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Assessment of phytoconstituents composition and anti hemolytic activity of *Psidium guajava* Leaf extract against H_2O_2 Induced hemolysis in Chicken Erythrocytes

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Abstract
The aim of the current study was to assess *Psidium Guajava* ethanolic leaf extract for its phytochemical constituents and anti hemolytic activity against H_2O_2 Induced Hemolysis in chicken erythrocytes. The phytochemical investigation of ethanolic extract showed the presence of carbohydrates, flavanoids, alkaloids, and saponins. In this present study ethanolic leaf extract of *Psidium guajava* shown to have moderate anti hemolytic activity against H_2O_2 induced hemolysis in chicken erythrocytes. The Ethanolic extract showed antihemolytic activity in the range from 12.5% to 43.75%. At varying concentration of plant extracts 20, 40, 60, 80 and 100 mg /ml the percent of inhibition of hemolysis recorded were 12.50%, 18.75%, 25%, 37.50%, 43.75% respectively.

Keywords: *Psidium guajava*, Erythrocytes, Hemolysis, phytochemicals.

Introduction

Medicinal plants gaining lot of importance now a days because of efficacy they have been showing in the traditional healing [1]. Herbs are the source of magnificent inhibitors that could act on wide variety of diseases. One of the great aspect of herbs is they show 100% results when comes to the healing. Herbs have all sorts of answers aginst various diseases [2]. The best source of drugs without lethal effects to human systems could be the plant source and this has been proved by the Traditional healing system and the recent studies conducted on the experimental animals [3]. The species *Psidium guajava* is an example of plants commonly used in popular medicine [4]. More recent ethnopharmacological studies show that *Psidium guajava* is used in many parts of the world for the treatment of a number of diseases, e.g. as an anti-inflammatory, for diabetes, hypertension, caries, wounds, pain relief and reducing fever [5]. The leaf extract of guava showed anticough activity by reducing the frequency of cough induced by capsaicin aerosol [6]. *P. guajava* leave methanol extract have cytotoxic effect on Human Cervical Cancer cells [7]. *Psidium gaujava* have the ability to inhibit the growth of the common oral flora with its abundant source of secondary metabolites [8]. The leaves and bark of *P. guajava* tree have long history of medicinal uses that is still employed today [9]. Flavonoid content of *Psidium guajava* leaves has been observed to have antibacterial activities [10]. Nutritional value of guavas are often included among superfruits, being rich in dietary fiber, vitamins A and C, folic acid; and the dietary minerals, potassium, copper and manganese [9]. In this study an attempt has been made to assess the phytochemicals present in leaf extract and its anti hemolytic activity against H_2O_2 induced hemolysis in RBC.

Materials and Methods

Materials

Leaves of *Psidium guajava* were collected from village seshammachruvu, Achanta mandalam, West godavari district. The plant was authenticated by Dr. Suryanayana raju, Department of Botany, S V K P & Dr. K S Raju Arts and Science College, Penugonda. Leaves were dried in sunlight for a week and then powdered using blender to get coarse powder. Chicken blood was collected from the local market in EDTA bottle.

Extraction process

Preparation of extract was done according to the procedure done by Sharmila *et al*., (2011) [11]. 25g of Leaf powder was packed in soxhlet extraction unit and exhaustively extracted using 100 ml of ethanol at 60 ºC for 12 hours. The extract was completely dried in water bath at 40 ºC and subsequent stored at 4 ºC.

Phytochemical analysis

A preliminary phytochemical investigation was conducted for the detection of steroids, terpenoids, flavonoids, saponins, tannins, carbohydrates, and phenols by using standard procedures [12, 4].

Assessment of Anti-hemolytic Activity

Anti hemolytic activity against H_2O_2 induced hemolysis in chicken RBC determined by *in vitro* method described by Tavazzi *et al*., (2001) [13] and Thagriki Dluya., (2015) [14]. The chicken erythrocytes were separated by centrifugation at 200 rpm and washed with saline or isotonic sodium phosphate buffer (pH 7.4) until the supernatant is colourless. The erythrocytes were then diluted with saline or phosphate buffer to give a 4% suspension. Varying amounts of the plant extracts (20, 40, 60, 80 and 100 mg /ml) with saline or buffer was added

to 2 ml of the suspension of erythrocytes and the volume was made up to 3.5 ml with saline or buffer. This mixture was pre-incubated for 120 min and then 0.5 ml H_2O_2 solutions of appropriate concentration in saline or buffer was added. The concentration of H_2O_2 in the reaction mixture was adjusted so as to bring 90% hemolysis of blood cells after 120 min incubation. After 120 min of incubation tubes were centrifuged and the amount of hemolysis was determined by measurement of the absorbance at 540 nm corresponding to hemoglobin liberation. Anti-hemolytic activity was expressed as the inhibition percentage and was calculated using the following formula:

Anti-hemolytic activity (%)

$$= \frac{\text{Control 540 nm} - \text{Sample 540 nm}}{\text{Control 540 nm}} \times 100$$

Where, Sample 540 nm was the absorbance of the sample and Control 540 nm was the absorbance of the control.

