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In vitro Evaluation of Antioxidant Activity of *Michelia champaca* (L.) Flowers

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ABSTRACT

The aim of the present study was to investigate the antioxidant activity of methanolic extract of *Michelia champaca* flowers. Antioxidant activity was evaluated by using *in-vitro* assay models like Total antioxidant capacity, Nitric oxide radical scavenging assay, Reducing Power Assay and Hydrogen Peroxide Scavenging Activity. All the antioxidant activities were compared with standard antioxidant such as ascorbic acid. Methanolic extract of the plant showed effective free radical scavenging activity at 300µg concentration. The IC50 values of the ethanolic extract of *Michelia champaca* flowers were found to be 260 µg in total antioxidant activity, 150 µg in nitric oxide radical assay, 240 µg in reducing power assay and 280 µg in hydrogen peroxide assay. The results obtained in this study showed that the flowers of *Michelia champaca* have antioxidant properties which provide a basis for the traditional use of the plant.

Keywords: *Michelia champaca*, IC 50, antioxidant, free radical.

INTRODUCTION

Free radicals in the body contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS[1,2] . Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radical induced tissue damages. Besides, well known and traditionally used natural antioxidants from tea, wine fruits, vegetables, spices and many other plant species have been investigated in the search for novel antioxidants[3,4]. There is still the demand to find information concerning the antioxidant potential of more plant species.

Michelia champaca L. (Magnoliaceae) commonly known as Svarna champa, a tall handsome tree with yellow fragrant blossoms, is commonly used by many traditional herbal preparations. The plant is

also reported to have significant wound healing,[5] antimicrobial,[6] antidiabetic,[7] antitumor[8],anti-inflammatory,[9] antioxidant,[10] and antiinfective[11] properties.

MATERIALS AND METHODS

Collection of plant material

The *Michelia champaca* flowers were procured from the local areas of Udumalaipettai, Coimbatore District, Tamilnadu. The collected plant material was botanically identified and confirmed by Dr.S.John Britto, The Director, Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamilnadu. The herbarium specimens were preserved and submitted to Department of Biochemistry, S.T.E.T Women's College, Mannargudi, Thiruvarur District, Tamilnadu for further reference (Voucher no. 001).

Preparation of the extract

The flowers were chopped into small pieces, shade-dried and coarsely powdered by using a pulverizor. The coarse powders were then subjected to successive extraction with methanol by Soxhlet method[12]. The extracts were then collected and distilled off on a water bath at atmospheric pressure and stored at 4ºC

Preliminary Phytochemical Screening

Phytochemical screening of methanol extract of *Michelia champaca* flower was carried out using standard qualitative methods[13,14].

Total antioxidant capacity

Total antioxidant capacity was measured by spectrophometric method. 0.1 ml of the extract (10 mg/ml) dissolved in water was combined in eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid[15].

The total anti oxidant activity was calculated according to the following equation

$$\% \text{ of Total antioxidant capacity} = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

A control is the absorbance of control; A test is the absorbance of sample

Nitric oxide radical scavenging assay

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which was measured by Griess reagent. The reaction mixture (3 ml) containing 10 mM sodium nitroprusside in phosphate buffered saline, and the fractions at different concentrations (100–300 µg/ml) were incubated at 25°C for 150 min. About 0.5 ml aliquot of the incubated sample was removed at 30 min intervals and 0.5 ml Griess reagent was added. The absorbance of the chromophore formed was measured at 546 nm. Inhibition of the nitric oxide generated was measured by comparing the absorbance values of standard ascorbic acid[16].

The nitric oxide radicals scavenging activity was calculated according to the following equation

$$\% \text{ of NO Radical Scavenging activity} = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

Reducing Power Assay

A 1ml of the extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The reaction mixture was incubated at 50°C

for 30 min. The reaction was stopped by adding 2.5 mL of 10% trichloroacetic acid and the mixture was centrifuged for 10 min at 3000 rpm.A 2.5 mL supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The activity was compared with ascorbic acid as standard[17] .

