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Research Article

ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF *MICHELIA CHAMPACA* L. FLOWERS

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ABSTRACT

Michelia champaca L. is an evergreen, small to medium sized tree. It is commonly known as Swarna champa or Kanak champa. Present study was conducted to test the antioxidant potential and phytochemical analysis of its flowers. Antioxidant activity was evaluated *in-vitro* by DPPH radical scavenging assay and reducing power assay. Highest activity was shown by methanol extract of flowers at 150µg/ml concentration in both DPPH assay and reducing power assay. Inhibition concentration 50% value of methanol was found to be 1.73 µg/ml and which is similar to standard ascorbic acid. A good correlation was also found to exist between percentage inhibition and concentration of extract with value $r^2 = 0.9951$ and $r^2 = 0.9357$ in DPPH and reducing power assay respectively. Phytochemical analysis of the methanol extract showed the presence of carbohydrates, alkaloids, flavonoids, triterpenoids, steroids and tannins.

Keywords: Antioxidant, Medicinal plants, *Michelia champaca* L., Phytochemical

INTRODUCTION

Medicinal plants are used as medicines from ancient times. Medicinal plant parts generally having phenolic compounds such as flavonoids, phenolic acids, tannins, coumarins, lignans and lignins in abundance. These compounds have several biological properties along with antioxidant activity.[1] Out of the major causative factors for the initiation of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases oxidative stress is one of them.[2] Oxidative stress is the condition, where the production of reactive oxygen species becomes greater than that of antioxidant defence molecules in the living system. The most effective path to diminish the action of free radicals which causes the oxidative stress is antioxidative defence mechanisms. The substances that either interrupt or inhibit the oxidation of molecules like lipids by inhibiting the initiation of oxidative chain reactions thus, preventing and repairing the damage caused by the reactive oxygen species to various cells are called as antioxidants.

Phytochemicals in plants especially flavonoids and polyphenols have been reported to prevent the free radical chain reactions, to save human body from various ailments.[3] In recent years the role of some secondary metabolites as protective dietary constituents has become an increasingly important area of human nutrition research. Antioxidant activity in medicinal plants is chiefly because of the presence of secondary metabolites.[4]

Michelia champaca L. is an evergreen or semi-deciduous tree from family Magnoliaceae. It is called Son champa or Swarna champa in common because of its golden yellow flowers. Flowering occurs in summer chiefly in April month and fruiting in winter season. Root and root bark of the plant are useful in the cure of abscesses, inflammation, constipation, amenorrhoea and dysmenorrhoea and are strong laxative and emmenagogue. On the other hand flower, flower buds and fruits have various properties like astringent, acrid, refrigerant, cardiotonic, haemostatic, digestive, carminative, depurative, anthelmintic, diuretic, expectorant, stimulant and antipyretic etc. according to the book "The Tretise of Indian Medicinal plants"[5]

Preliminary phytochemical analysis of various extracts of *Michelia champaca* L. leaves and flowers showed the presence of alkaloids, tannins, glycosides, carbohydrates, amino acids, flavonoids and sterols.[6] Antibacterial and free radical scavenging activity of *Michelia champaca* L. was done on its flowers. In this study hexane and ethyl acetate extract showed strong antioxidant activity with IC_{50} values of both extracts were found to be 10µg/ml and 250 µg/ml respectively.[7] Studies on antioxidant activity of its flowers showed effective free radicle scavenging activity at 300µg concentration.[8] The present study was undertaken to investigate the *in-vitro* antioxidant activity and phytochemical screening of *Michelia champaca* L. flowers.

MATERIAL AND METHOD

Plant collection, identification and extraction

Fresh flowers of *Michelia champaca* L. were collected from Rishabh Dev Udyaan, Shahpura, Bhopal. Plant was identified by senior botanist of Bhopal and confirmed by Botanical Survey of India, Allahabad with specimen code 1111-4.01-05. Collected flowers of *Michelia champaca* L. were shade dried at room

temperature. Dried plant material was grinded in mixer grinder into fine powder, and then 50g powdered plant material was extracted with 230ml of solvents (methanol, ethanol, aqueous) by Soxhlet apparatus for 72 hours. After this extracts were placed in hot air oven at 30-40°C. Remaining powdered form of the extracts was placed in refrigerator for further analysis.

