



Research Article

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Evaluation of antibacterial, antioxidant and lipid degradation potential of *Psidium guajava* leaf extracts

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ABSTRACT

Hyperlipidemia, hyperlipoproteinemia, dyslipidemia or hyperlipidemia is the presence of raised or abnormal levels of lipids and/or lipoproteins in the blood. Plants represent a vast source of potentially useful dietary supplements for improving blood glucose control and preventing long term complications. The present work is aimed at exploring the antibacterial, antioxidant and anti-hyperlipidemic activities of leaf extracts of *Psidium guajava*. The antibacterial activity of the extract was tested against various bacterial pathogens by agar well diffusion method. All the extracts have shown good antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Petroleum ether extract of the plant showed least activity against *K. pneumoniae*. The ethyl acetate, petroleum ether and methanol extracts were studied for their antioxidant activities at different concentrations (20, 40, 60, 80 and 100 µg/ml) using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. The free radical scavenging potential of the methanol extract exhibited the maximum activity of 81.24% in 100 µg/ml concentration. The lipid peroxidation inhibitory potential of the methanolic extract showed the maximum inhibitory activity of 94.32 %. By virtue of its antioxidant activity the methanolic extracts of *P. guajava* may show anti-hyperlipidemic activity.

Key words: *Psidium guajava*, antimicrobial, antioxidant, lipid peroxidation inhibition, anti-hyperlipidemic

INTRODUCTION

Cholesterol is a complex biomolecule that is synthesized from acetyl co-A through a chain of reactions. HMG-CoA is a key precursor of cholesterol. In extra hepatic cells cholesterol is derived from *de novo* synthesis, whereas hepatic cells derive cholesterol via receptor mediated uptake of plasma lipoproteins such as Low Density Lipoproteins (LDL) [5]. Lipid is a derivative of cholesterol that are deposited underneath adipose and muscle tissues, which serve as an energy source. Lipids are oxidized to CO₂ and H₂O. The emulsification of lipids occurs in intestine by bile acids. Lipids are classified into two major types: 1. Phospholipids 2. Glycolipids. Classifications of lipoproteins are: High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL), Very Low Density Lipoproteins (VLDL), Intermediate Density Lipoproteins (IDL) and Chylomicrons.

Lipids when accumulated underneath the tissues for a prolonged period of time leads to various disorders like Gaucher's disease, Tay Sachs disease, Neimann- pick disease, Fabry's disease, Hyperlipidemia etc. These are collectively called as lipidoses.

Hyperlipidemia is a condition in which, elevated levels of LDL and VLDL are seen in the peripheral stream. This in turn affects the HDL presence inversely leading to various adverse effects. So, when the LDL and VLDL biomolecules are seen excess, eventually there arises a chance for the accumulation of

these biomolecules leading to coronary heart disease, cardiovascular disease (CVD), myocardial infarction, stroke, peripheral vascular disease etc.

The current anti-hyperlipidemic therapy includes principally statins and fibrates, the former correct the blood lipid profile by inhibiting the biosynthesis of cholesterol and latter act by enhancing the clearance of triglycerides rich proteins [2].

Psidium guajava commonly called guava belongs to the family *Myrtaceae*. The leaves possess α - pinene, β - pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β -bisabolene, caryophyllene oxide, β -copanene, farnesene, humulene, selinene, cardinene and curcumene [1, 3, 4]. *Psidium guajava* commonly called guava contains ascorbic acid and other bio constituents that has a significant antioxidant activity which could be further screened for anti-hyperlipidemic activity [9].

EXPERIMENTAL SECTION

Preparation of plant extract:

The plant material *Psidium guajava* were collected from the natural locations of Chennai, Tamil Nadu in India.

Leaves of *Psidium guajava* were washed with water and allowed to shade dry for two weeks at room temperature. The shade dried leaves were grounded into coarse powder using commercial blender. The leaves extracts was prepared by cold percolation method [8]. The coarse powder was allowed to macerate in the following solvents: ethyl acetate, methanol and petroleum ether separately and kept in dark for 72 hours incubation. The extracts were filtered using Whatmann no.1 filter paper. The collected filtrate was condensed to dryness at room temperature.

Qualitative screening of bio constituents [11]:

The extracted plant samples were subjected to preliminary screening for the detection of active bio-components.

(i) Detection of alkaloids

Solvent free extract (50mg) was stirred with 2 mL of dilute hydrochloric acid (1mL HCl + 1mL H₂O) and filtered. The filtrate was tested carefully with various alkaloid reagents by Mayer's test in which 2 mL of filtrate, a drop or two of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate indicates the presence of alkaloids.

(ii) Detection of phenolic compound

The extract (50 mg) was dissolved in 5mL of distilled water. Ferric chloride test was carried out in which few drops of neutral 5% ferric chloride solution were added. A dark green color indicates the presence of Phenol.

