Saudi Journal of Biological Sciences (2013) **20**, 213–225

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Antioxidative, antimicrobial and cytotoxic effects of the phenolics of *Leea indica* leaf extract

Md. Atiar Rahman [a,b,*], Talha bin Imran [b], Shahidul Islam [a]

[a] *Department of Biochemistry, School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal (Westville Campus), Durban 4000, South Africa*
[b] *Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong-4331, Bangladesh*

Received 8 June 2012; revised 17 November 2012; accepted 24 November 2012
Available online 20 December 2012

KEYWORDS

Leea indica;
Radical scavenging;
Antibacterial;
Cytotoxic;
Probit

Abstract This study investigated the phytochemical, antioxidative, antimicrobial and cytotoxic effects of *Leea indica* leaf ethanol extract. Phytochemical values namely total phenolic and flavonoid contents, total antioxidant capacity, DPPH radical scavenging effect, $FeCl_3$ reducing power, DMSO superoxide scavenging effect and Iron chelating effects were studied by established methods. Antibacterial, antifungal and cytotoxic effects were screened by disk diffusion technique, food poison technique and brine shrimp bioassay, respectively. Results showed the total phenolic content 24.00 ± 0.81 g GAE/100 g, total flavonoid content 194.68 ± 2.43 g quercetin/100 g and total antioxidant capacity 106.61 ± 1.84 g AA/100 g dry extract. Significant ($P < 0.05$) IC_{50} values compared to respective standards were recorded in DPPH radical scavenging (139.83 ± 1.40 μg/ml), $FeCl_3$ reduction (16.48 ± 0.64 μg/ml), DMSO superoxide scavenging (676.08 ± 5.80 μg/ml) and Iron chelating (519.33 ± 16.96 μg/ml) methods. In antibacterial screening, the extract showed significant ($P < 0.05$) zone of inhibitions compared to positive controls Ampicillin and Tetracycline against Gram positive *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, and *Staphylococcus aureus* and Gram negative *Salmonella typhi*, *Salmonella paratyphi*, *Pseudomonas aeroginosa*, *Shigella dysenteriae*, *Vibrio cholerae*, and *Escherichia coli*. Significant minimum inhibitory concentrations compared to tetracycline were obtained against the above organisms. In antifungal assay, the extract inhibited the growth of *Aspergillus flavus*, *Candida albicans* and *Fusarium equisetii* by 38.09 ± 0.59, 22.58 ± 2.22, and 22.58 ± 2.22%, respectively. The extract showed a significant LC_{50} value compared to vincristine sulfate in cytotoxic assay. The results evidenced the potential

* Corresponding author at: Department of Biochemistry, School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal (Westville Campus), Durban 4000, South Africa. Tel.: +27 31 260 8982; fax: +27 31 260 7942.
E-mail addresses: atiarh@yahoo.com, 211560713@stu.ukzn.ac.za (M.A. Rahman).

Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2012.11.007

antioxidative, antimicrobial and cytotoxic capacities of *Leea inidica* leaf extract to be processed for pharmaceutical use.

1. Introduction

The investigation of medicinal properties of various plants attracted an increasing interest since last couple of decades due to their potent pharmacological activities, convenience to users, economic viability and low toxicity (Chew et al., 2012; Prashant et al., 2008). Recently, there has been an upsurge of finding natural antioxidants, from plant materials to replace synthetic antioxidants because the former ones are accepted as green medicine to be safe (Chanwitheesuk et al., 2005) for health management whereas the latter ones are quite unsafe and their toxicity is a problem of concern (Vinay et al., 2010). Natural antioxidants belonging to the higher plants especially the typical compounds, such as vitamins, carotenoids and phenolics exhibit antioxidant activity and they reduce disease-associated chronic health problems (Duarte et al., 2005). It has been reported that there is an inverse relationship between antioxidative status and incidence of human diseases such as cancer, aging, neurodegenerative disease, and atherosclerosis (Morales et al., 2008).

In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Janovská et al., 2003). Apart from this, most of the synthetic antimicrobial agents have various adverse effects on human health. On the contrary, the plant-derived antimicrobial agents are not associated with side effects and they have a prospective therapeutic benefit to heal many infectious diseases (Gulcin et al., 2004). This situation forced scientists to search for new antimicrobial agents from various sources like medicinal plants which are good sources of novel antimicrobial drugs (Karaman et al., 2003). For the same, current global populations are as well turned to plant medicines as their first line therapy for combating diseases and for routine health management (Perumal Samy et al., 2008).

Leea indica (Burm.f.) Merr (Leeaceae), an evergreen large shrub growing up to 2–3 m in height, is locally known as Kukur jiwa, Achila gach or Arengi. They grow in disturbed areas of lowland and upland rain forest in Asia–Pacific islands. In Bangladesh, it grows in hilly forests of Chittagong and Sylhet. *L. indica* has a long history of traditional medications by the tribes of Bangladesh. They prescribed the use in a combination of root paste of *L. indica*, *Oreocnide integrifolia*, and *Cissus repens* for bubo and boils (Yusuf et al., 2008). *L. indica* flowers have also been studied for anti-microbial, anti-oxidant, anti-inflammatory, hypo-glycemic, and phosphodiesterase inhibitory activities (Srinivasan et al., 2009). Recently researchers have reported the sedative and anxiolytic effect (Raihan et al., 2011), mitochondria mediated apoptosis effect of cancer cells (Wong and Abdul Kadir, 2012), growth inhibitory effect of Ca Ski cervical cancer cells (Wong and Abdul Kadir, 2011), and nitric oxide inhibitory effects (Saha et al., 2004) of *L. indica*. It is also used as an ingredient in the preparation of leucorrhea, intestinal cancer and uterus cancer treatment. The leaf decoction is consumed by women during pregnancy and delivery, for birth control or to treat obstetric diseases, and body pain (Srithi et al., 2009). Numbers of known chemical compounds including phthalic acid, palmitic acid, 1-eicosanol, solanesol, farnesol, three phthalic acid esters, gallic acid, lupeol and ursolic acid were identified from the leaves of *L. indica*.

In this study, we reported the further progress whereby the ethanol extract of *L. indica* was subjected to an analysis of phytochemical status, total phenolic content, total flavonoid, total antioxidant capacity, DPPH radical scavenging effect, $FeCl_3$ reducing effect, DMSO superoxide scavenging effect and Iron chelating effect. This study also reported the antibacterial, antifungal, and cytotoxic activities of the leaf extract using reference standards in each case.

2. Materials and methods

2.1. Chemicals and reagents

Absolute ethanol (99.50% v/v), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, and NBT were purchased from Sigma–Aldrich, Munich, Germany. Ascorbic acid (BDH, England), tetracycline disk (50 μg/disk), and ampicillin disks (50 μg/disks) were procured from Oxoid, England. Quercetin, curcumin, and vincristine sulfate were purchased from Merck, Germany.

2.2. Collection and identification of plant

The plant *L. indica* was selected by Md. Atiar Rahman, Assistant Professor, Department of Biochemistry & Molecular Biology, University of Chittagong and collected from Chittagong University hilly forest. The plant was identified by Dr. Shaikh Bokhtear Uddin, Taxonomist and Associate Professor, Department of Botany, University of Chittagong. A voucher specimen (Accession No. 36789) that contains the identification characteristics of the plant has been preserved in the Bangladesh National Herbarium for future reference.

2.3. Preparation of plant extract

The fresh leaves of *L. indica* were washed immediately after collection and chopped into small pieces, air dried at room temperature (25 ± 2 °C) and ground (Moulinex Blender AK-241, Moulinex, France) into powder (40–80 mesh). A 1475 g powder was let to soak in 6 L pure ethanol for 7 days at room temperature with occasional stirring. The extract was filtered through a cheese cloth followed by filter paper (Whatman No. 1). The whole filtrate was concentrated under reduced pressure at 50–55 °C through a rotatory vacuum evaporator (RE200, Bibby Sterling Ltd., England). The concentrated extract (79.0 g blackish-green crude, yield 5.4% w/w) was collected in a plastic Petri dish (90 × 15 mm) and allowed to air dry for the complete evaporation of solvent.

