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Original Article

Antioxidant activity in leaf extracts of *Michelia champaca* L.

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ABSTRACT

Michelia champaca L. belongs to the family *Magnoliaceae*. It is commonly known as swarnachampa or kanakchampa. The plant is traditionally used for the treatment of fever, colic, leprosy, cough, and rheumatism. Reactive oxygen species-induced oxidative stress is associated with the chronic diseases such as cancer, coronary heart disease, and osteoporosis. Antioxidants are molecules that inhibit free-radical reactions and prevent cellular damage. In the present study, antioxidant activity of *M. champaca* L. leaves was undertaken with three extracts, i.e., ethanol, methanol, and aqueous. Results revealed that ethanol extract of *M. champaca* L. has potent antioxidant ability of 49.14% at 150 µg/ml concentration in DPPH and the inhibition concentration 50% value of ethanol extract was found to be lowest at 5.41 µg/ml as compared to standard 4.59 µg/ml. A good correlation was also found to exist between concentration of extract and percentage inhibition with values $r^2 = 0.998$. In reducing power assay percentage inhibition of ethanol extract was 39.12% at 150 µg/ml concentration and a good correlation was found to exist between concentration of extract and percentage inhibition with value $r^2 = 0.967$.

Keywords: Medicinal plant, *Michelia champaca* L., oxidative stress, reactive oxygen species, swarnachampa

Introduction

Michelia champaca L. is an evergreen tree from family *Magnoliaceae* with various medicinal and traditional uses. Plant parts are useful in nausea, burning sensation, hemolysis, skin diseases, leprosy, ulcers, gout, cough, bronchitis, dysmenorrhea, malarial fever, etc. Its common names are kanakchampa, swarnachampa, champaka, and golden champa.

Medicinal plants play a pivotal role in the health care of ancient and modern cultures. Ayurveda, the Indian system of medicine mainly uses plant-based drugs or formulations to treat various human ailments because they contain the components of therapeutic value.[1]

Reactive oxygen species such as superoxide anion, hydroxyl radical, and hydrogen peroxide play a crucial role in the development of various ailments such as arthritis, asthma, dementia, mongolism, carcinoma, and Parkinson's disease. The free radicals in the human body are generated through aerobic respiration or from exogenous sources.[2]

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Antioxidants are phytochemicals, vitamins, and other nutrients that protect our cells from damage caused by free radicals. It helps fight oxidation, a normal chemical process that takes place in the body every day. Antioxidants with radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated.[3]

Several researchers have evaluated the antioxidant potential of plants using 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) and reducing power assay. In DPPH assay, the antioxidant potential is expressed as inhibition concentration 50% (IC_{50}) value. It is the concentration of extract required to scavenge 50% of DPPH molecules. The extract which shows lowest IC_{50} value is considered to be most potent antioxidant. In reducing power assay antioxidant potential is expressed as percentage (%) inhibition, i.e., how much inhibition of free radicals is found at different concentrations of the plant extract.

Kumar *et al.* performed DPPH radical scavenging activity and reducing power assay on flowers of *M. champaca* L.[4] The antioxidant activity of extracts increases with increase in concentration. Ethanol extract of flowers has the highest percentage inhibition 90.20% in comparision with Gallic acid and ascorbic acid. Ananthi and Chitra investigated that the methanolic extract of *M. champaca* L. flowers showed effective free-radical scavenging at 300 µg/ml.[5] Hasan *et al.* studied on 15 Bangladeshi medicinal plants which are traditionally used in different ailments are evaluated for antioxidant potential. *M. champaca* L. was one of the 15 plants, IC_{50} value reported here was at 22.43 µg/ml.[6]

Antioxidant activity of leaf of *M. champaca* L. was also done earlier in 2009. The IC_{50} values of extract and ascorbic acid were found to be 30.07 and

15.42 µg/ml, respectively. Percentage scavenging activity of DPPH radical was found to rise with increasing concentration of the crude extract.[7]