(iii) Detection of glycosides

50 mg of extract was hydrolyzed with 5mL of concentrated hydrochloric acid for 2h on a water bath, filtered and the hydrolysate was subjected to Borntrager's test in which 2 mL of filtrate hydrolysate, 3mL of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Pink color indicates the presence of glycosides.

(iv) Detection of terpenoids

Terpenoids were detected by performing Salkowski test. To 2mL of chloroform 0.5g of the extract was added and then concentrated H₂SO₄ (3ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

(v) Detection of flavonoids

0.5g of extract was dissolved in 5mL of Distilled water and filtered. Dilute ammonia (5mL) was added to 1mL of the extract filtrate. Concentrated sulphuric acid (1mL) was added. Yellow colorations that disappear on standing indicate the presence of flavonoids.

(vi) Detection of tannins

About 0.5 g of the extract was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

(vii) Detection of reducing sugars

The extract (100mg) was dissolved in 5mL of water and filtered. The filtrate was subjected to Fehling's test. 1 mL of filtrate was boiled on water bath with 1mL each of Fehling's solution I and II. A red precipitate indicated the presence of sugar.

ANTIBACTERIAL ACTIVITY

The antibacterial activity was performed by well diffusion method [7]. The test organisms used were *Staphylococcus aureus* and *Klebsiella pneumoniae*. Nutrient agar was prepared and poured in the sterile Petri dishes and allowed to solidify. 24 h old bacterial cultures *Staphylococcus aureus* and *Klebsiella pneumoniae* were swabbed on it. Then, five wells (8mm diameter) were made by using a sterile cork borer. The four different concentrations (250µg, 500µg, 750µg and 1000µg) of the test sample were loaded in the wells. Tetracycline was used as the control. The plates were then incubated at 37°C for 24h. After incubation the inhibition diameter was measured.

ANTIOXIDANT ASSAY

The Radical Scavenging Activity of different extracts was determined by using DPPH assay [10] with small modification. The decrease of the absorption at 517nm of the DPPH solution after the addition of the antioxidant was measured in a cuvette containing 2960µl of 0.1 mM ethanolic DPPH solutions mixed with 40µL of 20 to 100 µg/mL of sample extract and vortexed thoroughly. The setup was left at dark in room temperature and the absorption was monitored after 20 minutes. The ability of the sample extract to scavenge DPPH radical was calculated by the following equation:

$$\% \text{ of DPPH Radical Scavenging Activity (\% RSA)} = \frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \times 100$$

Abs. control is the absorbance of DPPH radical + ethanol; Abs. sample is the absorbance of DPPH radical + sample extract.

OXIDATIVE DEGRADATION OF LIPID**THIOBARBITURIC ACID ASSAY [6]**

Two milliliters each of 20% trichloroacetic acid and 0.67% thiobarbituric acid were mixed with 1mL of 2.51% linoleic acid and 1mL of plant extract. The solution was maintained in boiling water bath for 10min. Upon cooling, the solution was centrifuged at 3000rpm. The absorbance of the supernatant was measured at 532 nm. The percentage inhibition of the plant against the products in later stages of lipid peroxidation was evaluated with reference to the standard solution of butylated hydroxyl toluene (BHT).

$$\% \text{ inhibition} = \frac{A_{532} \text{ control} - A_{532} \text{ extract}}{A_{532} \text{ control}} \times 100$$

RESULTS AND DISCUSSION

The extracts were screened for the presence of phytochemicals and results were given in table 1, where all the extracts showed positive results for the presence of reducing sugars, flavonoids, alkaloids and terpenoids. Glycosides were present only in ethyl acetate extract. Methanol and petroleum ether extracts showed positive results for phenols. Petroleum ether extracts showed positive results for presence of methanol. Tannin is present only in methanolic extract of the plant.