The reducing power assay was calculated according to the following equation

$$\% \text{ of Reducing power assay} = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

Hydrogen Peroxide Scavenging Activity

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectro photometrically from absorption at 230 nm in a spectrophotometer. Extracts (100–300 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after ten minute against a blank solution containing in phosphate buffer without hydrogen peroxide [18]. The percentage of scavenging of hydrogen perox-ide of plant extract and standard ascorbic acid was calculated using the following equation:

$$\% \text{ of hydrogen peroxide scavenging activity} = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

Statistical Analysis

Results were expressed as Mean ± S.D

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening

The results of the phytochemical screening of flower extract of *Michelia champaca* as presented in Table 1

The total antioxidant activity of methanolic extracts were concentration dependent (100 µg, 200 µg, 300 µg), with the increasing concentration the activity is also increased. The IC 50 value for the extract was found to be 260 µg/ml. Standard ascorbic acid was found to be 280 µg/ml. The total antioxdant activity of methanolic extract nearer to the value of ascorbic acid.The results were showed in Fig 1.

The utility of antioxidant therapies in many diseases is well recognized. Cellular damage arising from an imbalance between free radical generating and scavenging systems has been implicated in the pathogenesis of a wide range of disorders including cardiovascular diseases, cancer and aging[19].

Antioxidant activity of plant is the most efficient way of combating tissue injuries undesired transformations and preventing health risks[20]. Total antioxidant capacity of methanolic extract of *Michelia champaca* may serve as a significant indicator of its potential antioxidant activity. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[21]. Flavonoids and tannins seem to be a most promising polyphenolic compounds[22]. Carotenoids like ß-carotene and lycopene present in plants exert antioxidant functions such as quenching of singlet oxygen and other electronically excited molecules and progression of many degenerative diseases[23].

Nitric oxide scavenging activity of the *Michelia champaca* flowers showed in Fig 2. IC 50 value for the flower extract and

ascorbic acid was found to be 150µg/ml, 100µg/ml. Flower extract showed maximum nitric oxide scavenging activity compared with standard ascorbic acid.

Nitric oxide (NO) is a potent pleiotropic mediator of physiological process such as smooth muscle relaxant, neuronal signaling, inhibition of platelet aggregation and activities. Although nitric oxide and superoxide radicals are involved in host defense, over production of these two radicals contributes to the pathogenesis of some inflammatory diseases. Moreover in the pathological conditions, nitric oxide reacts with superoxide anion and form potentially cytotoxic molecules, peroxynitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspect of inflammation and tissue damage seen in inflammatory diseases[24].

In presence of plant extract which is a scavenger the amount of nitrous oxide will decreases. The increased nitric oxide radical scavenging activity was observed in methanolic extract of tested plant. The nitric oxide scavenging potentiality may be due to antioxidant principle in the extract which competes with oxygen to react with nitric oxide and thus inhibit the generation of nitrites.

Reducing power of methanolic extract of *Michelica champaca* flowers were represented in Fig 3. Reducing power of methanolic extract of flowers were screened by using different concentration of plant extract. The maximum reducing power was observed at highest concentration (300 µg). IC 50 value of methanolic extract showed 240 µg/ml, ascorbic acid showed 230 µg/ml. The flower extract having potent reducing power when compared to ascorbic acid.

A reducing power is an indicative of reducing agent having the availability of atoms which can donate electron and react with free radicals and then convert them into more stable metabolites and terminate the radical chain reaction[25]. Accordingly, preliminary phytochemical analysis of *Michelia chamapca* showed the presence of flavonoids which may react with the free radicals to stabilize and terminate from free radical chain reaction.

The reducing ability of a compound generally depends on the presence of reductants[26], which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom[27].

Hydrogen peroxide scavenging activity of methanolic extract of *Michelia champaca* flowers were represented in Fig 4. Maximum H_2O_2 scavenging activity was observed in 300 µg. IC 50 value of methanolic extract showed 280µg/ml, ascorbic acid was showed 290µg/ml.