Antioxidant activity

2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of the plant extract was examined on the basis of the scavenging effect on the stable DPPH free-radical activity.[9] 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.[10]

$$\text{Percentage (\%) inhibition of DPPH activity} = [(AB - AA)/AB] \times 100$$

Where, AA and AB are absorbance values of the test and the blank sample, respectively. The IC50 value of each extract was also calculated using the line graph between concentration and % inhibition. It is the concentration of extract required to scavenge 50% of DPPH molecules.

Reducing power assay

The reducing power of extracts and positive controls were determined according to the following method.[11] 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% FeCl3 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.

Phytochemical Analysis

It has been proved in earlier studies that phytochemicals are responsible for the antioxidant activity in plants. Therefore, phytochemical screening of the most powerful extract in antioxidant activity testing out of three i.e. ethanol, methanol and aqueous was done to find out the different phytochemicals present in the extract or to determine the presence or absence of carbohydrate, protein, alkaloids, flavonoids, triterpenoids, steroids, and tannins. These phytochemicals have their specific role like carbohydrates are widespread and act as reserve food material like starch in tubers, grains and roots.[12] Proteins and amino acids function as catalyst for biological reactions while some act as structural materials. Alkaloids functions as detoxicating agents by removing those compounds whose accumulation might cause damage to the plants as well as they act as reservoirs of proteins synthesis.[13] Flavonoids are phenolic compounds and secondary metabolites abundantly present in plants. They can exert their antioxidant activity by inducing the activities of antioxidant enzymes and by scavenging free radicals. Triterpenoids, steroids and tannins mainly serve as protective function in repelling insect and microbial attack.[14]

RESULT

Methanol, Ethanol and Aqueous extracts of *Michelia champaca* L. flowers were used to investigate the antioxidant activity. In DPPH assay, antioxidant potential of methanol extract of flowers at 150µg/ml concentration was highest as compared to ethanol and aqueous extracts (Fig.1.). A good correlation was also found to exists between percentage inhibition and concentration of extract with $r^2 = 0.9951$. Inhibition concentration 50% value of methanol was found to be lowest i.e. 1.73µg/ml as compared to standard ascorbic acid i.e. 1.73µg/ml (Fig.2.). In reducing power assay, methanol extract of flower at 150µg/ml concentration shows highest antioxidant activity as compared to ethanol and aqueous extracts. Lowest activity was shown by aqueous extract of flowers. A good correlation was also exists between concentration of extract and % inhibition with value $r^2 = 0.9387$. (Fig. 3). The result of qualitative phytochemical screening of methanol extract of *Michelia champaca* L. flowers showed the presence of carbohydrate, alkaloids, flavonoids, triterpenoids, steroids and tannins (Table 1.)

DISCUSSION

DPPH is a stable free radical at room temperature. It has a characteristic absorption at 517nm, as antioxidants donate protons to these radicals, the absorbance decreases. This decrease in absorbance is taken as a measure of the extent of radical scavenging. From the above results it was found that the total antioxidant activity of methanol extract of flowers was concentration dependent i.e. antioxidant activity increases with the increasing concentration of the extract. Inhibition concentration 50% value (IC_{50}) of all the three extracts and standard are in the order of standard, methanol, ethanol and aqueous. 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.[15] Accordingly, preliminary phytochemical analysis of methanol extract of *Michelia chamapca L.* showed the presence of flavonoids which may react with the free radicals to stabilize and terminate from free radical chain reaction. This is similar to the earlier investigation[16], in which it has been proved that the presence of flavonoids in the methanol extract of the *Michelia champaca* L. flowers justifies the antioxidant potential of the plant which brings about its free radical scavenging potential. These in vitro results should be confirmed in vivo as this activity of the plant may be useful in combating various diseases related to oxidative stress, and to make an effective medicine from this plant.

