Research Article

Total Phenolic Content, Reducing Potential & Free Radical Scavenging Activity of Hydroalcoholic Leaf Extracts of *Psidium guajava* and *Persea americana*

Amith Kumar[1], Reshma Kumarchandra[*1], Rajalakshmi Rai[2], Suchetha Kumari N[3]
[1]Department of Biochemistry, Kasturba Medical College, Mangalore, Manipal University, Karnataka, India.
[2]Department of Anatomy, Kasturba Medical College, Mangalore, Manipal University, Karnataka, India.
[3]Department of Biochemistry, K S Hegde Medical Academy, Nitte University, Karnataka, India.
***Corresponding author's E-mail:** reshma.kc@manipal.edu

Accepted on: 27-05-2015; **Finalized on:** 30-06-2015.

ABSTRACT

In the present study, we have investigated the total phenolic content reducing property and antioxidant activity of hydroalcoholic leaf extract of *Psidium guajava* and *Persea americana*. *Psidium guajava* belongs to the family Myrtaceae, and is distributed throughout India. Earlier studies involving the leaves, roots and fruits of this plant, displayed ethno medicinal properties, and was used in treatment of various diseases. Tender leaves of *Psidium guajava* displayed antioxidant, antibacterial, anti-inflammatory, astringent and hypoglycemic activity. *Persea americana* belongs to family Lauraceae and is found abundantly in tropical and temperate regions of the world. The leaf extract significantly reduced the blood glucose level and helped in the management of diabetes. In view of this, hydro alcoholic leaf extracts of *Psidium guajava* and *Persea americana* were studied for their antioxidant properties like ABTS free radical scavenging activity and reducing property .Their total phenolic content was also estimated. Experimental results clearly unveil the antioxidant property of both plant extract. At 10 µg/ml concentration *Psidium guajava* and *Persea americana* leaf extract exhibited 28.48 ± 2.06 & 24.67 ± 3.4 % ABTS free radical activity,whereas standard ascorbic acid displayed 77.67 ± 0.28 respectively .The maximum phenolic content was found in *Psidium guajava* leaf extract (32.5 ± 4.33 mg GAE/g of extract) & minimum in (28.33 ± 2.88 mg GAE/g of extract) *Persea americana.* In the present study we observed that *Psidium guajava* leaf extract has maximum free radical scavenging, reducing potential and phenolic content when compared with *Persea americana*. Results indicated an increase in free radical scavenging activity and reducing property in both the plants in a dose dependent manner which was directly related to the phenolic content present in their leaf extracts.

Keywords: *Psidium guajava, Persea americana*, ABTS, Phenol, antioxidant.

INTRODUCTION

Active components present in plants have a role in providing various health benefits with minimum toxic side effects. Many of the herbs have a remarkably high content of antioxidants that was confirmed by its action on reactive oxygen and nitrogen species. Reactive oxygen and nitrogen species are generated in the body by various mechanisms and also by many external factors such as radiation, chemical carcinogens and atmospheric pollution which is responsible for the etiology of various interlinked diseases. Antioxidants produced in the body are not sufficient to counteract the free radicals generated in diseased state. Phenolic contents, flavonoids, alkaloids and terpinoids present in plants may provide additional protection to living systems from free radical induced damage and also help in the elevation of pre-existing antioxidant levels in the body[1]. The root and leaf extract of *Persea americana* is recommended for anemia and gastro duodenal ulcer[2]. The leaf, bark and fruit of *Persea americana* are used in treatment of various ailments such as hypertension[3], bronchitis & antimalerial[4] and diabetes[5]. Rural communities of Africa have used aqueous leaf extract of *Psidium guajava* for arthritis and inflammatory conditions. The phytoconstituents present in the leaf extract are reported to acquire antibacterial[6] and antioxidant[7] activities. In animal studies the leaf extract has proven its analgesic and anti-inflammatory activity[8]. Study done by Hui-Yin Chen[9] revealed the antioxidant potential of *Psidium guajava* leaf extract where the plant extract scavenged ABTS free radical, peroxyl radicals and superoxide anion in dose dependent manner and also inhibited linoleic acid oxidation. Considering the profound medicinal properties of these plants, an effort was made to investigate the reducing capacity, antioxidant property and phenolic content of leaf extracts of both plants with a forethought of using these plants as radiation protectors.