Hydrogen peroxide is a weak oxidizing agent and it is not very reactive, can cross biological membranes. Because of the possible involvement of hydrogen peroxide in the generation of hydroxyl radicals, this property places hydrogen peroxide in a more prominent role to initiate cytotoxicity than its chemical reactivity. Thus removing H_2O_2 is very important for the protection of living systems[28].

A wide variety of phenolic substances derived from edible plants have been reported to retain marked anti oxidant and anti-inflammatory activities, which contribute to their chemo preventive potential [29]. Hydrogen peroxide scavenging activity of tested plant may be due to their free radical scavenging properties which play an important role in decomposing hydrogen peroxide radicals.

Scavenging of H_2O_2 by extracts may be attributed to their phenolics which can donate electrons to H_2O_2, thus neutralizing it to water[30].

CONCLUSION

From the results obtained in the present study, it is concluded that methanolic flower extract of *Michelia champaca* which contains phenolics, flavonoids, tannins,

alkaloids, and carbohydrate exhibits antioxidant and free radical scavenging activities. These *in vitro* assays indicate that this plant extracts are a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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Table 1. Phytochemical Constituents of Flower Extract of *Michelia Champaca*

Phytochemicals	Flower Extract
Carbohydrates	+
Alkaloids	+
Terpenoids	+
Flavonoids	+
Tannins	+
Steroids	+
Protein	-
Aminoacids	-
Phenols	+

+ indicates presence whereas – indicates absence

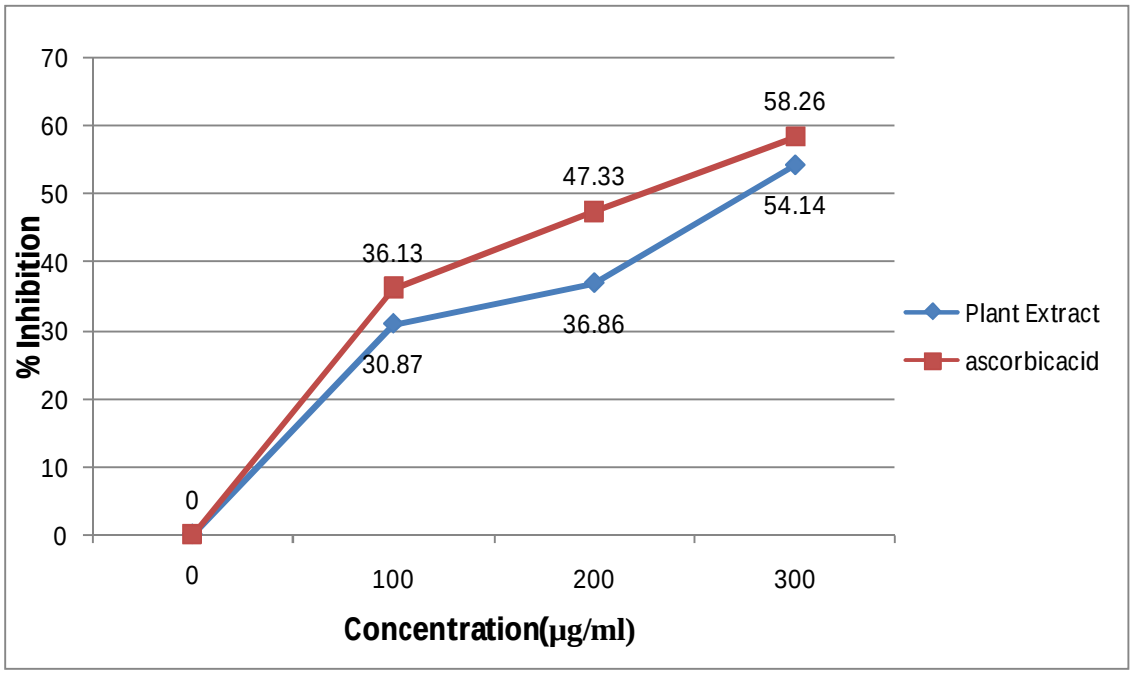

Figure 1. Total antioxidant capacity of Methanolic extract of *Michelia champaca* flowers

