

***In-vivo* antioxidant activity**

Antioxidant activity of aqueous extract of bark of *Psidium guajava* according catalase activity

Table 3 shows the activity of CAT measured against silymarin treated group were significantly normalized the values. When the rats treated with the aqueous extract of bark of *Psidium guajava* then the antioxidant enzyme were significantly higher**.**

Table 3: Effect of aqueous extract of bark of *Psidium guajava* on antioxidant activity in paracetamol induced rats.

S. No.	Groups	CAT
1	Normal control	46.03 ± 1.23**
2	Silymarin 100 mg/kg + Paracetamol	41.26 ± 1.09
3	Aqueous extract of bark of *Psidium guajava* 150 mg/kg + Paracetamol	40.02 ± 0.96**
4	Aqueous extract of bark of *Psidium guajava* 300 mg/kg + Paracetamol	43.33 ± 1.64**
5	Aqueous extract of bark of *Psidium guajava* 450 mg/kg + Paracetamol	45.42 ± 1.86**

Each value represents the mean, SD, ± SEM of six animals, one way analysis of variance (ANOVA) followed by Dunnett's test. Compared to standard **$p<0.01$.

DISCUSSION

Hydrogen peroxide is highly important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell by reacting with Fe^{2+} and possibly Cu^{2+} ions [24]. This assay shows the ability of *Psidium guajava* to inhibit hydrogen peroxide in the reaction mixture. From the results, it appeared that activities of the plant extract were nearly the same with the reference compounds. This could be due to the presence of phenolic compounds that donate electron to H_2O_2 and thus neutralizing it to water [25].

The enzyme antioxidant defense system is the nature protector against lipid peroxidation. For the *In-vitro* study hydrogen peroxide and for the *In-vivo* study CAT method is used respectively. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage [26].

Herbal plants are known to contain a variety of antioxidants. Numerous substances have been suggested to serve as antioxidants [27]. Ascorbic acid, known to act as antioxidant and it's used as standard. The extract of *Psidium guajava* bark showed good free radical-scavenging activity depending on the concentration used. The higher the concentration used the higher the free radical-scavenging effect. The antioxidant activities could be assayed by using several test systems.

Recent investigations showed differences between the test systems for the determination of antioxidant activity [28]. Oxidation of lipids can also be brought about by other mechanisms such as photooxidation. In recent years, accumulated evidence has demonstrated that UV induced oxidative damage occurs through the formation of free radicals which damage cellular components [29]. UV light can damage many tissue components including membrane phospholipids, proteins and nucleic acids. Cell membrane is the main target attacked by free radicals. The membranes of mitochondria and erythrocyte are easily oxidation induced by photodynamic and free radicals [30]. Therefore, antioxidants can be used to protect the cells from UV induced cellular damage by hydrogen peroxide scavenging activity and catalase activity. In this study, aqueous extract of bark of *Psidium guajava* showed the strong antioxidant activity and exhibited over 86.01% inhibition at 12 µg/ml concentration. In addition, the antioxidant activities of aqueous extract of bark of *Psidium guajava* shows correlated significantly with the H_2O_2 radical-scavenging activity ($r = 0.974$, $P < 0.01$).

CONCLUSION

The results of the present study showed that aqueous extract of bark of *Psidium guajava* exhibited potent antioxidant activity. In the *In-vitro* antioxidant study, the hydrogen peroxide radical scavenging activity shows the good response compare to Ascorbic acid. The *Psidium guajava* showed the strong antioxidant activity and exhibited over 86.01% inhibition at 12 µg/ml concentration. Similarly in the *In-vivo* antioxidant study, for catalase activity dose levels of 150, 300 and 450 mg/kg body weight were selected, in which 450 mg/kg body weight show more significant antioxidant activity than the 150 mg/kg and 300 mg/kg.so that the *Psidium guajava* is use for the further industrial studies.

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