MATERIALS AND METHODS

Plant Material

The tender leaves of *Psidium guajava* were collected from Soans nursery, Alangar, Moodbidri, Mangalore, Karnataka, India & *Persea americana* from Madikeri, Karnataka, India. The plant specimen was authenticated by Mr. Surendranath Joshi, Botanist, St Aloysius College, Mangalore, Karnataka, India. The collected tender leaves were washed with running tap water and shade dried.

Chemicals and Reagents

Chemicals such as 2, 2-azinoibis – (3-ethylbenzothiazoline-6-sulphonate) (ABTS) procured from Sigma Aldrich (St Louis, Missouri, USA). Potassium ferricyanide, Trichloroacetic acid, Gallic acid, Ascorbic

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

acid, Folin Ciocalteu's reagent and Ferric chloride from Sisco research laboratories (SRL) PVT. Ltd. (Mumbai, India) & all other reagents used were of analytical grade.

Preparation of Extract

The tender leaves were washed properly and shade dried at room temperature. After drying the leaves were powdered by an electrical mixer.100g of dried powdered leaves were suspended in 500 ml of 50% methanol in water and refluxed at 50°C in a soxhlet apparatus for 72 hours. The crude extract was concentrated by Rotary vacuum flash evaporator. The concentrated crude extract was stored at 4°C for further usage.

Preliminary Phytochemical screening of Hydroalcoholic Leaf Extract of *Psidium guajava* and *Persea americana*[10-12]. The hydroalcoholic leaf extract of *Psidium guajava and Persea americana* were tested for the presence of phytoconstituents. Qualitative test were performed to display the chemical composition of each of these plants. Plants are the source of secondary metabolites. Different qualitative tests were performed to elucidate the natural chemical components present in the leaf extract.

ABTS Free Radical Scavenging Activity

ABTS Free radical scavenging activity of hydroalcoholic leaf extracts was determined by spectrophotometric method[13]. ABTS free radical cation ($ABTS^{+}$) were generated *in vitro* by treating ammonium persulphate (2.45mM) with ABTS (7mM) solution. The mixture was undisturbed for 12-16 hours. The mixture was kept in dark at room temperature. 0.5 ml of various concentrations of leaf extract (2-10 µg/ml) was treated with 0.3 ml of ABTS solution and the final volume was made up to 1ml with distilled water. The absorbance was read at 745 nm and experiments were done in triplicates. Ascorbic acid was used as standard for comparison. The percentage of ABTS free radical scavenging activity was calculated using the formula.

% scavenging = [(Absorbance of control – (Absorbance of sample – Absorbance of sample blank / Absorbance of control] x 100.

Total Phenolic Content

Total phenolic content of hydroalcoholic leaf extract was determined by Folin Ciocalteu method[14]. The polyphenols present in the plant extract undergo redox reaction by reducing Folin – Ciocalteu reagent, resulting in the development of blue color complex. Gallic acid was used as reference standard for comparision.0.5 ml of various concentration of leaf extract (20-100 µg/ml) was treated with 2 ml of (1:10) diluted *Folin ciocalteu* reagent. The mixture was neutralized with addition of 4 ml of sodium carbonate solution (7.5% w/v). The mixture was incubated at room temperature for 30 minutes with constant shaking for the development of blue color. The absorbance was read at 765 nm using spectrophotometer. The phenolic content of leaf extract was determined by standard curve of Gallic acid. Total phenolic content was expressed as milligram/gram Gallic acid equivalent (GAE) of dry extract. Following formula was used to calculate the total phenol content.

$$T = \frac{C \times V}{M}$$

T = total phenol content, mg GAE /gram dry weight of extract

C = concentration of Gallic acid mg/ml

V = volume of plant extract in ml

M = plant extract weight in grams.