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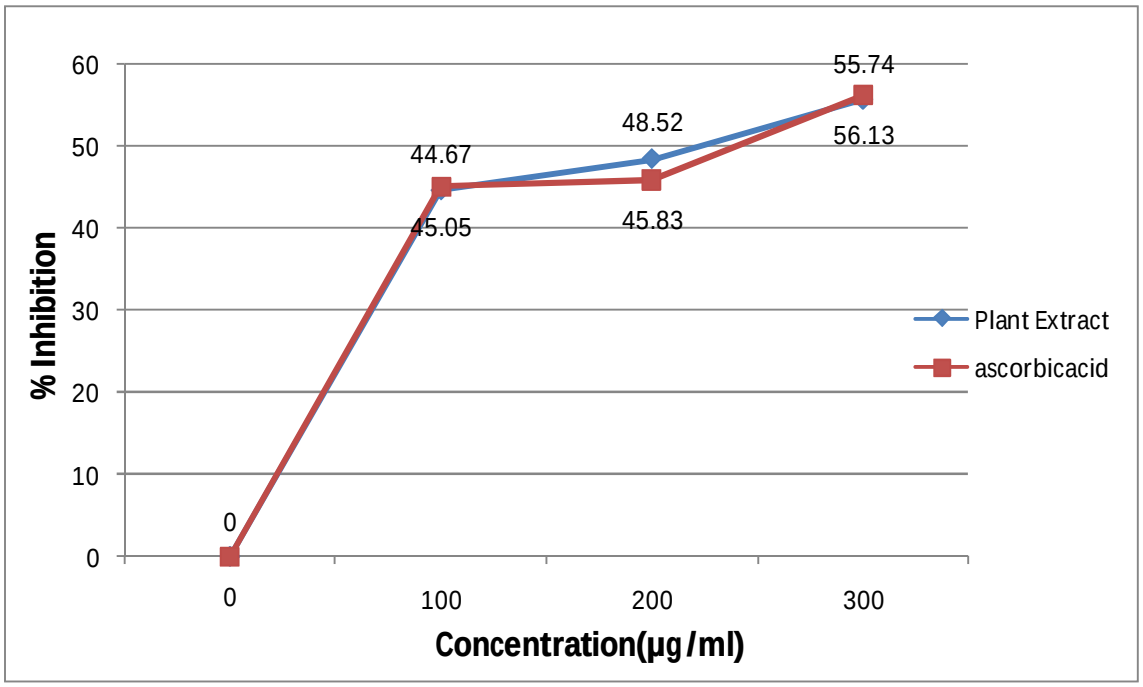

Figure 2. Reducing power assay of Methanolic extract of *Michelia champaca* flowers

