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Antioxidant, analgesic and cytotoxic activity of *Michelia champaca* Linn. Leaf

Md. Mokarram Hossain[1*], Rumana Jahangir[1], S. M. Raquibul Hasan[1], Raushanara Akter[1], Taksim Ahmed[2], Md. Imamul Islam[2], Abdullah Faruque[2].

Department of Pharmacy, Stamford University Bangladesh[1]
51 Siddeswari Road, Dhaka-1217
Department of Pharmacy, Jahangirnagar University [2]
Savar, Dhaka-1342

***Corresponding Author**

Md. Mokarram Hossain
Lecturer, Dept. of Pharmacy
Stamford University Bangladesh
Contact no.: +8801727348428
E-mail: smmh1123@yahoo.com

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ABSTRACT

Michelia champaca (Magnoliaceae) is a large medicinal plant which is traditionally used against a number of diseases including inflammatory conditions. In the present study crude methanol extract of *M. champaca* leaf was investigated for possible antioxidant, analgesic and cytotoxic activity. The extract showed remarkable antioxidant activity in DPPH radical scavenging activity, nitric oxide scavenging activity and total antioxidant capacity assays. In both DPPH radical and NO scavenging assay, the extract exhibited strong antioxidant activity and the IC_{50} values in DPPH radical scavenging and NO scavenging assays were found to be 30.07 µg/ml and 15.42 µg/ml, respectively while the IC_{50} values of ascorbic acid were 12.5 µg/ml and 4.07 µg/ml, respectively. Total antioxidant activity of the extract increased in a dose dependent manner. Analgesic activity of the crude extract was evaluated using acetic acid-induced writhing model of pain in mice. The crude extract at 200 mg/kg and 400 mg/kg b.w doses displayed significant ($p < 0.001$) reduction in acetic acid induced writhing in mice with a maximum effect of 68.7 % reduction at 400 mg/kg b.w. which is comparable to the standard, diclofenac sodium (78.2 %). The extract was also investigated for cytotoxic potential using Brine Shrimp lethality bioassay. In this bioassay the extract showed significant toxicity to Brine Shrimp nauplii with the LC_{50} value of 11.22 µg/ml. Results of the present study suggest that *M. champaca* leaf extract possesses strong antioxidant, analgesic and cytotoxic activity.

Key words: *Michelia champaca*, Magnoliaceae, Antioxidant, Analgesic, Cytotoxicity.

INTRODUCTION

In the past few years, there has been growing interest in the involvement of reactive oxygen species (ROS) in several pathological situations like cancer, chronic pain, cardiovascular diseases and arthritis. ROS produced *in vivo* include superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). H_2O_2 and $O_2^{\bullet-}$ can interact in the presence of certain transition metal ions to yield a highly-reactive oxidizing species, the hydroxyl radical ($OH^{\bullet}$) (Aruoma et al., 1989). The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease (Liao and Yin, 2000). Although the body possesses such defense mechanisms, as enzymes and antioxidant nutrients, which arrest the damaging properties of ROS (Halliwell et al., 1995; Sies, 1993), continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them, and cause irreversible oxidative damage (Tseng et al., 1997). Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (Soares et al., 1997). In this respect, polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity (Brown and Rice-Evans, 1998). Restriction is being imposed on the use of synthetic antioxidants because of their carcinogenicity, the need for natural antioxidants therefore become imperative and desirable (Behera et al., 2006; Grice, 1986; Wichi, 1988). Therefore as sources of natural antioxidants much attention is being paid to plants and other organisms. Thus interest in natural antioxidant, especially of plant origin, has greatly increased in recent years (Jayaprakash and Rao, 2000).

Michelia champaca (Family: Magnoliaceae), locally known as Swarna Champa, is a tree with golden-yellow fragrant flowers and aggregate fruits, grows wild in Chittagong Hill Tracts and planted in gardens in other areas of Bangladesh (Ghani, 2003). Previous investigations on the plant have revealed that it possesses anti-inflammatory, antimicrobial and leishmanicidal activity (Vimala et al., 1997; Khan et al., 2002; Takahashi et al., 2004). Recently a sesquiterpene lactone, parthenolide having anticancer activity has been isolated from ethanol extract of bark of *M. champaca* (Hoffmann et al., 1977). Flowers and fruit contain essential oil; bark contains alkaloids and beta-sitosterol (Ghani, 2003; Vimala et al., 1997). As part of our ongoing investigations on local medicinal plant of Bangladesh (Hossain et al., 2008) in this paper we are reporting antioxidant, analgesic and cytotoxic activity of the leaves of *M. champaca*.

