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In vitro antioxidant and antinociceptive potentialities of methanolic extract of *Litsea glutinosa*

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Abstract

The methanolic extract of *Litsea glutinosa* (Lauraceae) was evaluated for *in vitro* antioxidant activity by determination of hydrogen peroxide scavenging activity, total antioxidant capacity, assay of nitric oxide scavenging activity and reducing power test and *in vivo* antinociceptive effect in acetic acid induced writhing model in swiss albino mice. The results revealed the presence of pronounced antioxidant property as compared with ascorbic acid used as standard and a dose-dependent (250 and 500 mg/kg) analgesic effect. The antioxidant and antinociceptive properties observed seem to be in good accordance with the traditional uses of *Litsea glutinosa.*

Keywords: *Litsea glutinosa*; Lauraceae; Antioxidant; Antinociceptive; Swiss albino mice.

Introduction

The use of plants as a source of raw materials for medicine is an ancient one. The importance of plants in medicine remains even of greater relevance with the current global shift to obtain drugs from plant sources, as a result of which attention has been given to the medicinal value of herbal remedies for safety, efficacy and economy (Glombitza *et al.* 1993). Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants (Cox and Balick, 1994). The plants that are used in traditional medicines are likely and in some cases already known, to contain pharmacologically active compounds, which differ widely in terms of structures and therapeutic properties. Therefore considerable importance has been placed on the screening of these plants for active or lead compounds. Evaluation of the local flora exploited in traditional medicine for various biological activities is a necessary step in the isolation and characterization of the active principles and further leading to drug development (Rahman *et al.* 2011). A large number of plants have been screened as a viable source of natural antioxidants including tocopherols, vitamin C, carotenoids and phenolic compounds which are responsible for the maintenance of health, helping the human body to reduce oxidative damage and the protection from coronary heart diseases and cancer (Yanga *et al.* 2002). In view of these *Litsea glutinosa* was studied for its potential antioxidant and analgesic effects.

Litsea glutinosa (Common name: Indian laurel, Bengali name: Menda pata; Family- Lauraceae) is a moderately sized tree which grows to a height of about 20 to 30 feet. It is found mainly in India and South China to Malaysia, Australia and the Western pacific islands. Previous phytochemical studies revealed the presence of alkaloids (Hart *et al.* 1969; Bhakuni and Gupta, 1983; Holloway and Scheinmann, 1973), butanolides (Cheng *et al.* 2001; Chen *et al.* 1998), and sesquiterpenoids (Hoang *et al.* 2002; Zhang *et al.* 2001; Zhang *et al.* 2003a; Zhang *et al.* 2003b) in the genus *Litsea.* Few records are found in the literature with respect to the isolation of flavonoids from *Litsea* species (Wang *et al.* 2009). Its bark and leaves are used as a demulcent and mild astringent for diarrhea and dysentery and the roots are used to poultice sprains and bruises (Wang *et al.* 2010). In this paper, the antioxidant and the antinociceptive properties of the methanolic extract of *Litsea glutinosa* are being reported to justify the traditional use of this plant through *in vitro* and *in vivo* evaluation.

Materials and methods

Plant materials

Leaves of *Litsea glutinosa* were collected from Sylhet in June 2008 and were identified by Ms. Hosne Ara, Director,

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Bangladesh National Herbarium (BNH), Mirpur, Dhaka. A voucher specimen was prepared and deposited at BNH (DACB accession no: 32777).

Extraction of plant materials

About 140 g of dried and powered leaves was soaked in 1 L of 90% methanol for several days. The extract was filtered and concentrated to a gummy mass (6 g). Different chemical tests revealed the presence of alkaloids, flavonoids, saponins and tannins (Evans, 1989) in this extract.

Antioxidant properties

i) Qualitative assay

A suitably diluted stock solutions (sample solutions) were spotted on pre-coated Silica gel TLC (Thin layer chromatography) plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract and to choose the solvent system in which stock solutions run well. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the color changes (yellow on purple background) were noted (Sadhu *et al.* 2003).

ii) Quantitative assay

Free radical scavenging activity of the methanol extract was evaluated by determination of total antioxidant capacity, assay of nitric oxide scavenging activity and reducing power test. In all methods ascorbic acid is used as standard.

