# Antioxidant Activity of Different Extracts of Stem bark of *Millingtonia hortensis* Linn

**Nagaraja MS, Padmaa M Paarakh*.**

[1]Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore 560068, Karnataka, India
Corresponding Author: padmaparas@hotmail.com; 09880681532

**Summary**

The different extracts of *Millingtonia hortensis* Linn (Bignoniaceae) were evaluated for *in vitro* antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and reducing power determination method. Total phenol and total flavonoid content in each extracts were also determined. Gallic acid and ascorbic acid were used as reference standards. The extracts exhibited strong antioxidant DPPH radical scavenging activity with $IC_{50}$ value of 0.4358, 80.75, 54.07, 49.98, 26.22 and 39.07 µg/ml for gallic acid, petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of *M.hortensis* respectively. The absorbance for reducing power was found to be 0.504,0.064, 0.057, 0.076, 0.190 and 0.226 for ascorbic acid, petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of *M.hortensis* respectively. Total phenol content was found to be 16, 7.42, 28, 144 and 32 mg equivalent to gallic acid per gram of petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of *M.hortensis* respectively. Total flavonoid content was found to be 4.98, 21.56, 50.79, 64.92 and 19.67 mg equivalent to rutin per gram of petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of *M.hortensis* respectively. From the above data, it is clear that among all the extracts used, the methanol extract had strong antioxidant activity which could be due to the presence of flavonoids and phenols.

**Key words:** *Millingtonia hortensis* Linn.;antioxidant activity; DPPH; reducing power determination; gallic acid; ascorbic acid; total phenol content; total flavonoid content.

**Correspondence address: Dr. Padmaa M Paarakh**
**Principal and HOD, Department of Pharmacognosy**
**The Oxford College of Pharmacy**
**6/9, I Cross, Begur Road, Hongasandra, Bangalore 560068**
**Phone no.:09880681532, Fax No. : 080-30219829**
**E-mail ID:** padmaparas@hotmail.com

## Introduction

*Millingtonia hortensis* Linn (Bignoniceae) commonly known as Cork tree is important medicinal plant in Southern Asia ranging from India, Burma, Thailand and South China. The stem bark is used traditionally as mainly lung tonic, anti asthmatic and antimicrobial[1]. The scientific activities reported so far from the plants are antifungal[2], larvicidal[3, 4] and antiproliferative[5,6] activities.

Free radicals are reactive molecules involved in many physiological processes and human diseases such as cancer, aging, arthritis, Parkinson syndrome, ischemia, toxin induced reactions, alcoholism, liver injury etc. Research in finding a natural antioxidant from the plant source is therefore important as plants are potential source of immense chemicals for the treatment of number of aliments. With this view, the present study was undertaken to evaluate the different extracts of *Millingtonia hortensis* Linn for *in vitro* antioxidant DPPH free radical scavenging activity and reducing power capacity. Total phenol and total flavonoid content in each extracts were also determined.

## Materials and Methods

### Chemicals

1, 1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, rutin and ascorbic acid were purchased from Loba Chemie Pvt Ltd., Mumbai. All the chemicals and reagents used were of analytical grade.

### Plant Material

The fresh stem bark of *Millingtonia hortensis* Linn were collected from Bengaluru region, identified and authenticated by Dr Shiddamallayya N (SMPU/NADRI/BNG/ 2010-11/304) at National Ayurveda Dietetics Research Institute, Bengaluru, Karnataka. A voucher specimen was deposited in the Herbarium of Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore.

### Preparation of extracts

The dried stem bark powder were coarsely powdered and subjected to successive extraction by soxhlation. The extraction was done with different solvents in their increasing order of polarity such as petroleum ether, benzene, chloroform, methanol and distilled water. Each time the marc was dried and later extracted with other solvents. All the extract were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 1.44, 0.52, 0.61, 15.91 and 2.33 % w/w respectively with reference to the air dried plant material.

## Evaluation of Antioxidant Activity

### Antioxidant activity

### DPPH Radical Scavenging Activity

The free radical scavenging activity of the different extracts of stem bark of *Millingtonia hortensis* were measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH)[7]. Briefly, 0.1 mM solution of DPPH in ethanol was prepared. 1 ml of the solution was added to 3 ml of different extracts of *Millingtonia hortensis* in methanol at different concentration. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a spectrophotometer (UV- VIS Shimadzu). Reference standard compound being used were gallic acid. The experiment was done in triplicate. The $IC_{50}$ value is the concentration of sample required to inhibit 50 % of the DPPH free radical. The $IC_{50}$ value for the sample was calculated using log-dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated using the following equation:

DPPH scavenging effect (%) = 100 x $A_1$ / $A_0$

Where $A_0$ was the absorbance of the control reaction and $A_1$ was the absorbance in presence of the standards or samples.