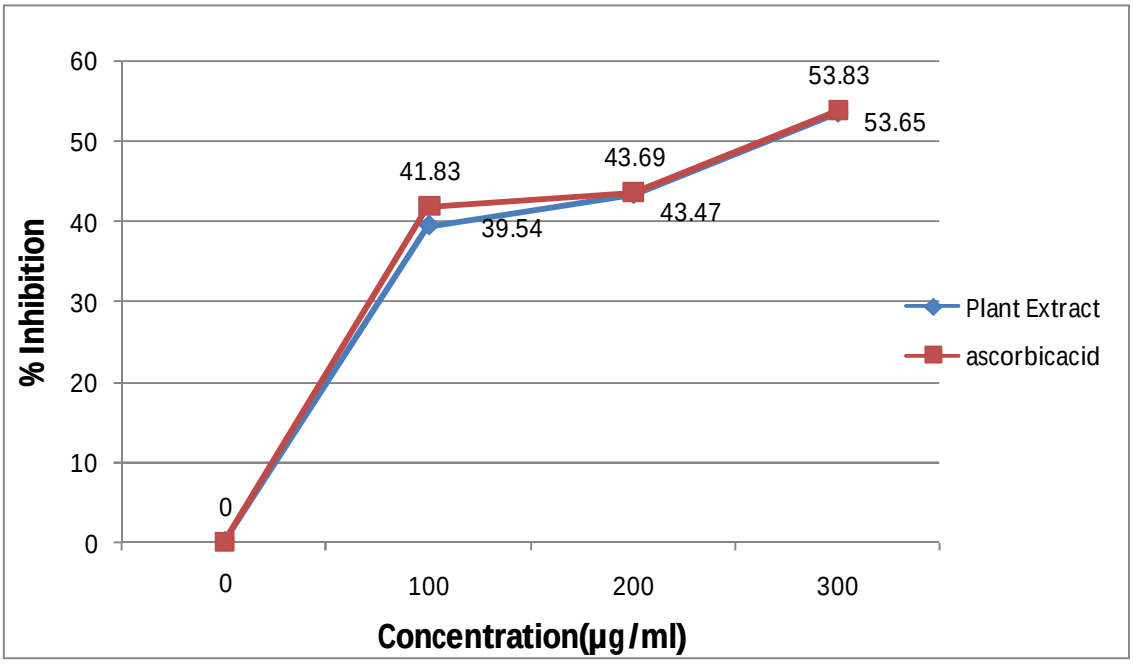

Figure 3. Hydrogen Peroxide radical scavenging of Methanolic extract of *Michelia champaca* flowers

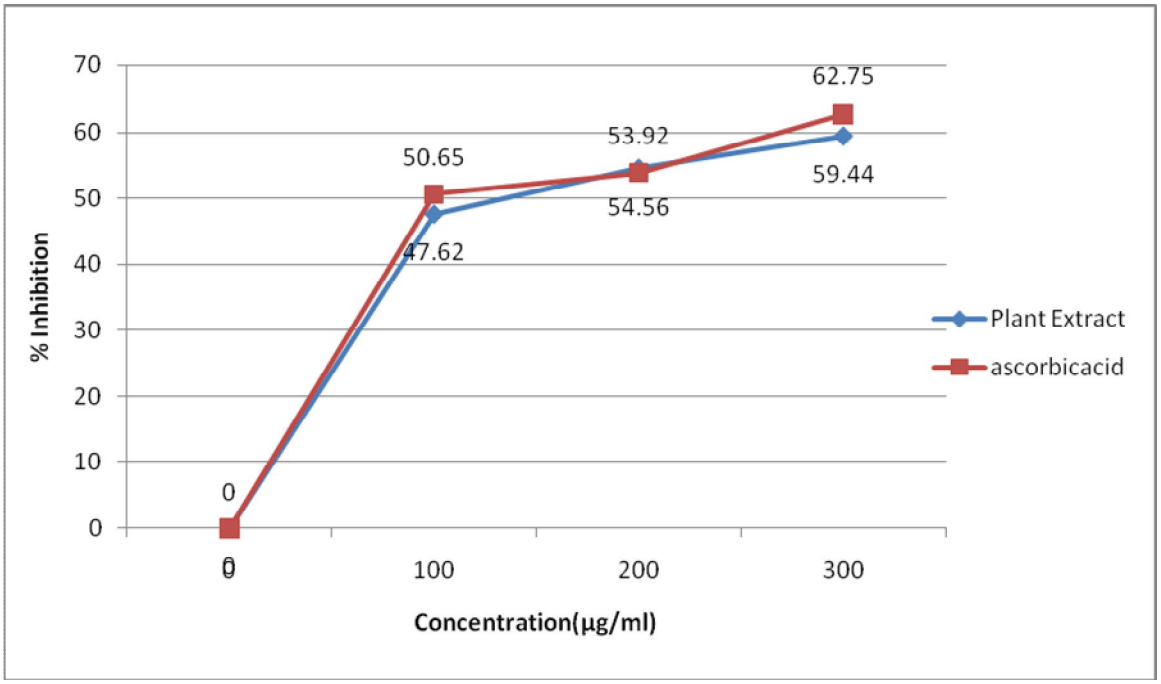

Figure 4. Nitric oxide scavenging of Methanolic extract of *Michelia champaca* flowers