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Preliminary Antioxidant Activity Analysis of Brazilian Pepper Tree (Schinus terebinthifolius) Extracts via TLC, FRAP, and DPPH

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Graphical Abstract

Fig. 1. Braxilian Pepper Tree: leaves, bark and berries from the Organic Garden at St. Thomas University.

Abstract. In the present study, Schinus Terebinthifolius (Brazilian Pepper Tree) extracts were evaluated for antioxidant activity using free radical scavenging activity, and ferric reducing power. The plant was collected in the organic garden at St. Thomas University and the extracts were prepared by maceration of three parts of the plants, the leaves, the berries, and the bark. The extracts were made using varying proportions of ethanol and hexane solvents. All the samples were analyzed using thin layer chromatography (TLC). Multiple extract samples were submitted to DPPH (2,2-diphenyl-1-picrylhydrazyl) assay to determine the free radical scavenging (FRS) capacity, and absorbance was read at 517 and 520 nm in a plate reader. A control sample was prepared containing the same volume of solvent and DPPH without any extract and reference ascorbic acid. Percent scavenging activity of the DPPH free radical is expressed as an ascorbic acid (AA) equivalent antioxidant capacity (mg AA/100g). A Ferric reducing anti-oxidant power assay (FRAP) was performed and absorbance was measured at 700 nm to quantify the total antioxidant activity. FRAP and DPPH assays indicated that the bark has significantly higher free radical scavenging ability than any other part of the plant.

Introduction

Schinus Terebinthifolius has been widely used in South America in herbal remedies and was reported to have anti-bacterial and antioxidant properties. High levels of antioxidant capacity in plants are believed to decrease oxidative stress and free radicals in the body..

Background research of the Brazilian Pepper Tree indicated that certain parts of the plant exhibited higher antioxidant and anti-bacterial levels than others. In order to compare the antioxidant activity levels, the plant was categorized into leaves, bark, and berries for testing. Also be used to evaluate total phenolic content and concentrations of antioxidants in different parts of the vine.

Materials and Methods

Extractions of Samples

Fresh leaf samples were dried before placing in an oven for 48 hours. Then, were crushed into fine powder using a mortar and pestle. Different sample extracts were prepared, each with 5 grams of the powder in different percent mixtures of 50/50 ethanol to hexane, 75/25 ethanol/hexane and ethanol alone. Each solution was subjected to maceration at room temperature for 24 hours.

Thin Layer Chromatography

Plant extracts were analyzed by TLC (Thin Layer Chromatography) using polar and non-polar solvents, and the spots were developed and visualized with Iodine and UV light. Multiple TLC plates like the one shown in Figure 2 demonstrated a variety of nonpolar as well as slightly polar compounds within the plant extracts. The various colors displayed indicate presence of chlorophyll A B, carotene, and other pigments.

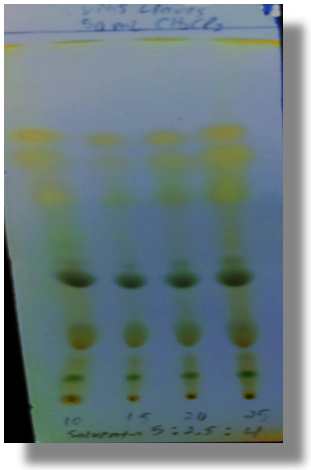


Fig.2. TLC of Muscadine grapes.

Ferric Reducing Power Assay (FRAP)

2.5 mL 0.2 M phosphate buffer, 2.5 mL potassium ferricyanide, 2.5 mL trichloroacetic acid, was added to 2.5 mL of extracts at different concentrations. The mixtures were incubated at 50°C for 20 min, and centrifuged at 3000rpm for 10 min. 2.5 mL of methanol and 0.5 mL of ferric chloride were added before absorbance values were taken at 700 nm.

Total Phenolic Content Assay (TPC)

2 mL of ethanolic extract solutions were mixed with 2.5 mL of 7.5% sodium bicarbonate ($NaHCO_3$), and 2.5 mL of 10% Folin-Ciocalteu reagent. The test tubes were placed in an incubator shaker for 45 min at 45°C before the absorbances were taken at 765 nm in the spectrophotometer.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging (FRS) Assay

This assay was used to determine radical scavenging activity of the extracts. 1mL of different extract concentrations were mixed with 1mL of DPPH. The test tubes were placed in a dark chamber for 30 min at room temperature. 0.2 mL of each solution were added into a well plate and read in a computer programming (Gen-5).