2.4. Phytochemical group tests of extract

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. These were identified by characteristic color changes using standard procedures described by Ghani (2003), Sofowara (1993), Trease and Evans (1989) and Harborne (1973). In each test 10% (w/v) solution of the extract was taken unless otherwise mentioned in the individual test.

2.5. Determination of total phenolic content (TPC)

TPC of the leaf extract was determined spectrophtometrically following the Folin–Ciocalteu method described previously with a minor modification (Iqbal et al., 2005). Briefly, 20 µl of sample or standard (2.5–50 mg/L gallic acid) plus 150 µl of diluted Folin–Ciocalteu reagent (1:4 reagent: water) was placed in each well of a 96-well plate, and incubated at room temperature for 3 min. Following an addition of 300 µl of sodium carbonate (2:3, saturated sodium carbonate: water) and a further incubation for 2 h at room temperature, the absorbance was read at 765 nm using a spectrophotometer (UV-1601 Shimadzu Corporation, Kyoto, Japan). The phenolic compound content was determined as gallic acid equivalents using the linear equation based on the calibration curve: $C = (c \times V)/m$, where, C = total content of phenolic compounds (mg/g plant extract in GAE), c = concentration of gallic acid obtained from calibration curve (mg/ml), V = the volume of the sample solution (ml), m = weight of the sample (g). All tests were conducted in triplicate.

2.6. Determination of total flavonoid content (TFC)

TFC of the leaf extract was determined using the method described by Kumaran and Karunakaran (2007) with slight modification. Briefly, 1.0 ml of extract solution (200 µg/ml) and standard (quercetin) at different concentrations were taken in test tubes. 3.0 ml of methanol followed by 200 µl of 10% aluminum chloride solution was added into the test tubes. Two hundred microliters of 1 M potassium acetate solution was added to the mixtures in the test tubes. Furthermore, each reaction test tube was then immediately diluted with 5.6 ml of distilled water and mixed to incubate for 30 min at room temperature to complete reaction. The absorbance of pink colored solution was noted at 415 nm using a spectrophotometer against blank methanol. TFC of the extract was expressed as quercetin equivalents (QE) after calculation using the following equation: $C = (c \times V)/m$, where, C = total flavonoid contents, mg/g plant extract in QE, c = concentration of quercetin obtained from calibration curve (mg/ml), V = the volume of the sample solution (ml), m = weight of the sample (g). All tests were conducted in triplicate.

2.7. Determination of total antioxidant capacity (TAC)

TAC of leaf extract was determined following the method described by Prieto et al. (1999). Three hundred microliters of extract (200 µg/ml) and standard (ascorbic acid) at different concentrations was taken in test tubes. 3.0 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added into the test tubes. The test tubes were incubated at 95 °C for 90 min to complete reaction. The absorbance of the solution was read at 695 nm using a spectrophotometer against blank after cooling to room temperature. Blank solution contained 3 ml of reagent solution and the appropriate volume (300 µl) of the same solvent was used for the sample, and it was incubated under the same conditions as for the rest of the sample solution. TAC is expressed as the number of equivalents of ascorbic acid following the equation below: $A = (c \times V)/m$, where, A = total antioxidant capacity, mg/g plant extract, in ascorbic acid equivalents, c = concentration of ascorbic acid obtained from calibration curve (mg/ml), V = the volume of the sample solution (ml) m = weight of the sample (g).

2.8. Assay of DPPH radical scavenging effect

The free radical scavenging effect of *L. indica* extract was assessed with the stable scavenger DPPH with slight modifications of the method described by Brand-Williams et al. (1995). Briefly, the concentrations (25, 50, 100, 200, 400, and 800 µg/ml) of extracts were prepared in ethanol. Positive control ascorbic acid solution was made with the concentration between 1 and 100 µg/ml. DPPH solution (0.004%) was prepared in ethanol and 5 ml of this solution was mixed with the same volume of extract and standard solution separately. These solutions were kept in dark for 30 min to read absorbance at 517 nm. The degree of DPPH-purple decolorization to DPPH-yellow indicated the scavenging efficiency of the extract. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. The scavenging activity against DPPH was calculated using the equation.

Percent of scavenging activity = $[(A - B)/A] \times 100$, where, A was the absorbance of control (DPPH solution without the sample), B was the absorbance of DPPH solution in the presence of the sample (extract/ascorbic acid).

2.9. Assay of $FeCl_3$ reducing power

The reducing power of *L. indica* extract was determined according to the method of Oyaizu (1986). 1.0 ml of extract solution (final concentration 50–500 µg/ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (10 g/L), and then the mixture was incubated at 50 °C for 20 min. Two and one-half, 2.5 ml of trichloro acetic acid (100 g/L) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (1.0 g/L) and absorbance measured at 700 nm in UV–Visible sspectrophotometer. Increased absorbance of the reaction mixture indicates an increase in reducing power. The increase of reducing power by the extract and standard was calculated as follows.

Percentage of increase of reducing power

$$= \left[\frac{A_{test}}{A_{control}} - 1\right] \times 100$$

where, A_{test} is absorbance of test solution; $A_{control}$ is absorbance of control. The antioxidant activity of the *L. indica* extract was compared with the standard ascorbic acid. A typical blank solution contained the same solution mixture

without plant extract or standard and it was incubated under the same conditions as for the rest of the sample solution.

2.10. Assay of superoxide radical scavenging activity by alkaline DMSO method

Superoxide scavenging activity of *L. indica* extract was determined by the alkaline DMSO method described by Madan et al. (2005) with slight modification. In this method, the concentration of superoxide in the alkaline DMSO system corresponds to the concentration of oxygen dissolved in DMSO. Briefly, superoxide radical was generated in non-enzymatic system. To the reaction mixture containing 0.1 ml of NBT (1.0 mg/ml solution in DMSO) and 0.3 ml of the extract (100–600 μg/ml) and standard (curcumin 5–50 μg/ml) in DMSO, 1.0 ml of alkaline DMSO (1.0 ml DMSO containing, 5 mM NaOH in 0.1 ml water) was added to give a final volume of 1.4 ml and the absorbance was measured at 560 nm. Control was prepared by mixing 300 μl of plain DMSO, 0.1 ml NBT solution and 1.0 ml alkaline DMSO. The decrease in the absorbance at 560 nm with antioxidants indicated the consumption of generated superoxide (Srinisavan et al., 2007; Reddy et al., 2008). The percentage of super oxide radical scavenging by the *L. indica* extract and standard scavenger curcumin were calculated as follows:

$$\text{Percentage of superoxide scavenging activity} \frac{\text{Test absorbance} - \text{control absorbance}}{\text{Test absorbance}} \times 100$$

2.11. Assay of iron chelating activity

Iron chelating activity of *L. indica* extract was determined according to the method described by Benzie and strain (1996). The reaction mixture containing 1.0 ml of O-Phenanthroline, 2.0 ml of $FeCl_3$ and 2.0 ml of extract at various concentrations ranging from 2 to 1000 μg/ml in a final volume of 5.0 ml was incubated for 10 min at ambient temperature. The absorbance at 510 nm was recorded. Ascorbic acid was added instead of extract and the absorbance obtained was taken as equivalent to 100% reduction of all ferric ions. Blank was carried out without extract. Experiment was performed in triplicate. The percentage of iron chelating activity by the *L. indica* extract and standard compound ascorbic acid was calculated as follows:

$$\text{Percent of iron chelating activity} = \frac{\text{Test absorbance} - \text{Control}}{\text{Test absorbance}} \times 100$$

2.12. IC_{50} value of the extract

Based on the screening results of the triplicate measurement of the extract, the inhibition concentration (IC_{50}) value was determined from the plotted graph of scavenging activity versus the concentration of extract (using linear regression analysis), which is defined as the amount of antioxidant necessary to reduce the initial radical concentration by 50%. Lower IC_{50} value indicates the higher scavenging effect (Chew et al., 2012).