Scavenging of hydrogen peroxide

The ability of the methanolic extract of *Litsea glutinosa* to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.* (1989). A solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm with molar absorbtivity 81 $(mol/l)^{-1}$ cm^{-1} in a spectrophotometer (Hach, DR-4000U). Extracts (50-250 mg/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. For each concentration, a separate blank sample was used for background subtraction. The percentage of scavenging of hydrogen peroxide of methanolic extract of *Litsea glutinosa* and standard compounds was calculated using the following equation:

Percent scavenged $[H_2O_2] = (A_0 - A_1)/ A_0 \times 100$

Where, A_0 was the absorbance of the control and A_1 was the absorbance in the presence of methanolic extract of *Litsea glutinosa* and standards (Gülçin *et al.* 2003).

b. Determination of total antioxidant capacity

The antioxidant activity of the extract 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 extract and subsequent formation of a green phosphate / Mo(V) complex at acid pH. 0.3 ml extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 ^oC for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer (Hach, DR-4000U) against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

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 (10 mM) in phosphate buffered saline was mixed with different concentrations of methanolic extract of *Litsea glutinosa* 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 the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1- naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm (Sreejayan and Rao, 1997).

Reducing power

The reducing power of methanolic extract was determined according to the method of Oyaizu (1986). Different

amounts of methanolic extracts (50 - 250 mg) in 1 ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 mol/l, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged (650 x g at room temperature) for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Antinociceptive properties

Evaluation of antinociceptive effect was performed by acetic acid induced writhing model in mice (Whittle, 1964). The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice. The test consists of injecting the 0.7% acetic acid solution intraperitoneally and then observing the animal for specific contraction of body referred as 'writhing'. A comparison of writhing was made with the positive control (Diclofenac-Na). Control and test samples are given orally 30 minutes prior to acetic acid injection. If the sample possesses analgesic activity, the animal that received the sample will give lower number of writhing than the control, i.e. the sample having analgesic activity will inhibit writhing.

Results and discussion

Antioxidant properties

i) Qualitative assay: The color changes (yellow on purple background) on the TLC plates were observed due to the bleaching of DPPH by the resolved bands.

Quantitative assay

Scavenging of hydrogen peroxide

Scavenging of H_2O_2 by extracts may be attributed to their phenolic constituents, which can donate electrons to H_2O_2, thus neutralizing it to water. The methanolic extracts of *Litsea glutinosa* were capable of scavenging hydrogen peroxide in a concentration-dependent manner (Fig. I).

Total antioxidant capacity

Total antioxidant capacity exerted by the extract is concentration dependent. It is observed that the extract is likely to have the capacity of reduction of Mo(VI) to Mo(V) by the antioxidant principle and the formation of a green phosphate / Mo(V) complex with a maximal absorption at 695 nm. The antioxidant activity is expressed as the number of equivalents of ascorbic acid (Table I).

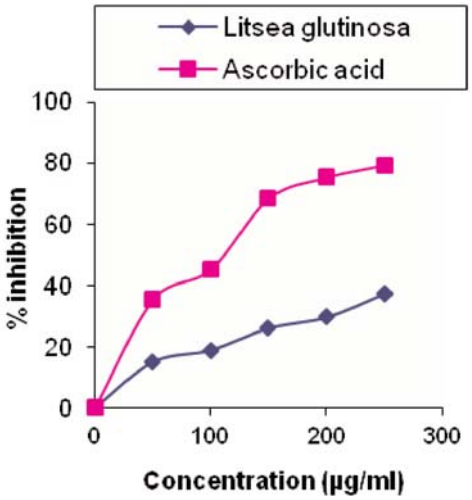

Fig. 1. H_2O_2 scavenging activity of methanolic extract of *Litsea glutinosa* Vs Ascorbic acid

Table I. Total antioxidant capacity of methanolic extract of *Litsea glutinosa*

Materials	Concentration (µg/mL)	Equivalent to ascorbic acid
Methanol extract of *Litsea glutinosa*	62.50	0.179±0.12
	125.0	0.257±0.03
	250.0	0.387±0.07
	500.0	0.501±0.16
	1000.0	1.031±0.11

Nitric oxide scavenging activity

From Fig. II, it is observed that the extract is likely to have concentration dependent nitric oxide scavenging activity. The leaves may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health. Nitric oxide is also implicated for inflammation, cancer and other pathological conditions (Moncada *et al.* 1991).

