FREE RADICAL SCAVENGING ACTIVITY AND TOTAL PHENOLIC CONTENT OF METHANOL EXTRACT OF *Dillenia retusa* FRUITS

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The *Dillenia retusa* (Dilleniaceae), "Godapara", is an endemic plant in Sri Lanka and extensively used in traditional medicines against a plethora of human ailments. The antioxidant compounds can either completely prevent or slow down cell damages caused by free radicals avoiding inflammation and other relevant diseases. In this study, methanolic extract of *D. retusa* fruits was evaluated for antioxidant activity and total phenolic content using ascorbic acid as the positive control. The total phenolic content of the methanol extract was determined by the Folin–Ciocalteu assay, and the result was expressed as a ratio of gallic acid equivalents to dry weight of the crude extract (GAE/DW). The antioxidant activity of the methanol extract was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The methanol extract exhibited a significant antioxidant activity, with an IC50 value of 3.46 ± 0.65 ppm compared to the positive control, Ascorbic acid (IC50 value of 37.26±4.34 ppm). These results suggest that the methanol extract of *D. retusa* fruits has a high potential to function as a natural antioxidant. The total phenolic content of the extract was 340.44 mg GAE/g DW which is relatively high compared to other fruit extracts found in the literature. The high phenolic content indicates that the extract contains a significant number of phenolic compounds that may contribute to its antioxidant activity. Overall, these investigations showed that the methanol extract of *D. retusa* fruits has a potent antioxidant activity and a high total phenolic content. The results suggest that the extract may be used as a natural alternative for synthetic drugs of antioxidant. The active compounds responsible for these properties have yet to be isolated and identified.

Keywords: DPPH, Gallic Acid Equivalent, Folin–Ciocalteu, Natural alternative for synthetic drug

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INTRODUCTION

Dillenia retusa, commonly known as Godapara, is an endemic plant found in Sri Lanka. It has a long history dating back to thousands of years in indigenous medicine of Sri Lanka. The fruits of *D. retusa* have been used for various medicinal purposes, including treatment of digestive disorders, skin diseases, and as an anti-inflammatory agent. In recent years, there has a growing interest in the potential antioxidant properties of plant extracts, including those from *D. retusa*, due to their ability to scavenge free radicals and protect against oxidative damage. Antioxidants are compounds that can neutralize or scavenge free radicals, which are highly reactive molecules that can damage biomolecules such as proteins, lipids, and DNA, leading to various diseases and aging processes. Natural antioxidants derived from plants have gained considerable attention due to their potential health benefits and their nutraceuticals, and pharmaceuticals application. Phenolic compounds, which are abundant in many plant extracts, have been reported to possess strong antioxidant activity. The aim of this study was to investigate the antioxidant activity and total phenolic content of the methanol extract from *D. retusa* fruits and compare it with ascorbic acid as a positive control.

METHODOLOGY

Collection and preparation of plant material: *D. retusa* fruits were collected from Kalawana, Sri Lanka. The fruits were cleaned, air-dried, and coarsely powdered using a grinder.
Extraction of methanol extract: The dried fruit powder (100 g) was extracted with pure methanol using slightly modified ultra sonic extraction method [**1**]. The extract was filtered and concentrated under reduced pressure using a rotary evaporator. The resulting methanol extract was stored at -20°c until further analysis.

Determination of antioxidant activity: The antioxidant activity of pure methanol extract was determined using the slightly modified 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay [**2**][**3**]. Briefly, a series of concentrations of the methanol extract (31.25-0.244 ppm) and ascorbic acid (positive control) were prepared using half dilution method methanol as the solvent. DPPH (1.2 mg) was dissolved in methanol (10 mL). Triplicates from each concentration (150 µL per well) prepared previously were loaded to a microplate and was added freshly prepared DPPH solution (60 µL). Triplicates of the control were loaded using methanol (150 µL per well) and added with freshly prepared DPPH solution (60 µL). For the sample control single well per each concentration was loaded with relevant concentration (150 µL per well) and methanol (60 µL) and for the control blank single well, loaded with methanol (210 µL) The loaded plate was then incubated in the dark for 30 minutes at room temperature, and the absorbance was measured at 517 nm using a microplate spectrophotometer. The percentage of DPPH radical scavenging activity was calculated using the following formula:

DPPH scavenging activity (%) = [(absorbance of control - absorbance of sample)/absorbance of control] x 100

The concentration of methanol extract required to scavenge 50% of DPPH radicals (IC 50 value) was calculated using non-linear regression analysis.

