

Determination of *In-vitro* antioxidant activity of *Ougeinia oojeinensis* leaves

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Abstract

In this investigation, the *In-vitro* antioxidant Activity of extracts of leaves of *Ougeinia oojeinensis* was evaluated. The antioxidant activity *in vitro* was measured by means DPPH, Reducing power and hydroxyl radical scavenging activity. DPPH radical scavenging activity of ethyl acetate extract of *Ougeinia oojeinensis* exhibited percent inhibition 66.774% and its IC₅₀ value was found to be 43.503µg/ml. DPPH radical scavenging activity of methanol extract of *Ougeinia oojeinensis* exhibited percent inhibition 76.267% and its IC₅₀ value was found to be 25.722µg/ml. Ascorbic acid was used as a reference compound which exhibited percent inhibition 89.428% and showed IC₅₀ value of 15.615 µg/ml.

Similarly hydroxyl radical scavenging activity of ethyl acetate extract of *Ougeinia oojeinensis* exhibited percent inhibition 62.183% and its IC₅₀ value was found to be 69.341 µg/ml. Similarly, hydroxyl radical scavenging activity of methanol extract of *Ougeinia oojeinensis* exhibited percent inhibition 75.84% and its IC₅₀ value was found to be 33.360µg/ml. Ascorbic acid was used as a reference compound which exhibited percent inhibition 87.749% and showed IC₅₀ value of 9.904 µg/ml. The methanolic extract showed strong reducing capacity as compared to ethyl acetate extract of *Ougeinia oojeinensis*.

Keywords: *Ougeinia oojeinensis*; Antioxidant; DPPH; IC₅₀; Reducing power; Hydroxyl radical scavenging activity; Ascorbic acid.

1. Introduction

Medicinal plants have been utilized as a form of drug for a long time. They contain many phytochemicals which are helpful in biological functions and as pharmaceutical drugs. Secondary metabolites which are produced by plants play an essential role in many diseases.

Ougeinia oojeinensis is a multipurpose medicinal plant of the family Fabaceae. The common names of the species are Tinsa, Sandan, and Panjan (Sharma, 2001;) (Singh, 2002;). The phytochemical screening of *Ougeinia oojeinensis* in methanolic extract of leaves is used for the detection of various phytochemicals. The phytochemical studies confirm the presence of alkaloids, carbohydrates, terpenoids, flavonoids, tannins and phenolic compounds, saponins, and glycosides in the extracts of *Ougeinia oojeinensis* leaves. Further phytochemical studies showed the presence of betulin, hydroxylupeol, luepeol, and isoflavanones such as homoferreirin, dalbergioidin, and ougenin (Mukherjee, 1963;) (Ghosh, 1965;) (Balakrishna,1965;).

The extract of the whole plant *O.oojeinensis* shows antioxidant, antispasmodic action, anti-inflammatory and analgesic, anthelmintic, antidiabetic, hepatoprotective, hypoglycemic, weak CNS depressant effect, and wound healing activity. It is used for the treatment of diarrhea, dysentery, diabetes, leprosy, leukoderma, jaundice, hemorrhages, fevers, urorrhagia, ulcers, verminosis, etc. (Gunasekaran, 2011; Verma, 2012; Velmurugan, 2013; Samyal, 2014; Samyal, 2014).

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Substances that inhibit the oxidation process and neutralize free radicals & their action are known as Antioxidants. Antioxidant plays a vital role in keep from harm our body from disease by reducing the oxidative stress to cellular component caused by Reactive Oxygen Species. It stimulates the production and activity of bone marrow stem cells, macrophages, T-cells, spleen & thymus gland showing enhanced function. It increases the cell nuclease activity and DNA repair and hence, it has a possible role in cancer treatment. Thus, consuming a diet rich in antioxidant plant foods will give photochemical, non-nutritive substances in plants that have health-protective effects. The main photochemical antioxidants with anticancer activity include alkaloids, saponins, vitamins (e.g., A, C, E, and K), carotenoids, terpenoids, flavonoids, polyphenols, enzymes, minerals, polysaccharides, lignins, xanthenes, and certain pigments. (Singh, 2011 ;) As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity (Kokate, 2002; Sahu RK, 2008; Sahu RK, 2009;).