Reducing Property of the Plant Extract

The reducing potential of *Psidium guajava* and *Persea americana* was determined by spectrophotometric method[15]. 2.5 ml of various concentration of plant extracts was treated with 2.5 ml of phosphate buffer pH 6.6 (0.02 M).To the above mixture 1 % of 2.5 ml of potassium ferricyanide was added. The solution was boiled at 50°C for 20 minutes. 2.5 ml of 10 % trichloroacetic acid was added after boiling. The solution was centrifuged at 3000 rpm for 15 minutes.5 ml of the supernatant was treated with 1 ml of 0.1 % $FeCl_3$. The absorbance was read at 700 nm. Quercetin was used as standard for comparison.

Statistical Analysis

The values are expressed as mean ± SD for three triplicate observations. Analysis of data was done by one way analysis of variance (ANOVA) followed by Post Hoc test, using IBM SPSS Statistics 20. $p \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis revealed the presence of flavonoids, saponins, terpinoids, tannins and glycosides in the hydroalcoholic leaf extract. The details of Phytoconstituents present are listed in Table 1.

Table 1: Phytochemicals detected in *Psidium guajava* and *Persea americana* leaf extract (+ = present, - = absent). Qualitative test for glycosides, terpinoids, saponins, tannins & flavonoids were positive in both leaf extracts.

Phytoconstituents	*Psidium guajava*	*Persea americana*
Flavonoids	+	+
Tannins	+	+
Saponions	+	+
Terpenoids	+	+
Glycosides	+	+

ABTS Free Radical Scavenging Activity

ABTS is a stable free radical which is used extensively to study the antioxidant activity of natural products. High molecular weight phenolics present in the leaf extract quench the free radicals. The effectiveness of the phytoconstituents depends upon higher molecular

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

weight, total number of aromatic rings & hydroxyl group substitution. $ABTS^{+}$ Radicals are scavenged by antioxidants through the mechanism of electron/hydrogen donation. Hydro alcoholic leaf extract of *Psidium guajava* and *Persea americana* were used to study antioxidant activity. In this experiment we found that *Psidium guajava* leaf extract displayed maximum free radical scavenging activity 28.48 ± 2.06 % when compared with *Persea americana* 24.67 ± 3.4. Whereas standard ascorbic acid displayed (77.67 ± 0.28) scavenging activity. When both plant extract were compared with a standard ascorbic acid the p value was significant (p>0.05). In our earlier studies we compared hydroxyl radical scavenging activity and iron chelating capacity of *Psidium guajavaand Persea americana*. Both plants displayed antioxidant property in which *Psidium guajava* leaf extracts displayed better activity when compared to *Persea americana*[16]. In earlier studies, there was a linear relationship between the antioxidant activity and phenolic content of *Psidium guajava* leaf extract & the linearity was due to the active compounds like ferulic acid and phenolic acid[9].

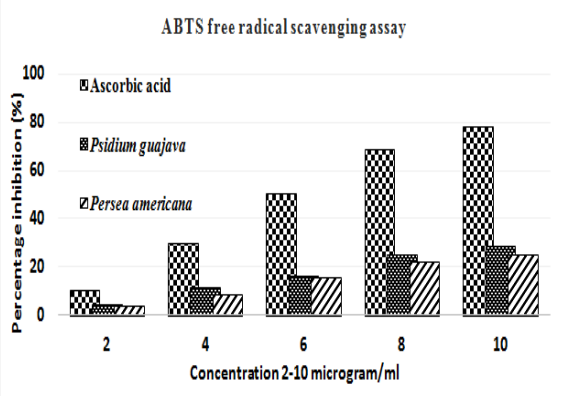

Figure 1: Percentage inhibition of ABTS free radical by *Psidium guajava*, *Persea americana* and standard ascorbic acid.