2.13. Antimicrobial (antibacterial and antifungal) activity of L. indica extract

2.13.1. Bacterial strains with stock ID

Gram positive *Bacillus subtilis* (*B. subtilis*; BTCC 18), *Staphylococcus aureus* (*S. aureus*; ATCC 6538) *Bacillus cereus* (*B. cereus*; BTCC 19), *Bacillus megaterium* (*B. megaterium*; BTCC 17), and Gram negative *Salmonella typhi* (*S. typhi*; AE14296), *Salmonella paratyphi* (*S. paratyphi*; CRL-ICDDR,B), *Pseudomonas aeruginosa* (*P. aeroginosa*; CRL-ICDDR,B), *Vibrio cholerae* (*V. cholera*; AE14748), *Shigella dysenteriae* (*S. dysenteriae*; AE14612) and *Escherichia coli* (*E. coli*; ATCC25922) were used for antimicrobial screening. All the stock cultures were collected from ICDDR, B and the Department of Microbiology, University of Chittagong, Bangladesh.

2.13.2. Media preparation and maintenance of bacteria sample concentration

All of the bacterial strains were grown and maintained on Muller Hinton agar (Hi media, India) media at 37 °C and pH 7.3 ± 0.2. The bacteria were subcultured overnight in Muller Hinton broth which was further adjusted to obtain turbidity comparable to McFarland (0.5) standard when required (Sein et al., 2008). Three different concentrations 1.0, 2.0, and 3.0 mg/disk of the extract were used for antibacterial assay.

2.13.3. Antibacterial screening by disk diffusion technique

The antibacterial activity of the extract was determined by disk diffusion technique (National Committee for Clinical Laboratory Standards, NCCLS, 2002). The test microbes were taken from the broth culture with inoculating loop and transferred to test tubes containing 5.0 ml of sterile distilled water. The inoculums were added until the turbidity was equal to 0.5 McFarland standards. Cotton swab was then used to inoculate the test tube suspension onto the surface of Muller Hinton agar plate and the uniformly swabbed plates were then allowed to dry. On the dry inoculated surfaces were placed disks prepared as follows. Sterilized Whatman paper disks (6 mm in diameter) were prepared by placing 0.5 ml of the desired solution (1, 2 and 3 mg/disk) of the extract on (6 mm diameter) disks in 0.01- or 0.02-ml increments (4) and allowing the disks to dry at 40 °C after each application. The disks containing plant extract were placed with blunt-nosed thumb forceps on the inoculated plates at equidistance in a circle. These plates were kept for 4–6 h at a low temperature (< 8 °C) to allow for diffusion of the extract from the disk into the medium. The same was done for negative control (ethanol). Reference antibiotic disks, tetracycline and ampicillin (positive control), were purchased as ready disks (30 μg/disk, Oxoid, England). The plates were incubated at 37 °C for 24 h. The experiment was conducted in triplicates. Antimicrobial activity was determined by a measurement of the inhibition zone diameter (mm) around each test organism.

2.13.4. Minimum inhibitory concentration (MIC) determination

MIC was determined by the micro-dilution method using serially diluted (2 folds) plant extract according to the National Committee for Clinical Laboratory Standards (NCCLS) (Na-

tional Committee for Clinical Laboratory Standards, 2000). MIC of the extract was determined by the dilution of *L. indica* of various concentrations of 0.0–25, 0.0–50, 0.0–75, 0.0–100, 0.0–125, and 0.0–150 μg/ml respectively. Equal volume of each extract and nutrient broth was mixed in a test tube. Specifically 0.1 ml of standardized inoculum ($1–2 \times 10^7$ cfu/ml) was added in each tube. The tubes were incubated aerobically at 37 °C for 18–24 h. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and growth media without inoculum) and organism control (tube containing the growth medium, saline and the inoculum). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes was regarded as MIC.

2.14. Fungal strains and stock ID

Four human pathogenic fungal strains namely *Aspergillus flavus* (*A. flavus*; UCFT 02), *Aspergillus oryzae* (*A. oryzae*; UCFT 03), *Candida albicans* (*C. albicans*; UCFT 06) and *Fusarium equisetii* (*F. equisetii*; UCFT 08) were used for antifungal assay. The strains were collected from the Department of Microbiology, University of Chittagong, Bangladesh.

2.14.1. Determination of antifungal activity of L. aspera extract

The poisoned food technique (Grover and Moore, 1962; Mishra and Tiwari, 1992; Nene and Thapilyal, 2002) was used to screen for anti-fungal activity of the extract. Potato dextrose agar was used as a culture medium. For this, the required concentration of extract (10% sample solution) was taken in a sterilized pipette in a sterilized petriplate and then 15 ml of medium was poured into the petriplate to mix well and allowed to solidify. Inoculation was done at the center of each plate with 5 mm of mycelium block for each fungus. The mycelium block was prepared with the help of cork-borer from the growing area of a 5 day old culture of the test fungi on PDA. The blocks were placed at the center of each petriplate in an inverted position to get greater contact of the mycelium with the culture medium. The inoculated plates were incubated at (25 ± 1 °C). The experiment was repeated three times. Proper control (PDA without extract) was also maintained. The diameters of fungal colonies were measured after 5 days of incubation. The average of three measurements was taken as colony diameter of the fungus in millimeters. The percentage inhibition of mycelial growth of the test fungus was calculated by the formula: $I = \{(C - T)/C\} \times 100$, where, I = percent of inhibition; C = diameter of the fungal colony in control; T = diameter of the fungal colony in treatment. The antifungal effect was compared with the standard antifungal drug fluconazole (100 μg/disk).

2.15. Brine shrimp lethality bioassay

Brine shrimp lethality bioassay was carried out according to Meyer et al. (1982) to investigate the cytotoxicity of the extract. The dried extract preparation was redissolved in DMSO to obtain a solution of 10 mg/ml of the extract for toxicity test. Serial dilution was then carried out in order to obtain the concentration 20–1000 μg/ml of the extract. 5.0 ml of artificial sea water was added into all test tubes. Simple zoological organism (*Artemia salina*) was used as a convenient monitor for cytotoxic screening. The eggs of the brine shrimps were collected from the Institute of Marine Science and Fisheries, University of Chittagong, Bangladesh and hatched in artificial seawater (prepared by using sea salt 38 g/L and adjusted to pH 8.5 using 1 N NaOH) under constant aeration for 24 h under the light. The hatched shrimps were allowed to grow by 48 h to get shrimp larvae called nauplii. After 48 h, active nauplii were attracted to one side in a glass petri dish by using a micropipette. The nauplii were then separated from the eggs by aliquoting them into another glass petri dish containing artificial sea water and used for the assay. Suspension containing 20 nauplii was added into each test tube and was incubated at room temperature (25 ± 1 °C) for 12 h under the light. The tubes were then examined after 24 h and the number of surviving larvae in each tube was counted with the aid of a 3× magnifying glass. Experiments were conducted along with control in a set of three tubes per dose. The percentage of mortality was plotted against the logarithm of concentration. The concentration that would kill 50% of the nauplii (LC_{50}) was determined from Probit analysis as well as linear regression equation using the software "BioStat-2009".

2.16. Statistical analysis

All data are presented as mean ± standard deviation (SD). The data were analyzed by a statistical software package (SPSS, version 19.0, IBM Corporation, NY, USA) using Tukey's multiple range post hoc tests. The values were considered significantly different at $P < 0.05$.

3. Results

3.1. Phytochemical screening of *L. indica* extract

Phytochemical screening of *L. indica* leaf extract under this study showed the presence of medicinally active secondary metabolites alkaloid, glycoside, cardiac glycoside, terpenoids, flavonoids, steroid and tannin. Anthraquinones, glycosides, carbohydrates, phlobatanins and saponins were not detected in the extract (Table 1).

3.2. Determination of total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) of L. indica extract

The total phenolic contents, total flavonoid content and total antioxidant capacity in the examined plant extract are expressed in terms of gallic acid, quercetin and ascorbic acid equivalent respectively. The values obtained for the concentration of total phenol, total flavonoid and total antioxidant capacity were measured as 24.00 ± 0.81 g GAE/100 g, 194.68 ± 2.43 g quercetin/100 g and 106.61 ± 1.84 g AA/100 g of dry extract (Table 2).