The study done on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems.[8] Phenolic compounds from medicinal plants possess strong antioxidant activity and may help protect the cells against the oxidative damage caused by free radicals.[9]

Various phytochemicals were reported in the leaves of *M. champaca* L. It was observed that phytochemicals present in the ethanol extract of leaves are alkaloids, flavonoids, glycosides, carbohydrates, amino acids, and tannins.[10]

Materials and Methods

Collection of plant material

Leaves of *M. champaca* L. were collected from Rishabh Dev Udyan, Shahpura, Bhopal and identified by senior botanist of Bhopal with specimen code 1111-4.01-05 and confirmed by Botanical Survey of India, Allahabad.

Preparation of extract

M. champaca L. leaves were shade dried and grinded in mixer. The powdered leaves (50 g) were extracted with 230 ml of solvents (ethanol, methanol, and aqueous) by soxhlet apparatus for 3 days. Then, the extracts were placed in hot air oven at 30-40°C, for obtaining in powdered form. The powdered form of the extract was then stored in refrigerator until further examination.

Antioxidant assays

Antioxidant activity of plant material was tested by following two methods, which are as discussed below.

Scavenging assay by DPPH

The antioxidant activity of the plant extract was examined on the basis of the scavenging effect on the stable DPPH free-radical activity.[11] Ethanolic solution of DPPH (0.05 mM) was added to 40 µl of extract solution with different concentrations. Ethanol 96% (2.7 ml) was added, and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance at zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following formulae.[12]

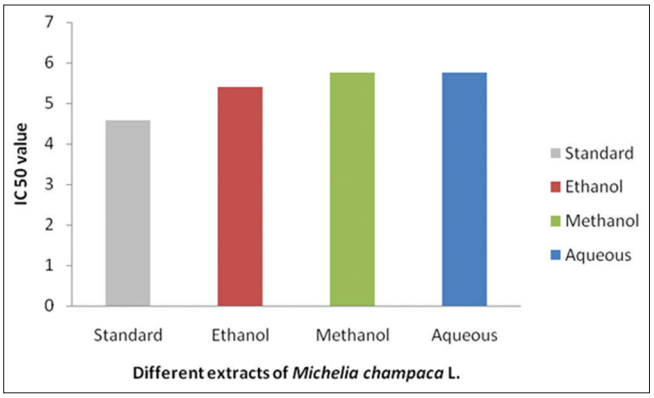
Percentage (%) inhibition of DPPH activity = $[(A_B - A_A)/A_B] \times 100$

Where, A_A and A_B are absorbance values of the test and the blank sample, respectively.

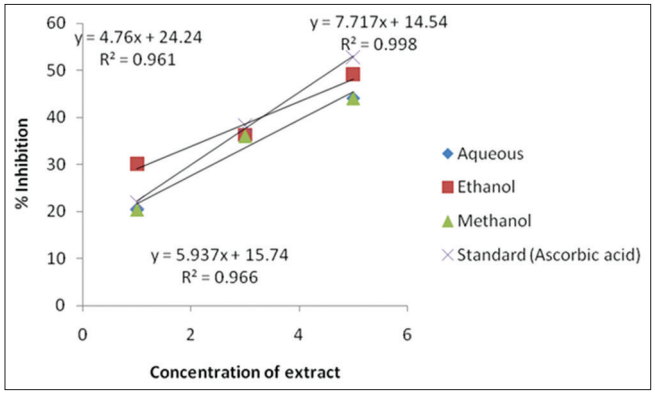
The IC_{50} value of each extract was also calculated using the line graph between concentration and % inhibition.