CONCLUSION

From the above study, it is concluded that the *Michelia champaca* L. flower extracts showed strong antioxidant activity in DPPH and reducing power assays. Percentage inhibition was found directly proportional to concentration of the plant extract. Phytochemical screening showed the presence of flavonoids in the methanol extract of flowers, which indicate and supports its potent antioxidant activity. Therefore, methanol extract proved to be superior and most powerful antioxidant as compared to aqueous and ethanol. These *in vitro* results should be confirmed *in vivo*.

Table 1. Phytochemical screenings of Methanol extract of *Michelia champaca* L. flowers.

Phytochemicals	Result
Alkaloids	Positive
Terpenoids	Positive
Flavonoids	Positive
Tannins	Positive
Steroids	positive
Protein	negative
carbohydrate	positive

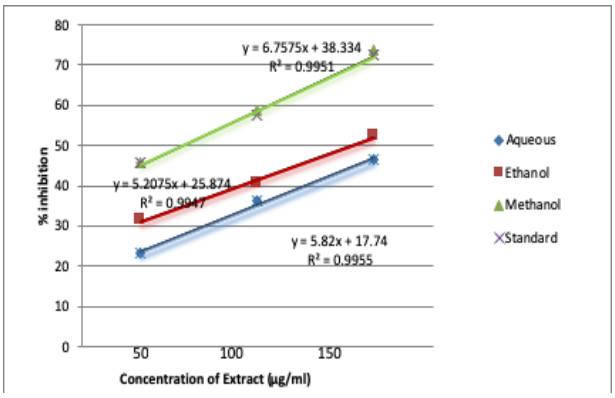

Fig. 1. Antioxidant activity of different extracts of *Michelia champaca* L. flower in DPPH radical scavenging assay.

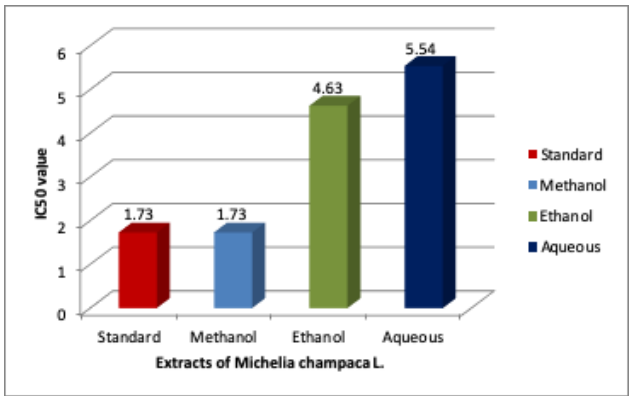

Fig.2. Comparison between the IC_{50} values of different extracts of *Michelia champaca* L. flowers

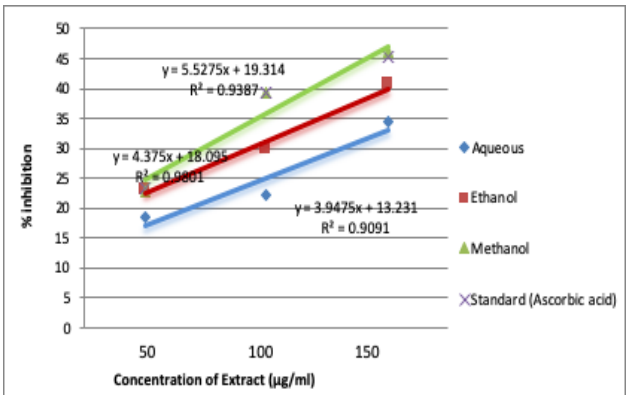

Fig.3. Antioxidant activity of different extracts of *Michelia champaca* L. flower in reducing power assay.

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