3.3. Antioxidant activity of L. indica extract

3.3.1. Assay of DPPH free radical scavenging effect

The results for DPPH free radical scavenging effect of the extract shown in Fig. 1(A) indicated that there was a significant ($P < 0.05$) difference of mean percentage scavenging effect between all the tested concentrations of the extract and reference

Table 1 Observation on phytochemical screening of *L. indica* extract.

Phytochemicals	Name of the test	Observation	Result
Alkaloids	Mayer's test	Creamy white precipitate	+ +
	Hager's test	Yellow crystalline precipitate	+ +
	Wagner's test	Deep brown precipitate	+ +
Glycosides	General test	Yellow color	+ +
Cardiac glycosides	Legal's test	Pink to red color	+ +
	Baljet's test	Yellow orange color	+ +
Anthraquinone glycoside	For O-glycoside	No rose pink, red or violet color in the aqueous layer	− −
	For C-glycoside	No rose red color in aqueous layer	− −
Terpenoids	Salkowsky test	A reddish brown coloration	+ +
Carbohydrates	Molisch Test	No red-violet layer at the interface of acid (bottom) and aqueous (upper) layers	− −
	Fehling's Test	No red precipitate	− −
Flavonoids	Specific test	Orange to red color	+ +
Steroids	Libermann-Burchard's test	Greenish color	+ +
Tannins	$FeCl_3$ test	Brownish green color	+ +
Phlobatanins	General test	No red precipitate formation	− −
Saponins	Frothing test	No change is observed	− −

Double plus (+ +) and minus (− −) ensures the presence and absence, respectively.

antioxidant ascorbic acid. The extract showed the highly significant radical scavenging activity (80.98 ± 0.42%) compared to that (98.36 ± 0.45%) of ascorbic acid. The inhibition concentration (IC_{50}) of the extract was determined by plotting a graph (Fig. 1E) of scavenging activity against the log concentration. The IC_{50} value of the extract (139.83 ± 1.40 μg/ml) was statistically significant ($P < 0.05$) compared to that (1.46 ± 0.06 μg/ml) of ascorbic acid. This value suggested that the radical scavenging activity of *L. indica* leaf extract was very high because the cutoff value is 1000 μg/ml. The value higher than this indicates that the extract or other synthetic antioxidant is not effective as radical scavenger.

3.3.2. FeCl3 reducing power

L. indica extract and ascorbic acid showed a dose dependent reducing activity (Fig. 1B) in $FeCl_3$ assay. Highest reduction was achieved 85.01 ± 0.22% by ascorbic acid and 27.25 ± 0.25% by *L. indica* extract at the concentration of 50 μg/ml. Regression analysis for reducing activity versus log concentration showed the IC_{50} value for *L. indica was* 16.48 ± 0.64 μg/ml, which was close to that (14.04 ± 1.20 μg/ml) of ascorbic acid indicating the potent $FeCl_3$ reducing power of *L. indica* extract (Fig. 1F).

3.3.3. Superoxide radical scavenging activity by alkaline DMSO method

Super oxide radical was formed by alkaline DMSO which reacted with NBT to produce colored diformazan. The ethanolic extract of *L. indica* scavenges super oxide radical and thus inhibits formazan formation. Super oxide scavenging activity of *L. indica* extract and reference compound curcumin showed a dose dependent activity (Fig. 1C). *L. indica* extract showed the largest superoxide scavenging effect 49.65 ± 0.51% which was significant to that (60.48 ± 0.53%) of curcumin, a standard superoxide scavenging agent. IC_{50} value (676.08 ± 5.80 μg/ml) of *L. indica* extract was highly significant compared to that of curcumin (Fig. 1G).

3.3.4. Iron chelating activity

The iron chelating effect of *L. indica* extract and standard antioxidant ascorbic acid is shown in Fig. 1D. However, regression analysis showed the IC_{50} value of *L. indica* extract was 519.33 ± 16.96 μg/ml which is not as promising a chelating agent like ascorbic acid which had the IC_{50} value of 8.81 ± 0.90 μg/ml (Fig. 1H).

3.4. Antibacterial activity of L. indica extract

Antibacterial activity results of *L. indica* ethanolic extract are given in Fig. 2. The mean zone of inhibition produced by the reference antibiotics, tetracycline and ampicillin was between 16 and 20 mm which was larger than that (9.0–12.0 mm) produced by the extract. The extract at three different concentrations 1, 2, and 3 mg/disk showed significant ($P < 0.05$) zone of inhibitions against Gram positive *B. subtilis* (9 ± 0.50, 11 ± 0.25, 12 ± 1.00 mm), *B. cereus* (9 ± 0.6, 10 ± 1.4, 11 ± 1.0) *B. megaterium* (15 ± 2.0, 16 ± 1.0, 19 ± 1.0 mm), and *S. aureus* (9 ± 1.0, 11 ± 2.0, 11 ± 1.0) and Gram negative *S. typhi* (10 ± 0.0, 10 ± 1.0, 10 ± 1.0 mm), *S. paratyphi* (8 ± 2.0, 10 ± 1.0, 11 ± 1.5 mm), *P. aeroginosa* (9 ± 1.5, 10 ± 1.0 10 ± 0.0 mm), *S. dysenteriae*

Table 2 Total phytochemical content in *L. indica* leaf extract.

Phytochemical	Regression equation	Content
Total phenolic	Y = 45.2x + 0.026	24.00 ± 0.81 g of GAE/100 g dry extract
Total antioxidant	Y = 0.004x + 0.131	194.68 ± 2.43 g of AA/100 g dry extract
Total flavonoid content	Y = 0.009x − 0.075	106.61 ± 1.84 g Quercetin/100 g dry weight

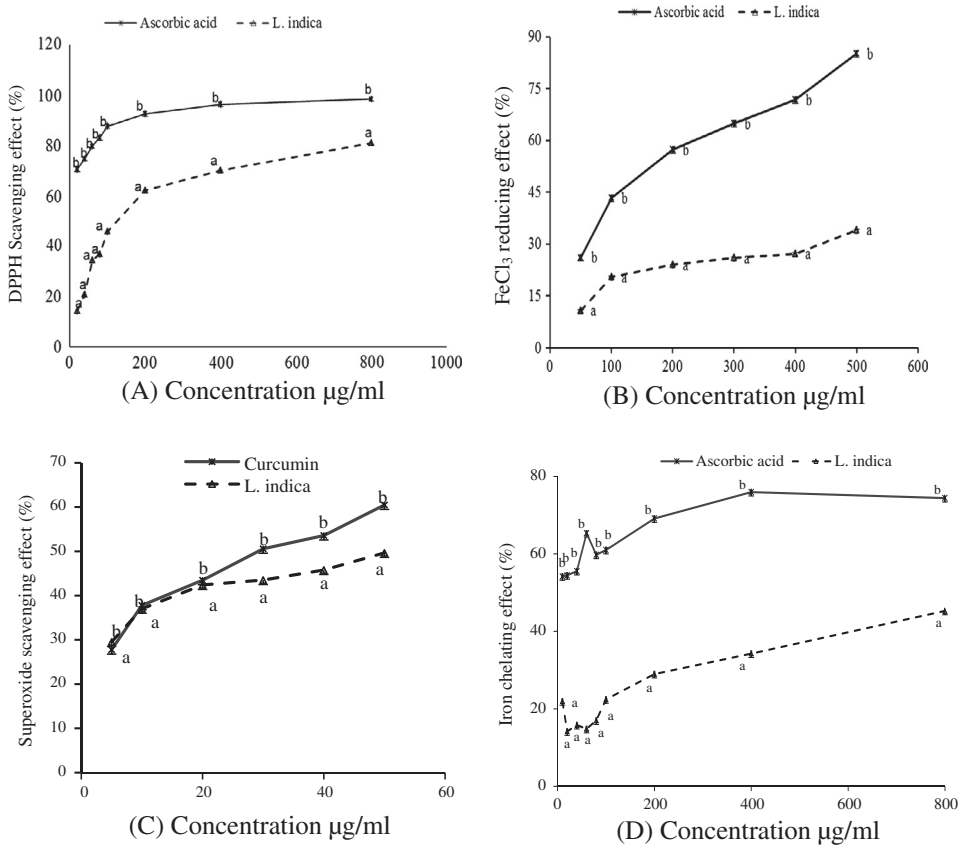

Figure 1 Comparative antioxidative effect (A–D) and IC_{50} values (e–h) of *L. indica* extract. Data are presented as mean ± SD for triplicate. Data leveled letters a and b shown on the graph lines indicate that the values are significantly different (Tukey's post hoc test for multiple comparisons, SPSS for windows, version 18.0, $P < 0.05$) from each other.