Recent studies suggest that the plant-origin antioxidants with free-radical scavenging properties may have great medicinal importance in free radical-mediated diseases like cancer, cardiovascular diseases, diabetes, gastrointestinal diseases, neurodegenerative diseases, arthritis, and aging processes. The antioxidant activity of plant extracts was determined by different *in vitro* methods such as the DPPH, Reducing power, and hydroxyl radical scavenging activity. In this investigation, the *In-vitro* antioxidant effect of *Ougeinia oojeinensis* extracts was evaluated.

2. Material and methods

2.1. DPPH Radical Scavenging Activity

2.1.1. Preparation of DPPH reagent

0.1 mM solution was prepared by dissolving of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol.

2.1.2. Preparation of Sample/Standard

Freshly 1 ml methanol solution of extracts was prepared. Take Different quantity of extracts/standard (20 – 100µl) from stock solution in a set of test tubes and add the methanol to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed completely and absorbance was recorded at 517 nm after 30 minutes incubation in dark at room temperature.

2.1.3. Preparation of control

For control, 3 ml of 0.1mM DPPH solution was taken and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm (Athavale *et al.*, 2012).

Percentage antioxidant activity of sample/standard was calculated by using formula:

$$\% \text{ Inhibition} = \left[\frac{\text{Ab of control} - \text{Ab of sample}}{\text{Ab of control}} \times 100 \right]$$

2.1.4. Reducing power assay

Preparation of standard solution

3 mg of ascorbic acid was dissolved in 3 ml of distilled water/solvent. Dilutions of this solution with distilled water were prepared to give the concentrations of 20, 40, 60, 80 and 100µg/ml.

2.2. Preparation of extracts

Stock solutions of extracts were prepared by dissolving 1mg of dried extracts in 1 ml of methanol to give a concentration of 1mg/ml. Then sample concentrations of 20, 40, 60, 80 and 100 µg/ml were prepared.

2.3. Protocol for reducing power

According to this method, the aliquots of various concentrations of the Ascorbic acid as a standard and extracts (20 to 100µg/ml) in 1.0 ml of deionized water were mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C in water bath for 20 min after cooling. Aliquots of 2.5 ml of (10%) trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 2.5 ml was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride

solution. The absorbance was measured at 700 nm in UV spectrometer (Systronic double beam-UV-2201). A blank was prepared without adding extract. (Quisumbing, 1978 ;).

2.3.1. Hydroxyl radical scavenging activity

Deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium. The reaction mixture containing FeCl₃ (100 μM), EDTA (104 μM), H₂O₂ (1 mM) and 2-deoxy- D-ribose (2.8 mM) were mixed with or without extract at various concentrations (20-100 μg) in 1 ml final reaction volume made with potassium phosphate buffer (20 mM, pH 7.4) and incubated for 1 hr at 37 °C. The mixture was heated at 95°C

in water bath for 15 min followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). Finally, the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. Absorbance of supernatant was measured at 532 nm. All readings were corrected for any interference from brown color of the extract or antioxidant by including appropriate controls. The negative control without any antioxidant or CPLL was considered 100% deoxyribose oxidation. The % hydroxyl radical scavenging activity of test sample was determined accordingly in comparison with negative control. Ascorbic acid was taken as the positive control (Ramakrishna *et al.*, 2012 ;).

$$\% \text{ inhibition} = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

3. Results and discussion

DPPH radical scavenging activity of ethyl acetate extract of *Ougeinia oojeinensis* exhibited percent inhibition 66.774% and its IC₅₀ value was found to be 43.503μg/ml. Similarly, DPPH radical scavenging activity of methanol extract of *Ougeinia oojeinensis* exhibited percent inhibition 76.267% and its IC₅₀ value was found to be 25.722μg/ml. Ascorbic acid was used as a reference compound which exhibited percent inhibition 89.428% and showed IC₅₀ value of 15.615μg/ml.

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The reducing ability of a compound may provide as a powerful indicator of its potential antioxidant activity. Dietary antioxidant such as ascorbic acid was used for comparison. Compounds with reducing capacity show that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can react as primary and secondary antioxidants. The methanolic extract showed strong reducing capacity as compared to ethyl acetate extract of *Ougeinia oojeinensis*.