Total Phenolic Content

Phenols and polyphenols are secondary metabolites which are produced by all types of plants. Consumption of high levels of fruits and vegetables leads to the high intake of phenolic compounds. The health benefits derived from the phenolic compounds is attributed to the antioxidant activity. The leaf extracts of *Psidium guajava* and *Persea americana* were analyzed for their total phenolic content and it was observed that *Psidium guajava* leaf extract displayed maximum phenolic content 32.5 ± 4.33mg GAE/g of extract when compared to *Persea Americana* 28.33 ± 2.88. Folin Ciocalteu method was used to estimate phenolic content and results are expressed as gallic acid equivalent/gram dry weight of plant extract.

Reducing Potential

The reducing property of leaf extract serves as a positive indicator of its potential antioxidant activity. These natural compounds donate an electron and thereby reduce the oxidized intermediates[17]. In this assay reducing agents present in the leaf extract converts fe^{3+}/ferricyanide to ferrous form. The development of pearl Prussian blue color determines fe^{3+} ion concentration.

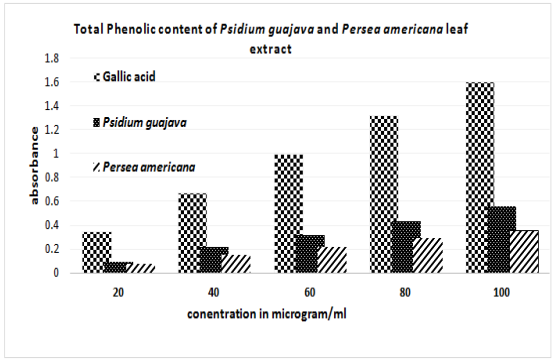

Figure 2: Total phenolic content of *Psidium guajava*, *Persea americana* and standard Gallic acid.

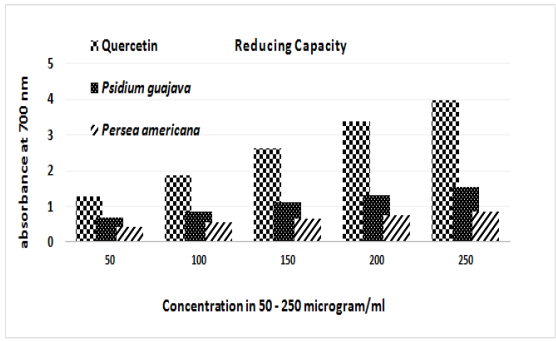

Figure 3: Reducing potential of *Psidium guajava, Persea americana* and standard Quercetin

The antioxidant activity of any compound depends on the action of reductones which exhibit the antioxidant activity by donating hydrogen atom and breaking free radial chain reaction. Antioxidants after donating an electron remains in a stable state[18]. *Psidium guajava* and *Persea americana* leaf extract displayed reducing property in dose dependent manner. Quercetin was used as a standard. Like antioxidant activity and total phenolic content, *Psidium guajava* leaf extract displayed maximum reducing ability (absorbance 1.555) than *Persea americna* (absorbance 0.864) however the activity was less than the standard Quercetin (absorbance 3.987). Plants are natural source of antioxidants as they contain natural chemicals such as Quercetin, Rutin, Morin, Ellagic acid, guaijavarin, guajiverine and guajivolic acid[19] which are responsible for reducing property.

CONCLUSION

Hydro alcoholic leaf extract of *Psidium guajava* and *Persea americana* displayed antioxidant activity by scavenging free radicals. This activity is attributed to the phenolic content present in the leaf extract. *Psidium guajava* leaf extract displayed maximum free radical scavenging property, reducing property, and had a higher phenolic content than *Persea americana*. Considering

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

their antioxidant property, further studies can be carried out with the active principles of these plants in order to study their pharmacological effects in general and radiation protection in particular.