(10 ± 1.5,11 ± 1.0,11 ± 2.0 mm), and *V. cholerae* (9 ± 0.5, 10 ± 3.0, 10 ± 0.25 mm). Thus the extract showed the largest zone of inhibition against the Gram positive *B. megaterium* and Gram negative *E. coli* at 3 mg/disk. Gram positive strains were found more sensitive than Gram negative organisms to the extract on average. However, *S. typhi* showed the lowest antibacterial activity to the extract.

3.5. Minimum inhibitory concentration

The minimum inhibitory concentrations of *L. indica* leaf extract for different bacterial strains ranged from 25 to 100 µl/ml. The arbitrary MIC trend to Gram positive bacteria (start at 25) is lower than that to Gram negative bacteria (start at 50). *Bacillus cereus* and Bacillus *megaterium* of the four gram positive bacteria and *Shigella dysenteria* and *Vibrio cholerae* of the six gram negative bacteria showed promising MIC values with *L. indica* extract.

3.6. Antifungal activity of L. indica extract

Antifungal activity results of *L. indica* ethanolic extract are given in Figs. 3 and 4. The percent inhibition achieved by the extract at 10 mg/disk was compared with that of standard antifungal drug Fluconazole at 100 µg/disk. The extract showed 38.09 ± 0.5, 22.58 ± 2.2, and 61.82 ± 2.7% of growth inhibition against *A. flavus*, *C. albicans*, and *F. equisetii*, respectively whereas fluconazole showed 67.01 ± 1.8, 40.00 ± 2.5, and 72.32 ± 2.3% of inhibition against those fungal strains. These inhibitions by the extract were significant ($P < 0.05$) compared to those by standard drug fluconazole. The extract showed no growth inhibition of *A. oryzae*.

3.7. Cytotoxic activity of L. indica extract

The results of brine shrimp nauplii testing are presented in Table 3 and Fig. 5. The LC_{50} value indicated the concentration

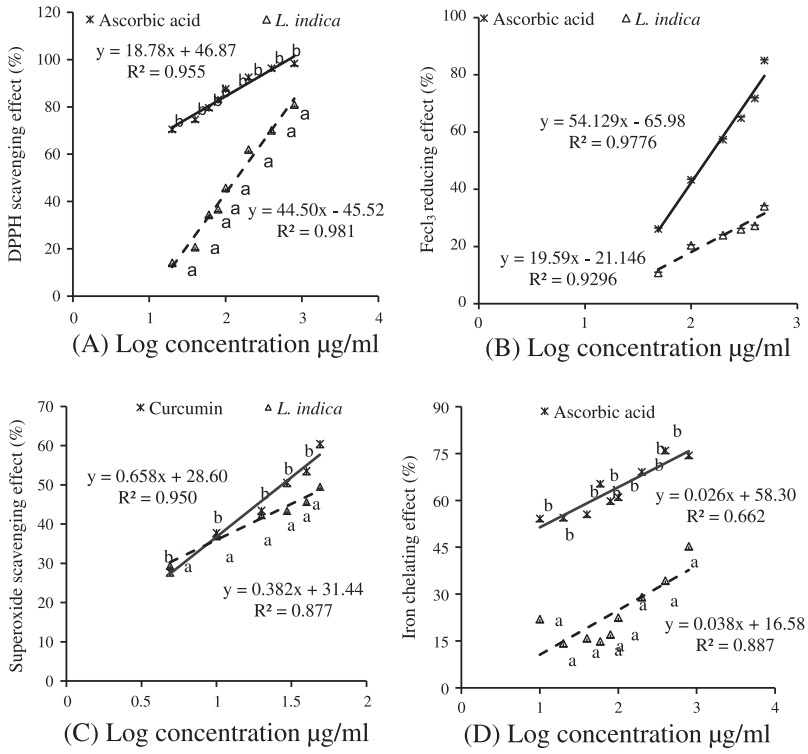

Figure 2 Comparative IC_{50} values (A–D) of *L. indica* extract. Data are presented as mean ± SD for triplicate. Data leveled letters a and b shown on the graph lines indicate that the values are significantly different (*Tukey's* post hoc *test* for multiple comparisons, SPSS for windows, version 18.0, $P < 0.05$) from each other.

by which 50% of the shrimps were killed. The effect of the extract was compared with vincristine sulfate (positive control). The *L. indica* extract had a LC_{50} value of 2.65 ± 0.16 µg/ml which was significantly ($P < 0.05$) different from that (0.76 ± 0.04 µg/ml) of positive control vincristine sulfate was (Fig. 5). Probit analysis (Table 3) showed that the "*Chi* square" value was 1.70 for the extract and 0.63 for vincristine sulfate (see Fig. 6 and Table 4).

4. Discussion

4.1. Phytochemical group tests

The secondary metabolites existing in the plant extract play a key role in the pharmacological actions of any plant or plant parts. This study was conducted to make an evidential approach in ascertaining the mentioned biological functions of *L. indica* extract. Alkaloids, flavonoids, terpenoids, steroids, tannins, phlobatannins, saponins and glycosides were present in the studied extract. These screened results were consistent with the previously conducted partial studies (Srinivasan et al., 2008).

4.2. DPPH radical scavenging effect

To evaluate the scavenging effect of the extract in this study, DPPH reduction was investigated against positive control ascorbic acid. The DPPH-stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts (Koleva et al., 2002; Suresh et al., 2008). The odd electron in

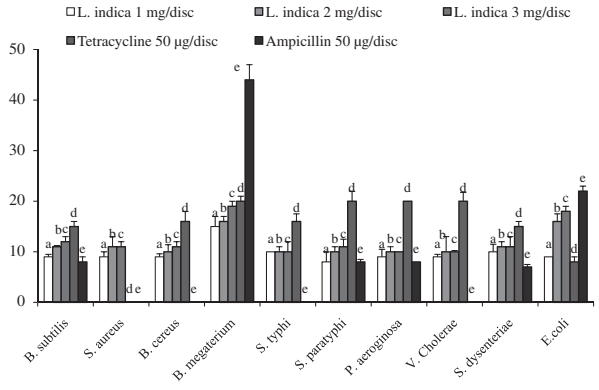

Figure 3 Comparative antibacterial effect of *L. indica* (three different concentrations), tetracycline and ampicillin expressed as zone of inhibition (diameter in mm). Data are shown as mean ± SD for triplicate of concentration. Superscript letters (a and b) shown in the bar line indicate that the values are significantly different (Tukey's post hoc test for multiple comparisons, SPSS for windows, version 18.0, $P < 0.05$) from each other.

the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color (Sarla et al., 2011). The color turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The more antioxidants occurred in the extract, the more DPPH reduction occurs.