Reducing power assay

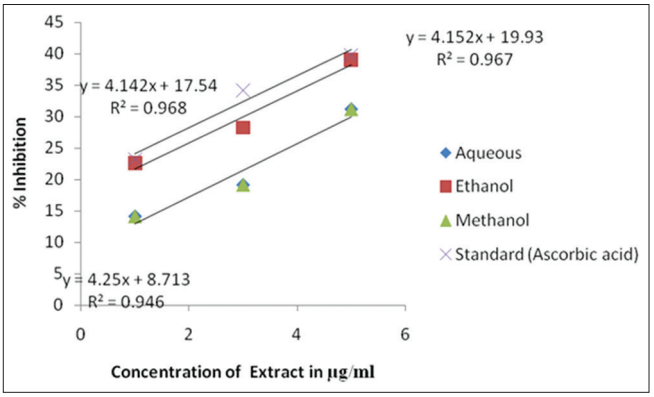
The reducing power of extracts and positive controls were determined according to the method of Yen and Chen.[13] They were each mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6, and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min before an equal volume of 1% trichloroacetic acid was added, and then, centrifuged at 5000 rotations/min (rpm) for 10 min. The upper layer of the solution was mixed with distilled water and 0.1% $FeCl_3$ with a ratio of 1:1:2 and the absorbance were measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.



Graph 2: Comparative graph of inhibition concentration 50% values of different extracts of *Michelia champaca* L.



Graph 1: Scavenging activity of different extracts of *Michelia champaca* L. in 2, 2'-diphenyl-1-picrylhydrazyl assay



Graph 3: Reducing power activity of different extracts of *Michelia champaca* L.

Results and Discussion

Free radicals are constantly generated resulting in extensive damage to tissues and biomolecules leading to various diseases such as neurodegenerative diseases, cancer, and AIDS. Medicinal plants are employed as an alternative source of medicine to mitigate the diseases associated with oxidative stress.

DPPH scavenging assay

DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging activity of extracts. As antioxidants donate proton to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. In this test, three extracts of leaves were used, i.e., aqueous, ethanol, and methanol. Concentrations of each sample used were 50, 100, and 150 µg. The total antioxidant activity of each extract was concentration dependent, i.e., with the increasing concentration of extract the activity also increased; it is shown in the Graph 1. Out of all the three extracts, highest percentage inhibition was shown by ethanol extract as compared to standard and lowest shown by both methanol and aqueous extracts with same values in all three concentrations. Graph 2 shows the amount of each extract that was required for 50% inhibition of DPPH, i.e., IC_{50} value. The IC_{50} values of different extracts were in the order: Standard, ethanol, methanol, or aqueous. Lowest IC_{50} value was shown by ethanol extract at 5.41 µg/ml which was very close to the IC_{50} of standard ascorbic acid at 4.59 µg/ml. Similarly, low and equal value of IC_{50} (5.76 µg/ml) was shown by methanol and aqueous extracts.

Reducing power assay

In reducing power assay, maximum % inhibition was shown by ethanol extract of *M. champaca* L. out of the three extracts as shown in Graph 3. Earlier phytochemical studies shows that the plant is rich in alkaloids, glycosides, carbohydrates, amino acids, flavonoids in ethanol extract and contains tannins, amino acid, flavonoids in aqueous extract.[14] Presence of these phytochemicals in the extracts, which are considered to be strong antioxidants, confirms the presence of antioxidant activity in the plant.

Conclusion

From the above results, it was concluded that the ethanolic, methanolic, and aqueous extracts of *M. champaca* L. exhibited antioxidant and free-radical scavenging activity. *In vitro* assays indicated that these plant extracts are significant source of natural antioxidants which will be beneficial for preventing the progress of various oxidative stress related diseases. Literature review has shown that very few studies on antioxidant potential of *M. champaca* L. leaves has been undertaken on these three extracts, and to the best of our knowledge till now, no such study on antioxidant potential in leaf of *M. champaca* L. by DPPH assay and reducing power assay has been undertaken. Among the three extracts studied, the IC_{50} value of ethanol extract was found to be lowest and it was very close to the IC_{50} value of standard. As far as the IC_{50} value of leaves is concerned in these extracts, it is the lowest value (i.e., minimum amount of extract was able to scavenge 50% of DPPH molecules) reported till now. These *in vitro* results should be confirmed *in vivo*.

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