MATERIALS AND METHODS

Chemicals

DPPH (1, 1-diphenyl, 2-picrylhydrazyl) was obtained from Sigma Chemical Co. USA. Ascorbic acid was obtained from SD Fine Chem. Ltd., Biosar, India Ammonium molybdate and sodium nitroprusside were purchased from Merck, Germany and ethylene diamine tetra acetic acid (EDTA), sodium phosphate, sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride from BDH, England.

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Plant material

Leaves of *M. champaca* were collected from Siddeswari, Dhaka in June 2008 and identified by the experts of National Herbarium, Mirpur, Dhaka, Bangladesh (Accession No. 32615) where a voucher specimen is deposited for future reference. Immediately after collection, the leaves were thoroughly washed with water and dried under shade at room temperature.

Extraction

The dried leaves were coarsely powdered. 90 gm powder of leaves was extracted with methanol by a Soxhlet apparatus at 65◦C. The solvent was completely removed and obtained 15 gm (yield 16.67 %) dried crude extract. This crude extract was used for investigation.

Test Animals

Male Swiss albino mice (20–25g) were used in this investigation. Animals were obtained from the animal house of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions and had free access to feed and water ad libitum. They were acclimatized to laboratory conditions for one week before experimentation. Experiments on animals were performed based on animal ethics guidelines (Zimmermann, 1983) and approved by Institutional Animal Research Ethics Committee.

Antioxidant activity

DPPH radical scavenging activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical was determined by the method described by Braca et al. (2001). Plant extract (0.1 ml) was added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517nm was determined after 30 min, and the percentage inhibition activity was calculated from [(A0–A1)/A0] x100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/ standard. IC50 values for the extract and standard were obtained from probit analysis of the respective percentage scavenging of DPPH radical.

Assay of Nitric oxide scavenging activity

The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM) in phosphate buffered saline was mixed with different concentrations of methanol extract of M. champaca was dissolved in methanol and incubated at room temperature for 150 min. The same reaction mixture without the methanol extract but the equivalent amount of methanol served as the control. After incubation, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H3PO4 and 0.1%

N-(1-naphthyl) ethylenediamine dihydrochloride was added. The absorbance of the chromophore formed was read at 546 nm (Garat, 1964).

Determination of total antioxidant capacity

The total antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto et al. (1999). The assay is based on the reduction of Mo (VI)–Mo (V) by the extracts and subsequent formation of a green phosphate/Mo (V) complex at acid pH. 0.3 ml extracts were combined with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 1C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Antinociceptive activity

Acetic acid-induced writhing test

The 'acetic acid-induced writhing' test used in this study was described earlier by Koster et al. (1959). Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III, group-IV, consisting of 5 mice in each group. Each group received a particular treatment i.e. control, standard and the two doses of the extract. Group-I served as the control and received 1% tween-80 in distilled water, Group-II received diclofenac–Na (10 mg/kg b.w., i.p), the standard drug to compare the antinociceptive activity of the extract. The last two groups i.e. Group-III & Group-IV were administered the crude extract suspension 200 mg/kg and 400 mg/kg, b.w., p.o., respectively. Thirty minutes after drug treatment each group was treated with intraperitoneally administered 0.2 ml of 0.7% acetic acid solution (Koster et al., 1959). Five minutes after acetic acid administration, the number of writhes (i.e., abdominal contractions and stretches) were counted for the next ten minutes and recorded. The recorded numbers of acetic acid-induced writhes that occurred in the standard and test group were compared with those in the control group.

Brine Shrimp lethality bioassay

For Brine Shrimp lethality bioassay the eggs of Brine Shrimp (*Artemia salina* Leach) were collected and hatched in a tank at a temperature around 37◦ C equipped with constant oxygen supply for 24 hours. Stock solutions of both the samples were prepared by dissolving required amount of extract in specific volume of pure dimethyl sulfoxide (DMSO). Thirty six clean vials were taken; eighteen of these were for the extract in nine concentrations (two vials for each concentration) and eighteen for control test. 4 ml of seawater was given to each of the vials. Then with the help of micropipette specific volumes of samples were transferred from the stock solutions to the vials to get final sample concentrations of 1.25, 2.5, 5, 10, 20, 40, 80, 160 and 320 µg/ml. The concentration of DMSO in these vials should not exceed 40µl per 4 ml of Brine Shrimp nauplii because above this concentration DMSO may become toxic to the nauplii (Meyer et al., 1982; Mclaughioin, 1990). In the control vials same volumes of DMSO (as in the extract vials) were taken. With the help of a Pasteur pipette 10 living nauplii were put to each of the vials. After 24 hours, the vials were observed and the numbers of survived nauplii in each vial were counted and the results were noted. From this observation, the percentage of lethality of Brine Shrimp nauplii was calculated for each concentration of the extract.