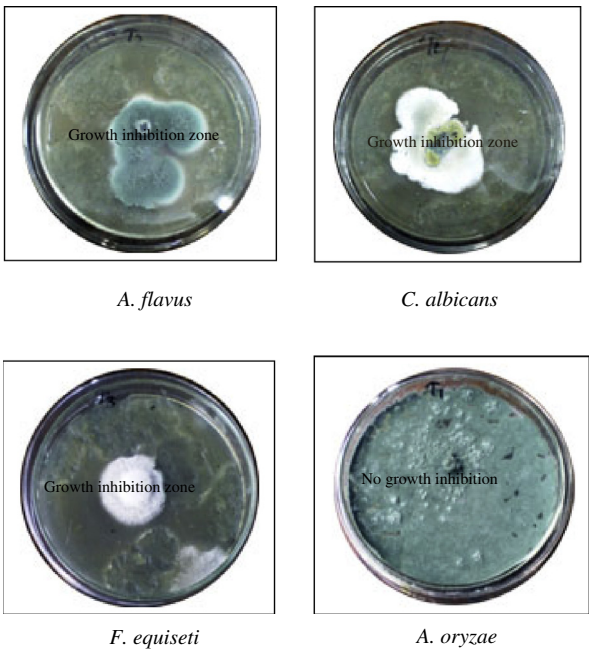

Figure 4 *In vitro* antifungal effect of *L. indica* leaf extract. Photographs shows the growth inhibitions of different fungal strains.

Table 3 Minimum inhibitory concentrations (MICs) of *L. indica* and tetracycline against tested bacterial strains.

Test organisms	MIC of *L. indica* extract (μg/ml)	MIC of tetracycline (μg/ml)
Gram positive bacteria		
B. subtilis	⩾50	⩾4
S. aureus	⩾75	⩾16
B. cereus	⩾25	⩾4
B. megaterium	⩾25	⩾4
Gram negative bacteria		
S. typhi	⩾75	⩾8
S. paratyphi	⩾100	⩾16
P. aeroginosa	⩾100	⩾16
S. dysenteriae	⩾50	⩾4
V. cholerae	⩾50	⩾8
E. Coli	⩾75	⩾8

The quantification of antioxidant in the extract is made by calculating the IC_{50} value. This study showed that the IC_{50} value of leaf extract, 39.83 ± 1.4 μg/ml, was statistically significant to that of ascorbic acid 1.46 ± 0.06, suggesting a high radical scavenging activity of *L. indica* leaf extract because the cutoff value of IC_{50} is 1000 μg/ml. The value higher than this indicates that the extract or other synthetic antioxidant is not effective as radical scavenger (Chew et al., 2012). However, the scavenging effects of different parts of a plant might vary from each other due to the varied concentrations of active phytochemicals responsible for antioxidants in those parts (Chew et al., 2012). Ascorbic acid is used as reference standard because ascorbic acid acts as a chain breaking scavenging agent that impairs the formation of free radicals in the process of intracellular substance formation throughout the body,

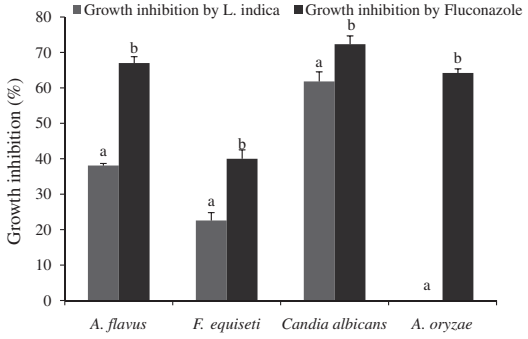

Figure 5 *In vitro* antifungal effect of *L. indica* leaf extract. Bar graph shows the comparative growth inhibitions by *L. indica* extract and atifungal drug fluconazole. Data are shown as mean ± SD for triplicate. Superscript letters a and b indicate that the values are significantly different (*Tukey's* post hoc *test for* multiple comparisons, SPSS for windows, version 18.0, $P < 0.05$) from each other.

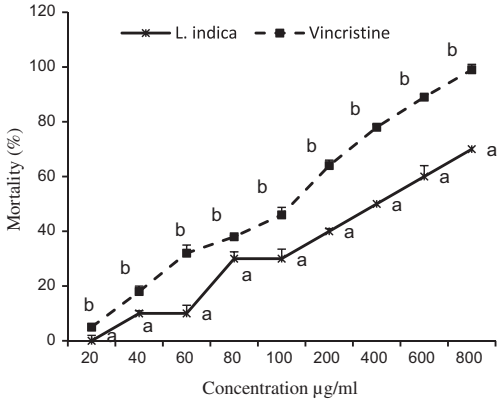

Figure 6 Lethality of *L. indica* (*) against 24-h-age brine shrimp (*A. Salina*) in comparison to standard vincristine sulfate (■) with the plotted concentration. Data are shown as mean ± SD of twenty shrimps for each concentration. Letters (a and b) shown in the plot indicate that the values are significantly different (Tukey's post hoc test for multiple comparisons, SPSS for windows, version 18.0, $P < 0.05$) from each other.

including collagen, bone matrix and tooth dentine (Aqil et al., 2006).

4.3. $FeCl_3$ reducing activity

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995). The reducing power of *L. indica* ethanol extract along with that of ascorbic acid at concentrations between 50–500 μg/ml showed that high absorbance indicates high reducing power (Roy et al., 2012). The reducing power of the plant extract was increased as the amount of extract concentration increases. This is because the presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form (Chung et al., 2002). In our study, the reducing power of extract was lower than that of ascorbic acid but the IC_{50} value

Table 4 Calculation of LC_{50} value, confidence limit and chi square value by probit analysis.

Sample	LC_{50} (μg/ml)	Range of confidence limit	Regression equation	*Chi* square
L. indica	2.65 ± 0.16	2.25–2.69 μg/mL	Y = 45.98*X + S32	1.70
Vincristine sulfate	0.76 ± 0.04	0.57–0.82 μg/mL	Y = 3.16 + 2.98X	0.63

of extract was close to that of ascorbic indicating that *L. indica* has a statistically significant ($P < 0.05$) reducing power.

4.4. Scavenging of superoxide radical by alkaline DMSO method

The scavenging activity of the extract against superoxide radical generated in the NaOH-alkaline DMSO-NBT system, resulting in the formation of the blue formazan was studied in this research. The generated superoxide remains stable in solution, which reduces nitro blue tetrazolium into formazan dye at room temperature. Superoxide scavenger capable of reacting inhibits the formation of a red dye formazan (Hagerman et al., 1998). The inhibition of formazan formation by the extract was reflected through the IC_{50} value for extract, 676.08 ± 5.80 μg/ml, which was significantly ($P < 0.05$) different compared to that of curcumin, 27.58 ± 1.58 μg/ml. This finding demonstrates that *L. indica* leaf extract is capable of non-enzymatically inhibiting the superoxide radical, produced in biological system, which is a precursor of many ROS and is shown to be harmful for various cellular components, although the enzyme superoxide dismutase possessed in aerobic and anaerobic organisms can catalyze the breakdown of superoxide radical (Shirwaiar et al., 2007).

4.5. Iron chelating effect

Ortho substituted phenolic compounds were found more active than unsubstituted phenol. Hence, these compounds may exert pro-oxidant effect by interacting with Iron. O-phenanthroline quantitatively forms complexes with Fe^{2+} which get disrupted in the presence of chelating agents (Mahakunakorn et al., 2004). The alcoholic extract interfered with the formation of a ferrous-O-phenanthroline complex, thereby suggesting that the extract has metal chelating activity (IC_{50}, 519.33 ± 16.96 μg/ml). As iron plays a major role in the formation of lipid peroxidation in the body, the effects of antioxidant phytochemicals in the biological systems depend on their ability to scavenge radicals, chelate metals, activate the antioxidant enzymes, and to inhibit the oxidases (Kulkarni et al., 2004).

4.6. Antibacterial activity of L. indica extract

Plants have long been a very important source of drug and many plants have been screened whether they contain compounds with therapeutic activity (Rosy et al., 2010). Therefore, it is vital to evaluate the antimicrobial activity of *L. indica*. The bacterial strains were chosen to be studied as they are important pathogens and rapidly develop antibiotic resistance with their increased uses. In disk diffusion technique, the mean zone of inhibition produced by the commercial antibiotic, tetracycline and ampicillin, was larger than that produced by ethanol extract. It may be attributed to the fact that the plant extract being in crude form contains a smaller concentration of bioactive compounds (Zuraini et al., 2007). In classifying the antimicrobial activity it would be generally expected that a much greater number would be active against Gram positive than Gram negative bacteria (Joshi et al., 2011). Apart from this, the higher MIC values are an indication that either the plant extracts are less effective on bacteria or that the organism has the potential of developing antibiotic resistance, while the low MIC values for bacteria are an indication of the higher efficacy of the plant extracts. The higher MIC values of *L. indica* extract for gram positive bacteria compared to those for gram negative bacteria indicate the greater efficacy of the extract to gram positive bacteria.