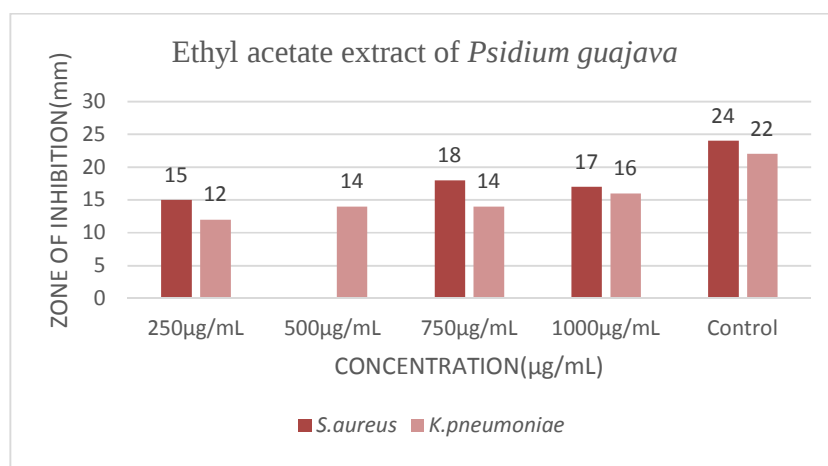
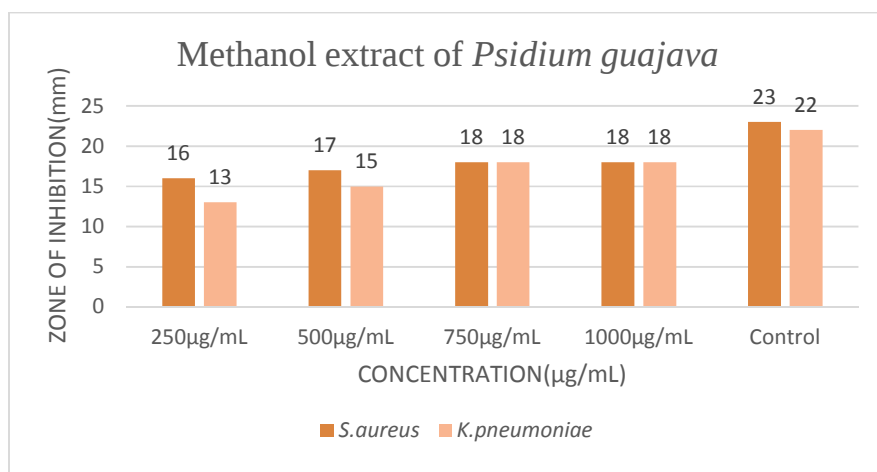
Table 1: Phytochemical screening of *Psidium guajava* plant extract

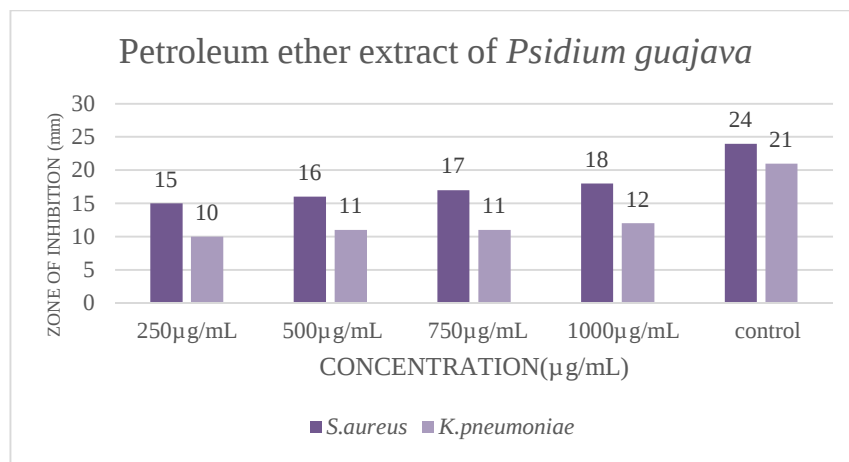
Phytochemicals	Ethyl acetate extract	Methanol extract	Petroleum ether extract
Reducing sugar	+++	+++	+++
Glycosides	+++	---	---
Tannins	---	+++	---
Flavanoids	+++	+++	+++
Phenols	---	+++	+++
Alkaloids	+++	+++	+++
Terpenoids	+++	+++	+++

(+++) indicates the presence and (---) indicates the absence of phytochemical.