Acknowledgement: The authors are thankful to Board of Research in Nuclear science (BRNS), Department of Atomic Energy, Bombay, Government of India for funding this project.

REFERENCES

1. Weiss JF, Landauer MR, Radioprotection by antioxidants, Ann N Y Acad Sci, 899, 2000, 44-60.
2. Pamplona R, Encyclopedia of Medicinal Plants ,2nd Edition, Vol. 1 and 2, Education and Health Library, The European Union, U. K, 128 – 150.
3. Gupta MP, Arias TD, Correa M, Lamba, J Crude Drug Res, 17, 1979, 115.
4. Gessler MC, Nkunya MH, Mwasumbi LB, Heinrich M, Tanner M, Screening Tanzanian medicinal plants for antimalarial activity, Acta Trop, 56(1), 1994, 65-77.
5. Ramirez VR, Mostacero LJ, Garcia AE, Megia CF, Pelaez PF, Medina CD, Miranda CH, Banco Agraro del Peru and Nacl, Univ Trujillo, Trujillo, 1988, Peru.
6. Caceres A, Figueroa L, Taracena AM, Samayoa B, Plants used in Guatemala for the treatment of respiratory diseases & Evaluation of activity of 16 plants against gram-positive bacteria, J Ethnopharmacol, 39(1), 1993, 77-82.
7. Qian H, Nihorimbere V, Antioxidant power of phytochemicals from *Psidium guajava* leaf, J Zhejiang Univ Sci, 5(6), 2004, 676-83.
8. Ojewole JA, Anti-inflammatory and analgesic effects of *Psidium guajava*leaf aqueous extract in rats and mice, Methods and Finding in Experimental and Clinical Pharmacology, 28, 2010, 441-446.
9. Hui-Yin Chen, Gow-Chin Yen, Antioxidant activity and free radical-scavenging capacity of Extracts from guava (*Psidium guajava* L.) leaves, 101(2), 2007, 686-694.
10. Sofowora A, Medicinal Plants and Traditional Medicines Africa, 2nd edition, Ibadan, Nigeria, Spectrum Books Ltd, 1993.
11. Trease GE, Evans WC, Pharmacognosy, A Physician's Guide to Herbal Medicine, 13th Edition, Bailliere Tindall London, 1989.
12. Harborne JB, Phytochemical methods, Chapman and Hall Ltd London, 1973, 49-188.
13. Shirwaikar A, Rajendran K, Kumar CD, Indian J Exp Biol, 42(8), 2004, 803-807.
14. Singleton SL, Rossi JA, Calorimetry of total phenolic with Phosphomolybdic-Phosphotungstic acid reagents, Am J Enology Vitic (16), 1965, 144.
15. Oyaizu M, Studies on product of Browning reaction prepared from glucosamine, Jap J Nutr, (44), 1986, 307.
16. Amith Kumar, Reshma Kumarchandra, Rajalakshmi Rai, Free radical scavenging & Iron chelating capacity of *Psidium guajava* and *Persea americana* leaf extract a comparative study, Journal of Pharmacy Research, 8(6), 2014, 728-732.
17. Chanda S, Dave R, *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview, African Journal of Microbiology Research, 3(13), 2009, 981-996.
18. Meir S, Kanner J, Akiri B, Hadar SP, Determination and involvement of aqueous reducing compounds in oxidative systems of various senescing leaves. J. Agric. Food Chem, 43, 1995, 1813-1817.
19. Joseph Boby, Priya Mini, Review on nutritional, medicinal and pharmacological properties of *psidium guajava* , International Journal of Pharmacy and Bioscience, 2, 2011, 53-69.

Source of Support: Nil, **Conflict of Interest:** None.

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.