In a research conducted by using the flowers of *L. indica*, Srinivasan et al. (2009) indicated that the essential oil of this plant showed good effect against *E. coli* and *S. dysenteriae* and moderate effect against *B. subtilis*, *B. cereus* and *S. aureus*. These results are partially consistent with those of our study although the sample preparation was different. However, this study implies that the secondary metabolites responsible for antibacterial activity are greatly dependent on solvent system and the way the metabolites are collected from the plant sources. Moreover, growth area also affects the chemical components of the plants and leads to the activity difference (Girish and Satish, 2008). Another fact is that some organisms have intrinsic resistance from a restrictive outer membrane barrier and transenvelope multidrug resistance pumps (MDRs) to show good susceptibility to plant materials (Girish and Satish, 2008).

Antimicrobial activities of the extract depend on the nature of phytochemicals present in the extract. Researchers showed that presence of terpenoids, flavonoids, tannins, alkaloids, steroids other compounds of phenolic nature or free hydroxyl group which are classified as active antimicrobial agents (Ramzi et al., 2008; Sule et al., 2011). Among these phytochemicals, tannins act by iron deprivation, hydrogen bounding or non-specific interactions with vital proteins such as enzymes (Scalbert, 1991), alkaloids are known to be a DNA intercalator and an inhibitor of DNA synthesis through topoisomerase inhibition (Guittat et al., 2003). Flavonoids form a complex with extracellular and soluble proteins and a complex with bacterial cell walls (Marjorie, 1999). All others are associated with antimicrobial action through different mechanisms (Govindappa et al., 2011). Whatever the mechanism, it is clear that this antibacterial activity may be attributed to the alkaloids, glycosides, steroids, terpenoids and flavonoids, since some of these secondary metabolites were detected in the extract (Nwadinigwe and Ogochukwu, 2011).

4.7. Antifungal effect of L. indica extract

Plant extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trails. Antifungal

activity of the crude extract was tested using poisoned food technique in our study (Grover and Moore, 1962; Mishra and Tiwari, 1992; Nene and Thapilyal, 2002). Percent inhibition of fungal mycelia growth showed that the extract had a very promising inhibitory effect on *C. albicans* compared to the reference antifungal drug fluconazone, while the extract showed moderate antifungal effect against *A. flavus* and *F. equisetii*. Usually the crude extract has a wide range of physiological activity of alkaloid, terpenoids, flavonoid, anthraquinone, steroid, and tannin. Of all these, flavonoid was found to be the biochemical constituent responsible for the antifungal action (Sule et al., 2011). Steroids were reported as a major component acting as antifungal secondary metabolite (Onwuliri and Wonang, 2005). These observations are also consistent with our findings suggesting that the antifungal effect of *L. indica* extract is probably due to the individual or synergistic effect of the secondary metabolites present in the extract.

4.8. Cytotoxic activity of L. indica extract

Brine shrimp lethality is a general bioassay which is indicative of cytotoxicity, antibacterial activities, pesticidal effects and various pharmacologic actions (Meyer et al., 1982). Therefore, the isolation of bioactive compounds from natural sources and the use of plant extracts require toxicity information on the constituent of interest in order to delineate the effect of toxicity on both the host cells and target cells of pharmacological uses. Lethal concentration (LC_{50}) from the regression and probit analysis (Bliss, 1934) in 24 h of our study showed that LC_{50} value of *L. indica* extract was 2.65 ± 0.16 μg/ml (confidence limit 95%) where the lower and upper limits were 2.2561, and 2.6981 μg/ml respectively. Comparison of this result with the standard vincristine sulfate (0.76 ± 0.04 μg/ml) indicated that the lethality of *L. indica* extract is statistically significant ($P < 0.05$) suggesting the notable clinical importance of the extract against tumor cells, pesticides etc. because the brine shrimp assay is considered as a convenient probe for a preliminary assessment of toxicity, detection of fungal toxins, pesticidal and anti-tumor effect and other pharmacological actions (Meyer et al., 1982). Apart from this, the LC_{50} value of the extract was less than 1000 μg/mL, which is the cutoff point in detecting cytotoxicity ascertaining that the extract is concluded to be very important to use in above mentioned actions. The "*Chi* square" value of the extract (0.76) rejects the null hypothesis making the result statistically more significant to remove any discrepancy between expected mortality rates and the actual mortality rates of freshly hatched *A. salina*.

The cytotoxic effect of plants is principally contributed by the presence of secondary metabolites like alkaloid, glycoside, steroid, tannin, phlobatannin, terpenoid and flavonoid in their extract (Ozçelik et al., 2011). This is also consistent with our observation because the phytochemical group analysis of the extract showed the presence of alkaloid, terpenoids, tannins, phlobatannins, steroids and flavonoids. Further toxicity studies could be conducted on individual cell lines to confirm the toxic effect of phytochemical groups.

5. Conclusion

In conclusion, the study demonstrates that ethanolic extract of whole *L. indica* has promising antioxidant and antimicrobial effects on human health. The extract also has very prominent cytotoxic effects to be used in many pharmacological as well as biological actions. However, further studies are suggested to investigate these effects for the isolated and identified pure compounds from *L. indica*.

Acknowledgements

Authors are grateful to the Chittagong University Research Cell for providing the research Grant (Ref No. 5193/Res/Dir/CU/2011) to conduct the research. The authors are also thankful to the Taxonomist and Associate professor, Dr. Shaikh Bokhtear Uddin, Department of Botany, University of Chittagong, for identifying all the plants.

References

Aqil, F., Ahmed, I., Mehmood, Z., 2006. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk. J. Biol. 30, 177–183.

Benzie, I.F.F., Strain, J.T., 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power, the FRAP assay. Anal. Biochem. 239, 70–76.

Bliss, C.I., 1934. The method of probits. Science 79, 38–39.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28, 25–30.

Chanwitheesuk, A., Teerawutgulrag, A., Rakariyatham, N., 2005. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. Food Chem. 92, 491–497.

Chew, A.L., Jessica, J.J.A., Sasidharan, S., 2012. Antioxidant and antibacterial activity of different parts of *Leucas aspera*. Asian Pac. J. Trop. Biomed. 2, 176–180.

Chung, Y.C., Chang, C.T., Chao, W.W., Lin, C.F., Chou, S.T., 2002. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. J. Agric. Food Chem. 50, 2454–2458.

Duarte, M.C.T., Figueira, G.M., Sartoratto, A., Rehder, V.L.G., Delarmelina, C., 2005. Anti-*Candida* activity of Brazilian medicinal plants. J. Ethnopharmacol. 97, 305–311.

Ghani, A., 2003. Medicinal plant of Bangladesh:Chemical constituents and uses. Asiatic Society of Bangladesh, Dhaka.

Girish, H.V., Satish, S., 2008. Antibacterial activity of important medicinal plants on human pathogenic bacteria – a comparative analysis. World Appl. Sci. J. 5, 267–271.

Govindappa, M., Naga Sravya, S., Poojashri, M.N., Sadananda, T.S., Chandrappa, C.P., 2011. Antimicrobial, antioxidant and in vitro anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc. J. Pharmacog. Phytother. 3, 43–51.

Grover, R.K., Moore, J.D., 1962. Toximetric studies of fungicides against brown rot organisms, *Sclerotia fructicola* and *S. laxa*. Phytopathology 52, 876–880.