3.1. DPPH Assay

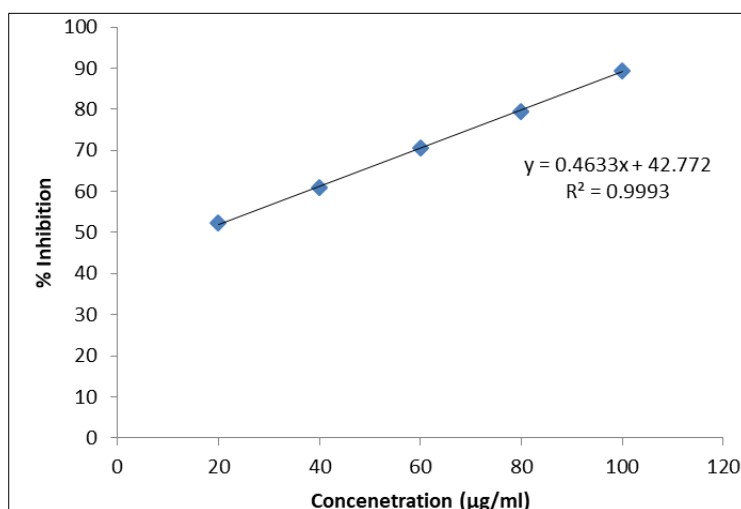


Figure 1 DPPH radical scavenging activity of Ascorbic acid

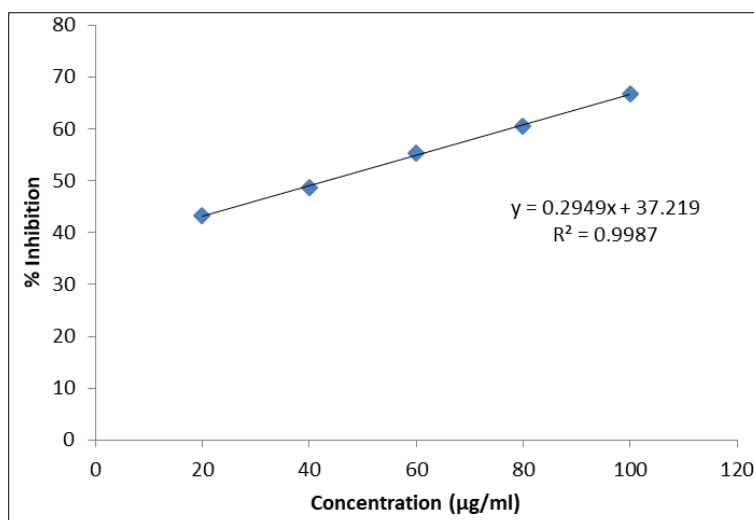


Figure 2 DPPH radical scavenging activity of Ethyl acetate extract of *Ougeinia oojeinensis*

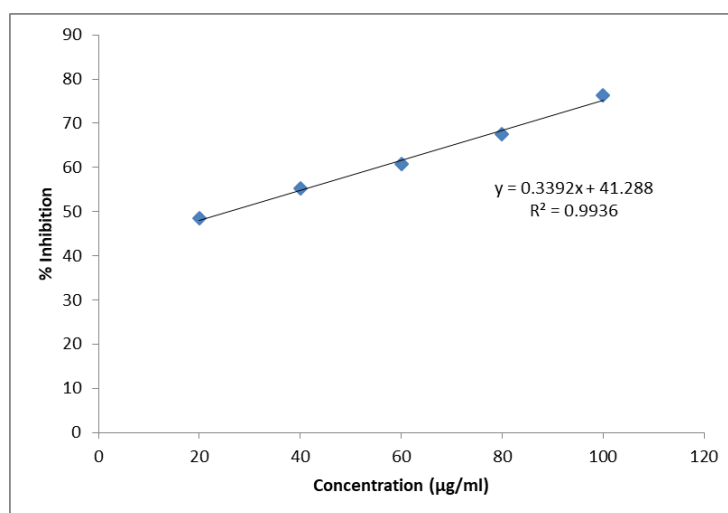


Figure 3 DPPH radical scavenging activity of Methanolic extract of *Ougeinia oojeinensis*