Results and Discussion

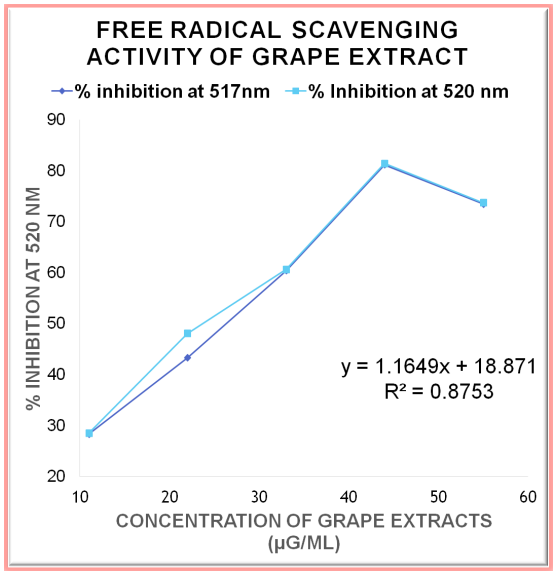
75 EtOH/ 25 Hex leaves Extract	Total Phenolic Content	FRP Total Antioxidant
sample 1	3507 ± 1348	0.474 ± 0.177
sample 2	8699 ± 3283	1.566 ± 0.156
sample 3	21355 ± 3804	3.419 ± 0.292
sample 4	33881 ± 3628	5.649 ± 0.401
sample 5	55036 ±4490	10.07 ± 0.911

75 EtOH/25 Hex Roots Extract	Total Phenolic Content	FRP Total Antioxidant
sample 1	1747.9± 812.8	0.295 ± 0.0319
sample 2	11366 ± 2203	1.455 ± 0.0337
sample 3	21272 ± 2045	3.524 ± 0.321
sample 4	34992 ± 4200	5.349 ± 0.174
sample 5	51703 ± 5292	9.353 ± 0.641

75 EtOH/25 Hex Grapes Extract	Total Phenolic Content	FRP Total Antioxidant
sample 1	252.2 ± 45.86	0.0549 ± 0.017
sample 2	1160 ± 39.29	0.264 ± 0.0533
sample 3	2589 ± 226.3	0.598 ± 0.0402
sample 4	4610 ± 272.2	1.007 ± 0.1044
sample 5	7219 ± 508.8	1.657 ± 0.1475

Table 1-3. Total Phenolic Content (mgGAE/g) and Ferric reducing Power (mgAAE/g)..Mean ± Standard Deviation

Conclusions



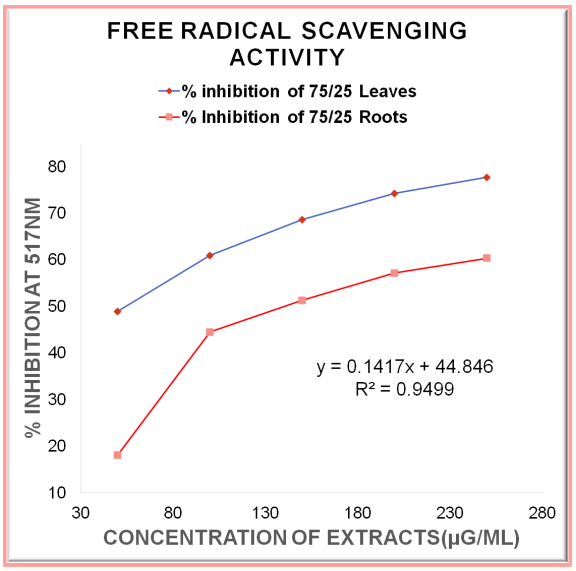


Fig 3. and **Fig 4**. DPPH free radical scavenging Activity (%) Inhibition of grapes, leaves, and roots extracts in 75/25 EtOH/hexane

FRP

According to **Table 1**, Vitis leaves exhibited the highest TPC and FRP of all the parts of the plant tested in this study (TPC= 55036 ±4490 mgGAE/g; FRP= 10.07 ± 0.911 mgAAE/g) . These values correspond to higher concentration of the leaf extracts, varying from 50 to 250µg/mL compare to the grapes with lower concentrations ranging from 10 to 54 µg/mL. Concentrations were very similar among the roots and leaves extracts. Both FRP and TPC measurements were very close in value. Grapes showed the highest value in the TPC with 7219 ± 508.8 mgGAE/g.

DPPH

The antioxidant activities of the extracts in terms of free scavenging activity were expressed as % inhibition ranging from 18 to 81. The graphs show a direct correlation between the concentration of the samples and their scavenging activity. As the concentration increased, the percent inhibition also increased. Results showed up that the 75/25 EtOH/hexane grape extract exhibited the greatest antioxidant activity with a value of 81.27 ± 0.180% compared to the leaves which were 77.80 ± 0.111% and the roots with 60.49 ± 0.204% scavenging activity. This findings indicates that both the Vitis leaves and grapes extracts have high antioxidant activities and they are the most potent sources of antioxidants

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