Determination of total phenolic content: the total phenolic content of the methanol extract was determined using the Folin–Ciocalteu assay [**4**]. A series of concentrations of gallic acid (standard) ranges from 0-0.06 ppm and methanol extract of *D. retusa* with unknown concentration were prepared dissolved in methanol, and triplicates from each concentration of gallic acid (65 µL per well and 10% and Folin–Ciocalteu reagent (105 µL per well) were loaded into a microplate and incubated for 3 mins at room temperature. 7.5% sodium carbonate solution (80 µL) was added per each well and incubated for another 30 mins in the dark at room temperature. The absorbance was measured at 765 nm using a microplate spectrophotometer. The total phenolic content of the methanol extract was expressed as gallic acid equivalents (GAE) in milligrams per gram of dry weight.

RESULTS AND DISCUSSION

Antioxidant activity: The antioxidant activity of the methanol extract of *D. retusa* fruits was determined by the DPPH radical scavenging assay. According to the Figure (01) graph, the methanol extract exhibits a significant antioxidant activity, with an IC50 value of 3.46±0.65 ppm, indicating that the extract has a strong antioxidant activity. Ascorbic acid, used as a positive control showed an IC50 value of 37.26±4.34 ppm, which was considerably lower than that of the methanol extract. These results suggest that the methanol extract of *D. retusa* fruits has a great potential to function as a natural antioxidant.

Total Phenolic content: The total phenolic content of the methanol extract of *D. retusa* fruits was determined by the Folin–Ciocalteu assay, and the result was expressed as gallic acid equivalents (GAE) in milligram per gram of dry weight. The total phenolic content of the extract was found to be 340.44 mg GAE/g DW, which is relatively high compared to other fruit extracts. The high phenolic content indicates that the extract contains a significant number of phenolic compounds that may contribute to its antioxidant activity. Phenolic compounds are known to possess various biological activities, including antioxidant, anti-inflammatory, and anticancer properties. The presence of these compounds in the methanol extract of *D. retusa* fruits suggests that the extract may have potential health benefits.