Results

Phytochemical analysis

The phytochemical investigation of ethanolic extract of leaves showed the presence of carbohydrates, flavanoids, alkaloids, and saponins (Table 1).

Table 1: shows the presence of phytochemicals in ethanolic extract of *Psidium guajava* leaves.

S. No	Phyto constituent	Presence of phyto chemical in Ethanolic extract
1.	Carbohydrates	+
2.	Flavanoids	+
3.	Alkaloids	+
4.	Tannins and Phenols	-
5.	Proteins	-
6.	Saponins	+
7.	Sterols	-

+ indicates presence; - indicates absence.

Anti-haemolytic activity

The ethanolic leaf extract of *Psidium guajava* shown to have moderate anti hemolytic activity. The Ethanolic extract showed antihemolytic activity in the range from 12.5% to 43.75%. At varying concentration of plant extracts 20, 40, 60, 80 and 100 mg/ml the percent of inhibition of hemolysis recorded were 12.50%, 18.75%, 25%, 37.50%, 43.75% respectively. (Figure 1).

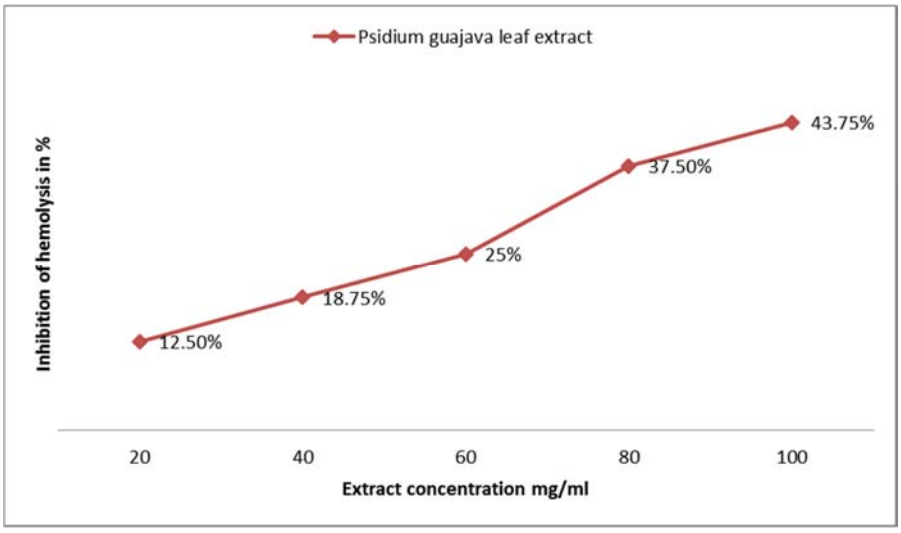

Fig 1: Anti hemolytic activity of *Psidium guajava* ethanolic leaf extract

Discussion

The phytochemical analysis revealed that the ethanolic leaf extract shown to contain carbohydrates, flavanoids, alkaloids, and saponins but tannins, proteins and sterols were found to be absent in this study. Earlier work have revealed the presence of alkaloids, flavonoids, sterols, terpenoids, quinone, oil and fat, phenol, starch, anthocyanin, protein, carbohydrate and cellulose present in the leaves [4]. These phytochemicals are naturally present in the plant extracts which could make the plant useful for treating different ailments and having a potential of providing useful and safe drugs and drug leads for human use [15, 16, 17]. The ethanolic leaf extract of *Psidium guajava* shown to have moderate anti hemolytic activity. Recent study by Chonthida *et al*., (2013) [18] revealed that *Psidium Guajava* leaf extract have potential inhibition capacity against ferrous ion induced hemolysis. But in this study anti hemolytic activity was moderate, this variation may be due to the place where it has raised. When red blood cells were treated

with H_2O_2 (toxicant), % hemolysis was found to be increased. This may be because of the oxidizing nature of H_2O_2 with respect to cell membrane degradation and release of haemoglobin from the cell [19]. H_2O_2 also cause mobilization of Fe2+ by Ca2+ via Fenton reduction stimulating the production of OH- radicals [20]. All these factors combinedly cause destabilization of cell membrane, which is probably the key event of the lysis of the cell [19]. Polyphenols possess many biological effects, mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelation of transition metals [21, 22].

Conclusion

Through this present study it can be concluded that the Traditional healing system has provided many important herbs have the potential to cure lethal diseases. The feasability of making efficient drugs from plant sources is very much near. Based on previous studies this plant *Psidium Guajava* accounts for various medicinal properties. Hence effective work should be done to isolate of various phyto inhibitors.

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