**Reducing Power Determination**

The reducing power of different extract was assayed by Oyaizu[8]. Different concentrations of the extracts were mixed with 2.5 ml phosphate buffer [0.2 M phosphate buffer; pH 6.6] and 2.5 ml of 1% potassium ferricyanide solution and incubated at 50C for 20 min. The above solution were cooled; mixed with 2.5 ml of 10 % trichloroacetic acid and the content were centrifuged at 1000 rpm for 10 min. 2.5 ml of supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % ferric chloride solution and allowed to stand for 10 min and absorbance was measured at 700 nm. Higher the absorbance, higher is the reducing power. Ascorbic acid was used as reference standard.

**Estimation of Total Phenol[TP] Content**

The total phenol content was determined by Folin-Ciocalteu assay[9]. Different concentration of extracts were made up to 3.5 ml, then 0.5 ml of Folin-Ciocalteu reagent followed by 2 ml of 7.5 % sodium carbonate solution. The above solution is incubated at room temperature for 10 min and absorbance was measured at 650 nm. Total phenolic content are expressed as gallic acid equivalent [mg/g] of the dried weight.

**Estimation of Total Flavonoid [TF]Content**

The total flavonoid content in the extracts were determined by method modified by Zhishen's method[10]. Different concentration of extracts in methanol[ 3 ml] was mixed with 0.1 ml of 10 % aluminum chloride followed by 0.1 ml of 1 M potassium acetate solution. Add 2.8 ml of water and kept for incubation at room temperature for 30 min. The absorbance was measured at 415 nm. The total flavonoid content are expressed as Rutin equivalent [mg/g] of the dried weight.

**Result and Discussion**

The model for scavenging the stable DPPH radical is widely used model to evaluate antioxidant activities in a relatively short time to compare with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accept an electron or hydrogen radical to become a stable diamagnetic molecule and therefore inhibit the propagation phase of lipid peroxide[11,12].

The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progresses, which results in scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants.

Table: 1 illustrate the percentage inhibition of DPPH radical by standard and different extracts. The $IC_{50}$ value was found to be 0.4358, 80.75, 54.07, 49.98,26.22 and 39.07 µg/ml for gallic acid, petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of *M.hortensis* respectively.

Reducing power assay measures the electron-donating capacity of an antioxidant. The reducing properties are generally associated with the presence of reductones, which have been shown to exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation[13]. Being good electron donors, phenolic compounds show the reducing power and have ability to convert the ferric ion $Fe^{3+}$ to ferrous ion $Fe^{2+}$ by donating an electron[14]. Increasing absorbance at 700 nm indicates an increase in reductive ability.

Table: 2 illustrate the absorbance of standard and different extracts. The absorbance for reducing power was found to be 0.504,0.064, 0.057, 0.076, 0.190 and 0.226 for ascorbic acid, petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of *M.hortensis* respectively.

The ability to reduce Fe(III) may be attributed to hydrogen donation from phenolic compounds, which is also related to the presence of reductant agent.

Total phenol content [ Table 3] was found to be 16, 7.42, 28, 144 and 32 mg equivalent to gallic acid per gram of petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of *M.hortensis* respectively.

Total flavonoid content [ Table 4] was found to be 4.98, 21.56, 50.79, 64.92 and 19.67 mg equivalent to rutin per gram of petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of *M.hortensis* respectively.

**Table 1: DPPH free radical scavenging activity of standard and extracts of *M.hortensis***

| Extract/standard | $IC_{50}$ value (µg/ml) |
|---|---|
| Gallic acid | 0.4358 |
| Petroleum ether extract | 80.75 |
| Benzene extract | 54.07 |
| Chloroform extract | 49.98 |
| Methanol extract | 26.22 |
| Aqueous extract | 39.07 |

**Table 2: Absorbance of reducing power of standard and extracts of *M.hortensis***

| Extract/standard [15 µg/ml] | Absorbance |
|---|---|
| Ascorbic acid | 0.504 |
| Petroleum ether extract | 0.064 |
| Benzene extract | 0.057 |
| Chloroform extract | 0.076 |
| Methanol extract | 0.190 |
| Aqueous extract | 0.226 |

**Table 3: Total phenol content of extracts of *M.hortensis***

| Extract | mg of gallic acid/ gm of extract |
|---|---|
| Petroleum ether extract | 16 |
| Benzene extract | 7.42 |
| Chloroform extract | 28 |
| Methanol extract | 144 |
| Aqueous extract | 32 |

**Table 4: Total flavonoid content of extracts of *M.hortensis***

| Extract | mg of rutin/ gm of extract |
|---|---|
| Petroleum ether extract | 4.98 |
| Benzene extract | 21.56 |
| Chloroform extract | 50.79 |
| Methanol extract | 64.92 |
| Aqueous extract | 19.67 |

Significant correlations were observed between DPPH radical scavenging and reducing power, TP and TF. Many supportive reports emphasize the positive correlation between phenolic content and antioxidant efficacy[15,16]. A positive correlation between antioxidant activity and polyphenol content was found, suggesting that the antioxidant capacity of the plant extracts is due to a great extent to their polyphenols[17,18] .

The strongest antioxidant activity of the methanol extract could be due to the presence of flavonoids and phenols.The components responsible for the antioxidant activity of the extract are unknown. Further research is therefore needed for the isolation and identification of the antioxidant components in the extract.

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