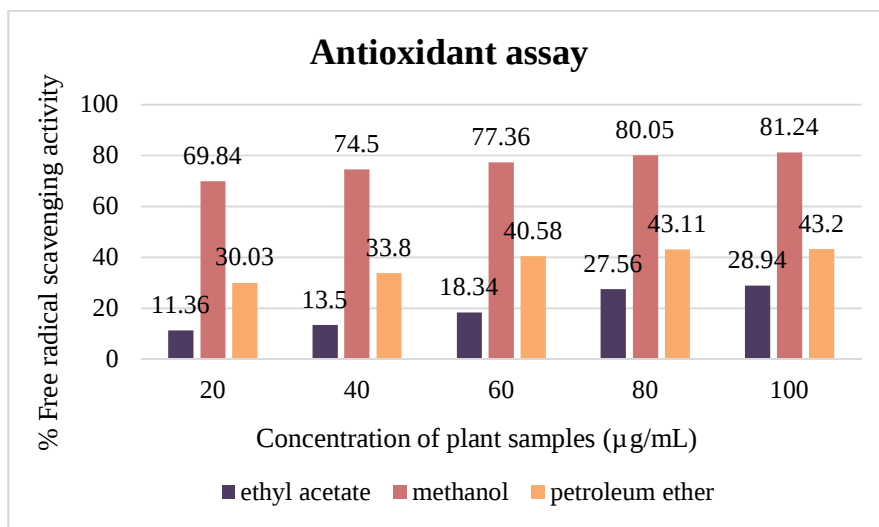
ANTIBACTERIAL ACTIVITY

The inhibition of ethyl acetate extract of *Psidium guajava* leaf at different concentrations towards *S. aureus* and *K. pneumoniae* were presented in the graph 1. 750µg/mL of sample concentration exhibited higher inhibition (18 mm) against *S. aureus*. 1000µg/mL of extracts showed maximum zone (17mm) against *K. pneumoniae*. *S. aureus* and *K. pneumoniae* were susceptible towards the control (Tetracycline), 24 mm and 22 mm respectively.

GRAPH 1: Antibacterial activity of Ethyl acetate extract of *Psidium guajava* against *S.aureus* and *K.pneumoniae*GRAPH 2: Antibacterial activity of Methanol extract of *P.guajava* against *S.aureus* and *K.pneumoniae*

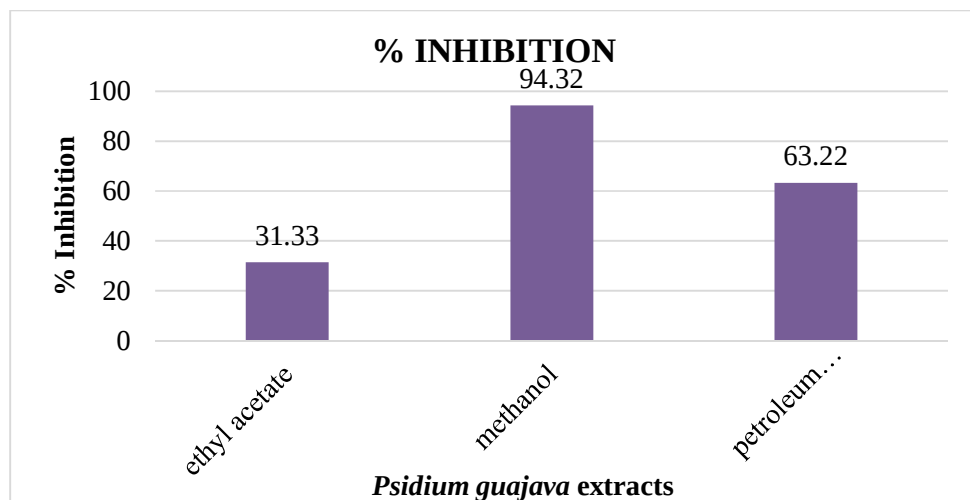
GRAPH 3: Antibacterial activity of Petroleum ether extract of *P.guajava* against *S.aureus* and *K.pneumoniae***ANTIOXIDANT ACTIVITY**

Antioxidant properties of the plant samples were performed by free radical scavenging assay. The antioxidant potential of the plant leaf extracts were presented in Graph 4. The results indicate that free radical scavenging potential increases with increase in concentration of plant extracts. The methanol extract of *P.guajava* showed a maximum antioxidant property (81.24%) at 100 µg/mL. In petroleum ether extract the maximum potential was seen in 100 µg/mL of 43.2%. Whereas the least activity (28.94%) was found in ethyl acetate extract.

GRAPH 4: Antioxidant potential of different extracts of *P.guajava* leaf**LIPID PEROXIDATION INHIBITION ASSAY**

The lipid peroxidation inhibition assay was performed by following a standard protocol. The final results showed in table 1. The results revealed the potential of the plant sample, in which methanolic plant extract showed maximum inhibition activity (94.32%) followed by petroleum ether extract (63.22%), and ethyl acetate extract (31.33%). The results were graphically represented in graph 5.

GRAPH 5: Inhibitory potential of plant extracts on lipid peroxidation



CONCLUSION

From the above results, it is clearly seen that *Psidium guajava* has cluster of bio-constituents that promotes the plant for treating diseases through indigenous methodology. This plant has the advantage of preventing wide range of side effects caused by synthetic drugs. The presence of bioactive components shows a good antibacterial potential against pathogenic bacteria. Methanolic extract of the plant showed higher antibacterial activity. The plant was subjected to antioxidant assay by DPPH assay through which the free radical scavenging property of the plant was understood, that methanolic extract exhibited higher antioxidant property. When the plant possesses antioxidant property, it was further preceded with lipid peroxidation inhibition assay, the results concluded that the leaf extracts of the plant has the potential to inhibit the lipid peroxidation. From this ideology, *Psidium guajava* methanolic extract has the maximum inhibition of lipid peroxidation than petroleum ether extract. Further *in vivo* studies are required for anti-hyperlipidemia an activity that involves in decrease in serum TG, TC, LDL, VLDL levels.

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