3.2. Reducing power scavenging activity

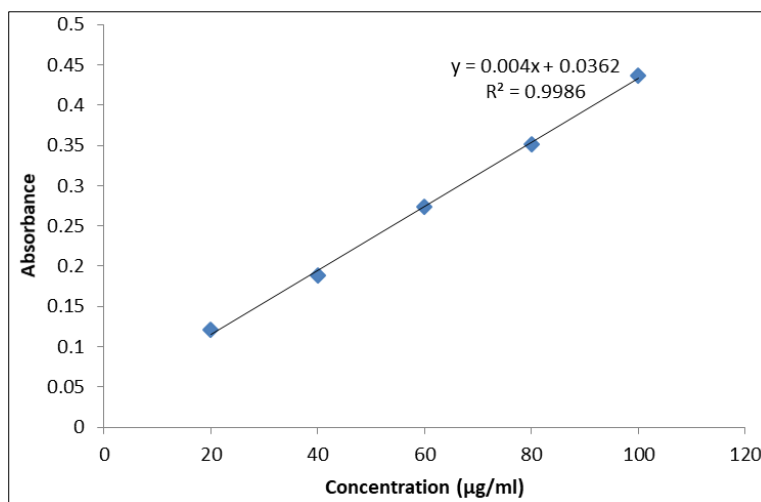


Figure 4 Reducing power scavenging activity of Ascorbic acid

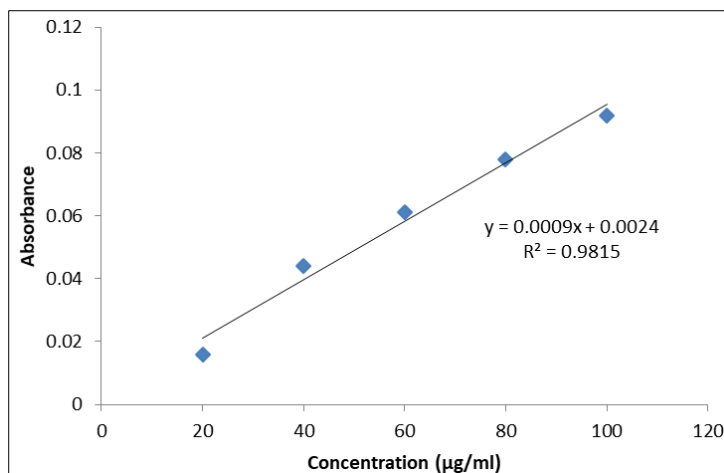


Figure 5 Reducing power scavenging activity of Ethyl acetate extract of *Ougeinia oojeinensis*

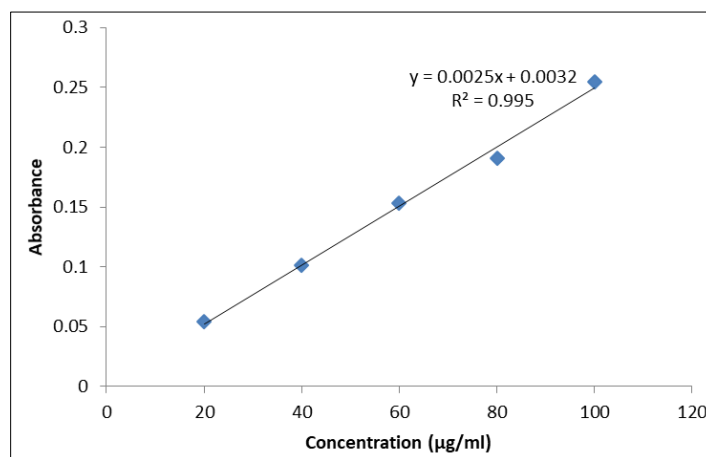


Figure 6 Reducing power scavenging activity of Methanolic extract of *Ougeinia oojeinensis*