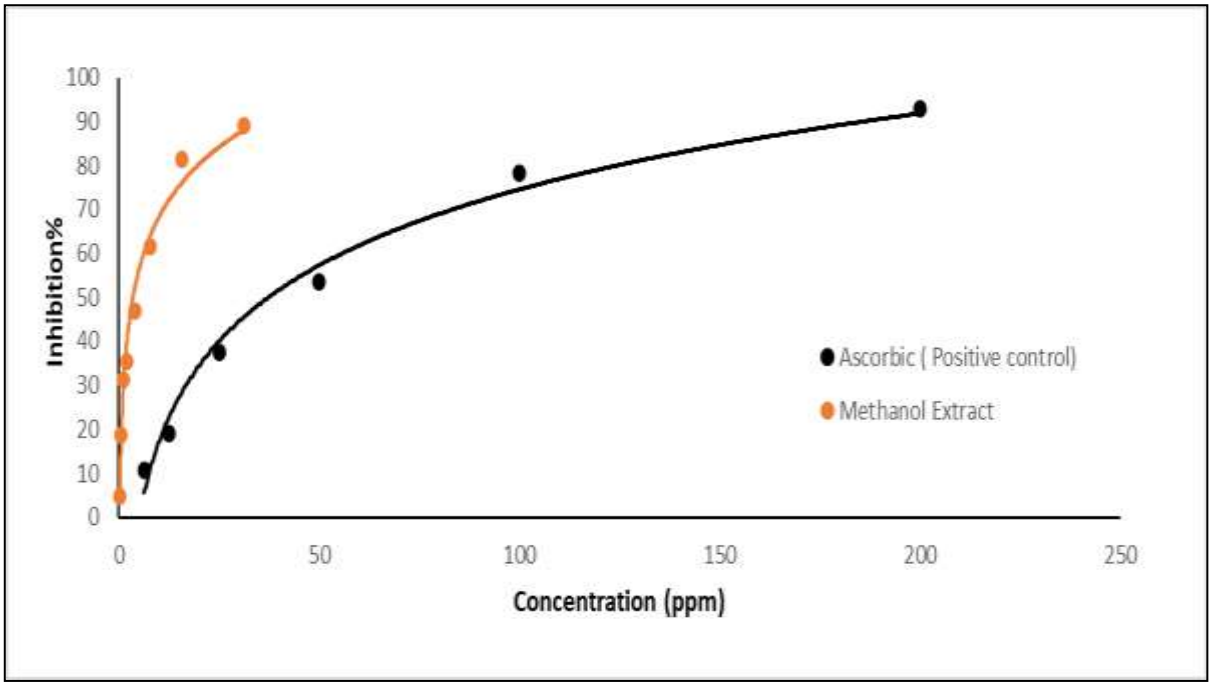

Fig.(01) DPPH radical scavenging activity of methanol extract (R^2 = 0.984) of *D.retusa* fruits and

Ascorbic (R^2 = 0.9857)

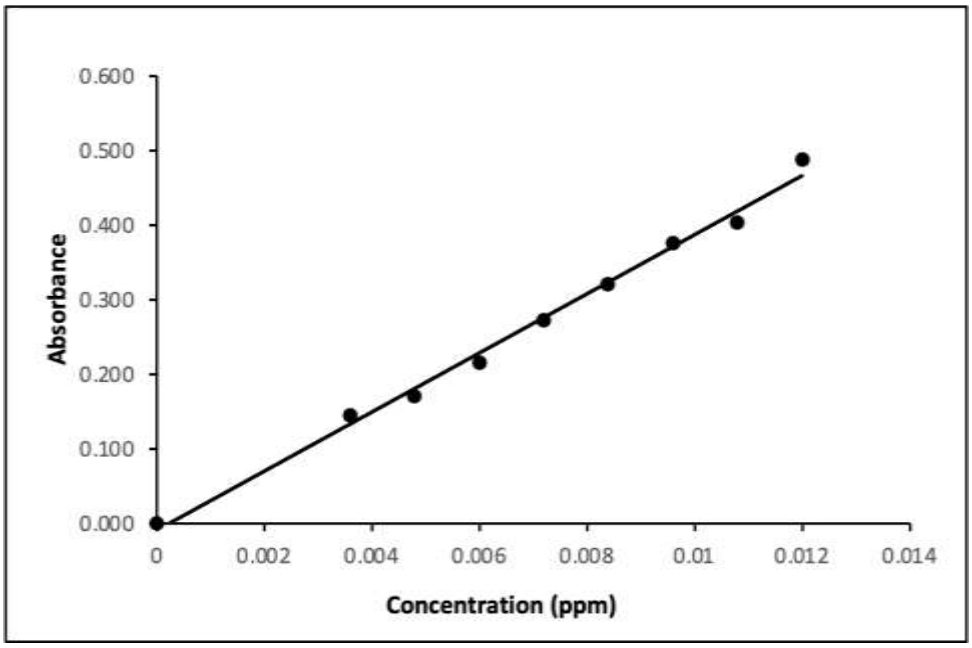

Fig.(02) Gallic acid standard curve (R^2 = 0.9932)

CONCLUSIONS/RECOMMENDATIONS

In conclusion, this study investigated the antioxidant activity and total phenolic content of the methanol extract of *D. retusa* fruits. This promising result shows that the extract exhibited a strong antioxidant activity and a high total phenolic content. These findings suggest that the extract may be used as a natural antioxidant for therapeutic purposes to reduce the oxidative stress of the body due to free radicals generating in the human body. The antioxidant activity of the extract can be attributed to the presence of phenolic compounds [**5**]. However, responsible active compound for this observed antioxidant activity has yet to be isolated and identified. In addition, further *in vivo* and *in vitro* studies are also required to evaluate the safety and efficacy of the extract for use in human health applications.

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