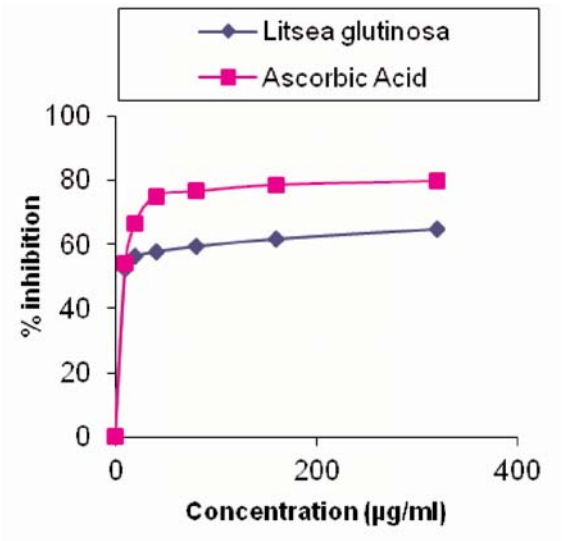

Fig. II. Nitric oxide scavenging activity of methanolic extract of *Litsea glutinosa*

Reducing power

Reduction ability of the extract has been investigated from the Fe^{+++} - Fe^{++} transformation using the method followed by Oyaizu (1986). Duh (1998) and Tanaka *et al.* (1988) have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductones (Duh, 1998) which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Fig. III shows the reduction ability of *Litsea glutinosa.*

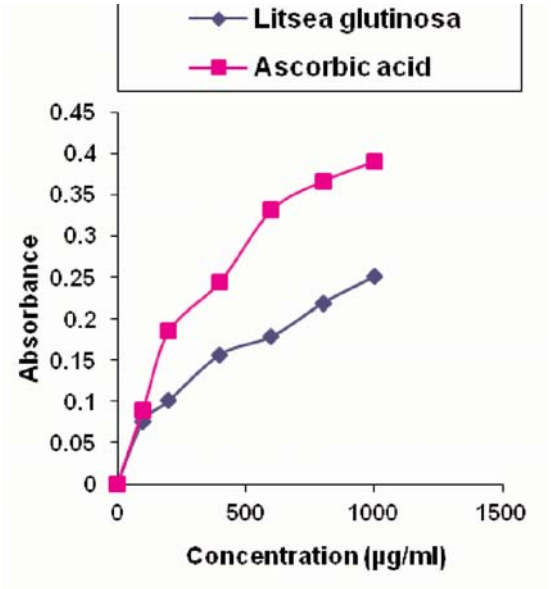

Fig. III. The reducing power of methanolic extract of *Litsea glutinosa*

Antinociceptive properties

In acetic acid induced mice, the methanolic extract produced 69.57% and 86.96% writhing inhibition at the doses of 250 mg/kg and 500 mg/kg body weight respectively, whereas Diclofenac sodium produced 45.65% inhibition at the dose of 25 mg/kg. Table II shows the antinociceptive effect of *Litsea glutinosa.*

Table II. Antinociceptive property of methanolic extract of *Litsea glutinosa* in acetic acid induced writhing in mice

Animal group/Treatment	Concentration (µg/mL)	Equivalent to ascorbic acid
Control 1% Tween-80 solution in water, 10 ml/kg, p.o. n=4	23 0.01[a] (100)	--
Positive control Diclofenac sodium 25 mg/kg, p.o. n=4	12.5 0.13[a] (54.35)	45.65
Test group I Methanolic extract 250 mg/kg, p.o. n=4	7 0.11[a] (30.43)	69.57
Test group II Methanolic Extract 500 mg/kg, p.o. n=4	3±0.04[a] (13.04)	86.96

Values are expressed as mean ± S.E.M.; a, indicates P<0.001 vs. control; p.o.: per oral.

A combination of antioxidants with analgesics normalized the oxidative stress which suggests that the administration of antioxidants in pain treatment may be employed to decrease the doses of analgesics (Rokyta1 *et al.* 2003). Antioxidant-

based pain killers may be a viable alternative in future to addictive medications such as morphine. Therefore, medicinal plants like *Litsea glutinosa* which possesses both antioxidant and antinociceptive properties can be used in the treatment of pain stimulated oxidative stress condition through their pharmacological validation.

Conclusion

Our present study demonstrates the antioxidant and antinociceptive properties of *Litsea glutinosa* and justifies its use in traditional medicine.

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