RESULTS

DPPH radical scavenging activity

Results of the DPPH radical scavenging assay is given in Figure 1. The IC50 values of the extract and Ascorbic acid were found to be 30.07 µg/ml and 15.42 µg/ml respectively. % scavenging of DPPH radical was found to rise with increasing concentration of the crude extract (Figure 1).

Nitric oxide radical inhibition assay

The scavenging of nitric oxide by the plant extract was increased in a dose-dependent manner as illustrated in Figure 2. 50% of nitric oxide generated by incubation was scavenged by the extract at a concentration of 15.42 µg/ml whereas the concentration of reference standard (Ascorbic acid) to scavenge 50% nitric oxide was 4.07 µg /ml.

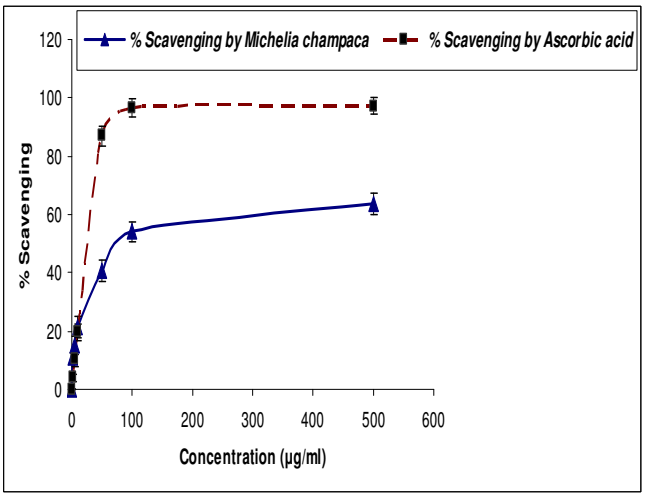

Figure 1: DPPH radical scavenging activity of crude methanol extract of *Michelia champaca* Leaf (Values are the average of duplicate experiments and presented as mean ±SD)

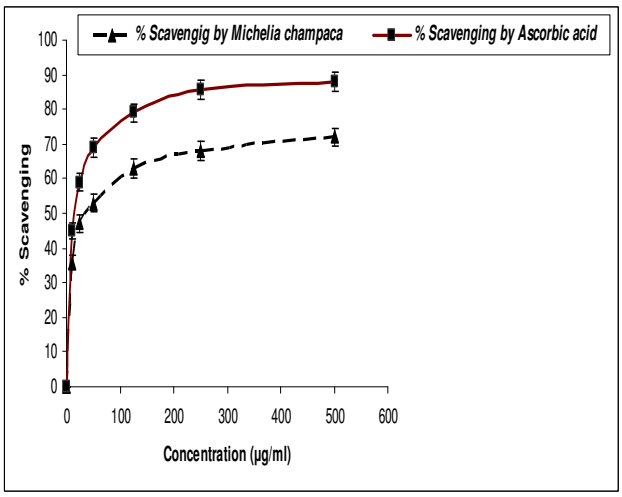

Figure 2: Nitric oxide scavenging activity of crude methanol extract of *Michelia champaca* Leaf (Values are the average of duplicate experiments and presented as mean ± SD)

Total antioxidant capacity

Total antioxidant capacity of the extract is given in Figure 3. Total antioxidant capacity is expressed as the number of equivalents of ascorbic acid (AAE).

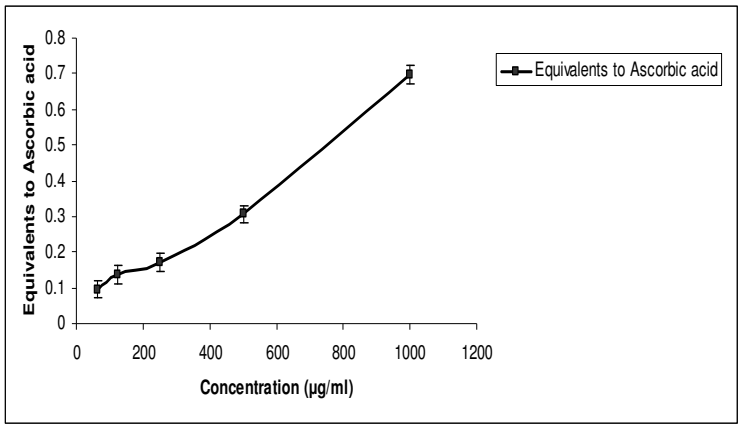

Figure 3: Total antioxidant capacity of crude methanol extract of *Michelia champaca.* Leaf (Values are the average of duplicate experiments and presented as mean ± SD)

Acetic acid-induced writhing test

Crude extract at both dose levels produced significant ($p < 0.001$) and dose dependent analgesic effect (Table 1). Maximum inhibition of writhing response was exhibited by the extract at 400 mg/kg was 68.7 % which was comparable to that of diclofenac sodium at 10 mg/kg (78.2%).