3.3. Hydroxyl radical scavenging activity

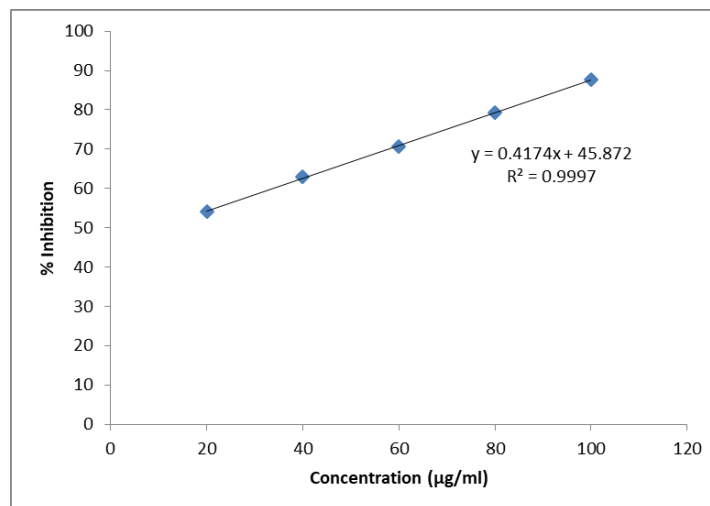


Figure 7 Hydroxyl radical scavenging activity of Ascorbic acid

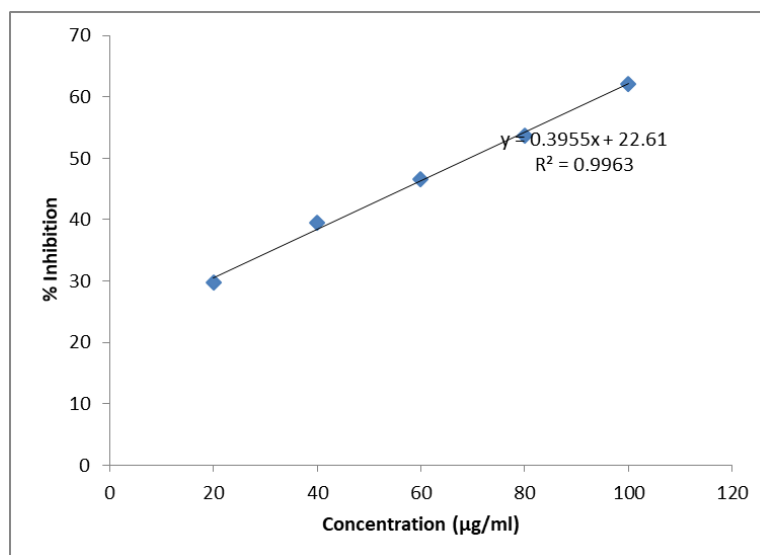


Figure 8 Hydroxyl radical scavenging activity of Ethyl acetate extract of *Ougeinia oojeinensis*

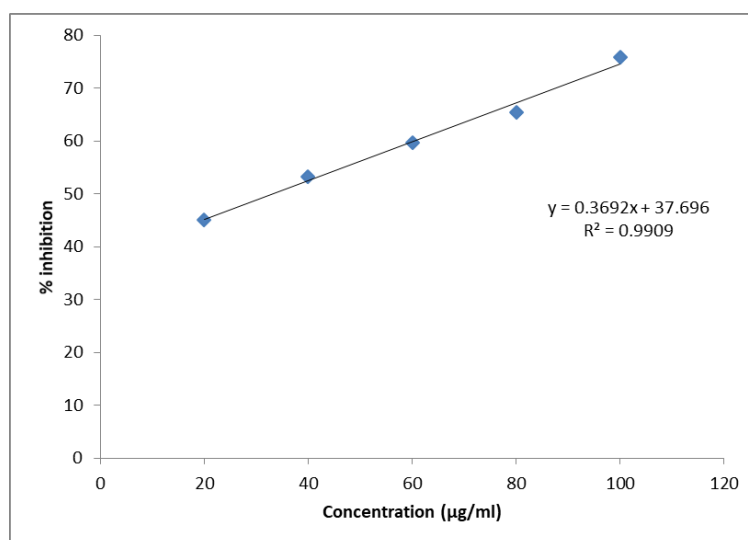


Figure 9 Hydroxyl radical scavenging activity of Methanolic extract of *Ougeinia oojeinensis*

4. Conclusion

The analysis of *Ougeinia oojeinensis* leaves revealed significant antioxidant activity, high phenolic and flavonoid content, and potential for medicinal and therapeutic applications. It is important to note that these *in vitro* findings should be further validated through *in vivo* and clinical studies to ascertain the antioxidant effects in living organisms and evaluate their potential health benefits. Further investigations are needed to explore the specific mechanisms and compounds responsible for any anticancer properties associated with these leaves. This research serves as a foundation for potential future studies focused on the plant's role in cancer prevention and treatment.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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