Table 1: Effect of *M. champaca* leaf extract on acetic acid induced writhing in

Treatment	Route of administration	No of Writhing	% Inhibition
Group-I	Per oral	42.2±2.51	--
Group-II	Intraperitoneal	9.2±1.92**	78.2
Group-III	Per oral	26.0±1.92**	38.4
Group-IV	Per oral	13.2±1.73**	68.7

Values are presented as mean ±SEM (n = 5); ** $p < 0.001$, Dunnet test as compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received Diclofenac-Na 10 mg/kg body weight, Group III and IV were treated with 200 and 400 mg/kg body weight of the methanol extract of *Michelia champaca*

Brine Shrimp lethality bioassay

The result of Brine Shrimp lethality bioassay is given in Table 2. *M. champaca* leaf extract displayed strong cytotoxic activity. LC50 value for the extract was found to be 11.22 µg/ml.

Table 2: Brine shrimp lethality bioassay of crude methanol extract of *Michelia champaca.* leaf

Test solution	Conc. (µg/ml)	Log Conc.	% Mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
Michelia champaca Leaf extract	1.25	0.097	30	11.22	199.53
	2.5	0.398	30		
	5	0.699	40		
	10	1	45		
	20	1.301	50		
	40	1.602	70		
	80	1.903	75		
	160	2.204	85		
	320	2.505	100		

DISCUSSION

Most of the free radical production within the body involves oxygen, and thus the free radicals are often referred to as reactive or reduced oxygen species. Presence of free radicals in the body may cause cell and tissue damage. This sort of damage is known as oxidative damage. (Halliwell and Gutteridge, 1989; Halliwell, 1996). Several mechanisms for the production of free radicals in the body have been proposed. The mitochondria and ischemia-reperfusion injury have been areas of focus. Free radicals cause cellular damage by reacting with the phospholipid bilayer of cellular membranes. This reaction results in the production of measurable end products, primarily malondialdehyde. The most effective way to eliminate free radicals is with the help of antioxidant nutrients such as ascorbic acid (vitamin C), alpha-tocopherol (vitamin E) and beta-carotene (vitamin A) which can be found in vast amounts in fruits and vegetables. Literature review about the plant confirms the presence of polyhydric phenolic compounds, flavonoids, sesquiterpine etc. Any of these phytoconstituents can be responsible for the antioxidant activity of the crude extract. Phenolic compounds are commonly found in both edible and inedible plants and they have been reported to have multiple biological effects, including antioxidant activity. Phenolic compounds and flavonoids have also been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals (Rice-Evans et al., 1997; Jorgensen et al., 1999). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994).

The writhing test is generally used for screening of antinociceptive effects (Koster *et al.*, 1959). With respect to the writhing test, the research group of Deraedt *et al.* (1980) described the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid. They found high levels of prostaglandins PGE_2 and $PGF_{2\alpha}$ during the first 30 min after acetic acid injection. Nevertheless, it was found that the intraperitoneal administration of acetic acid induces the liberation not only of prostaglandins, but also of the sympathetic nervous system mediators (Hokanson, 1978). The methanol extract of *M. champaca* showed significant inhibition on acetic acid-induced writhing response compared to reference drug diclofenac sodium (10 mg/kg) in mice. Diclofenac sodium reduces inflammation, swelling and arthritic pain by inhibiting prostaglandins synthesis (Small, 1989). Result of this test suggests that the extract might possess chemical constituent that have the capability to inhibit prostaglandin synthesis.

The extract also showed significant cytotoxicity on Brine Shrimp nauplii. This may be due to the fact that *M. champaca* contains parthenolide, a sesquiterpene, which was previously reported to possess antimicrobial and leishmanicidal activity (Takahashi et al., 2004; Khan et al., 2002).

CONCLUSION

The study clearly indicates that the extract possesses antioxidant and cytotoxic substances. At the same time its ability to suppress abdominal writhes confirms the analgesic property of the extract. These findings justify the traditional uses of this plant in the treatment of diabetes, wounds, inflammatory conditions, worms, infestations and malarial fever. Further research is necessary for elucidating the active principles.

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