Guittat, L., Alberti, P., Rosu, F., Van Miert, S., Thetiot, E., Pieters, L., Gabelica, V., De Pauw, E., Ottaviani, A., Roiu, J.-F., Mergny, J.L., 2003. Interaction of cryptolepine and neocryptolepine with unusual DNA structures. Biochimie 85, 535–541.

Gulcin, I., Uguz, M.T., Oktay, M., Beydemir, S., Kufrevioglu, O.I., 2004. Evaluation of the antioxidant and antimicrobial activities of clary sage (*Salvia sclarea* L.). Turk. J. Agric. For. 28, 25–33.

Hagerman, A.E., Riedel, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem. 46, 1887–1892.

Harborne, J.B., 1973. Phytochemical methods: a guide to modern techniques of plant analysis. Chapman and Hall Ltd., London, UK.

Iqbal, S., Bhanger, M.I., Anwar, F.A., 2005. Antioxidant properties and components of some commercially varieties of rice in Pakistan. Food Chem. 93, 265.

Janovská, D., Kubíková, K., Kokoška, L., 2003. Screening for antimicrobial activity of some medicinal plants species of traditional Chinese medicine. Czech J. Food Sci. 21, 107–110.

Joshi, B., Sah, G.P., Basnet, B.B., Bhatt, M.R., Sharma, D., Subedi, K., Pandey, J., Malla, R., 2011. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). J. Microbiol. Antimicrob. 3, 1–7.

Koleva, I.I., Van Beek, T.A., Linssen, J.P.H., de Groot, A., Evstatieva, L.N., 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Ana. 13, 8–17.

Karaman, I., Sahin, F., Gulluce, M., Ogutcu, H., Sengul, M., Adiguzel, A., 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L.. J. Ethnopharmacol. 85, 231–235.

Kulkarni, A.P., Aradhya, S.M., Divakar, S., 2004. Isolation and identification of a radical scavenging antioxidant–punicalagin from pith and carpellary membrane of pomegranate fruit. Food Chem. 87, 551–557.

Kumaran, A., Karunakaran, R.J., 2007. In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT - Food Sci. Technol. 40, 344–352.

Madan, M.P., Raghavan, G., Ajay Kumar Singh, R., Palpu, P., 2005. Free radical scavenging potential of *Saussarea costus*. Acta Pharm. 55, 297–304.

Mahakunakorn, P., Tohda, M., Murakami, Y., Matsumoto, K., Watanabe, H., 2004. Antioxidant and free radical scavenging activity of Choto-san and its related constituents. Biol. Pharm. Bull. 27, 38–46.

Marjorie, C., 1999. Plant products as antimicrobial agents. Clin. Microb. Rev. 12, 564–582.

Meir, S., Kanner, J., Akiri, B., Hadas, S.P., 1995. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J. Agric. Food. Chem. 43, 1813–1815.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45, 31–34.

Mishra, M., Tiwari, S.N., 1992. Toxicity of *Polyalthia longifolia* against fungal pathogens of rice. Indian Phytopath. 45, 56–61.

Morales, G., Paredes, A., Sierra, P., Loyola, L.A., 2008. Antioxidant activity of 50% aqueous-ethanol extract from *Acantholippia deserticola*. Biol. Res. 41, 151–155.

NCCLS, 2002. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Third ed. NCCLS, Wayne, PA.

Nene, Y., Thapilyal, L., 2002. Poisoned food technique of fungicides in plant disease control, third ed. Oxford and IBH Publishing Company, New Delhi.

Nwadinigwe, Ogochukwu, A., 2011. Antimicrobial activities of methanol and aqueous extracts of the stem of *Bryophyllum pinnatum* Kurz (Crassulaceae). Afr. J. Biotechnol. 10, 16342–16346.

Onwuliri, F.C., Wonang, D.L., 2005. Studies on the combined antibacterial action of Ginger (*Zingiber officinale* L) and Garlic (*Allium sativum* L.) on some bacteria. Nig. J. Bot. 18, 224–228.

Oyaizu, M., 1986. Studies on product of browning reaction prepared from glucoseamine. Jpn. J. Nutr. 44, 307–315.

Ozçelik, B., Kartal, M., Orhan, I., 2011. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. Pharm. Biol. 49, 396–402.

Perumal Samy, R., Ignacimuthu, S., Patric Raja, D., 2008. Preliminary screening of ethnomedicinal plants from India. Eur. Rev. Med. Pharmacol. Sci. 12, 1–7.

Prashant, K.R., Dolly, J., Singh, K.R., Gupta, K.R., Watal, G., 2008. Glycemic properties of *Trichosanthes dioica* leaves. Pharm. Biol. 46, 894–899.

Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269, 337–341.

Raihan, M.O., Habib, M.R., Brishti, A., Rahman, M.M., Saleheen, M.M., Manna, M., 2011. Sedative and anxiolytic effects of the methanolic extract of *Leea indica* (Burm. f.) Merr. leaf. Drug Discov. Ther. 5, 185–189.

Ramzi, A.A.M., Salah, A.A.A., Hasson, S., Althawab, F.M.N., Alaghbari, S.A.Z., Lindequist, U., 2008. Antimicrobial, Antioxidant and Cytotoxic Activities and Phytochemical Screening of Some Yemeni Medicinal Plants. eCAM, 0, 1–8.

Reddy, B.S., Reddy, R.K.K., Reddy, B.P., Ramakrishna, S., Diwan, P.V., 2008. Potential in vitro antioxidant and protective effects of *Soymida febrifuga* on ethanol induced oxidative damage in HepG2 cells. Food Chem. Toxicol. 46, 3429–3442.

Rosy, B.A., Joseph, H., Rosalie, 2010. Phytochemical, pharmacognostical, antimicrobial activity of *Indigofera spalathoids* Vahl. (Fabaceae). Int. J. Biol. Technol. 1, 12–15.

Roy, A., Khanra, K., Mishra, A., Bhattacharyya, N., 2012. General analysis and antioxidant study of traditional fermented drink Handia, its concentrate and volatiles. Adv. Life Sci. Appl. 1, 54–57.

Saha, K., Lajis, N.H., Israf, D.A., Hamzah, A.S., Khozirah, S., Khamis, S., Syahida, A., 2004. Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. J. Ethnopharmacol. 92, 263–267.

Sarla, S., Prakash, M.A., Apeksha, R., Subhash, C., 2011. Free radical scavenging (DPPH) and ferric reducing ability (FRAP) of *Aphanamixis polystachya* (Wall) Parker. Int. J. Drug Dev. Res. 3, 271–274.

Scalbert, A., 1991. Antimicrobial properties of tannins. Phytochemistry 30, 3875–3883.

Sein, T.T., Spurio, R., Cecchini, C., Cresci, A., 2008. Screening for microbial strains degrading glass fiber acrylic composite filters. Int. Biodeterior. Biodegradation 63, 901–905.

Shirwaiar, A., Shirwaikar, A., Punitha, I.S.R., 2007. Antioxidant studies on the methanol stem extract of *Coscinium fenestratum*. Nat. Prod. Sci. 13, 40–45.

Sofowara, A., 1993. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria.

Srinisavan, R., Chandrasekar, M.J.N., Nanjan, M.J., Suresh, B., 2007. Antioxidant activity of Caesalpinia digyna root. J. Ethnopharmacol. 113, 284–291.

Srinivasan, G.V., Ranjith, C., Vijayan, K.K., 2008. Identification of chemical compounds from the leaves of *Leea indica*. Acta Pharm. 58, 207–214.

Srinivasan, G.V., Sharanappa, P., Leela, N.K., Sadashiva, C.T., Vijayan, K.K., 2009. Chemical composition and antimicrobial activity of the essential oil of *Leea indica* (Burm. f.) Merr. flowers. Nat. Prod. Rad. 8, 488–493.

Srithi, K., Balslev, H., Wangpakapattanawong, P., Srisanga, P., Trisonthi, C., 2009. Medicinal plant knowledge and its erosion among the Mien (Yao) in northern Thailand. J. Ethnopharmacol. 123, 335–342.

Sule, W.F., Okonko, I.O., Omo-Ogun, S., Nwanze, J.C., Ojezele, M.O., Ojezele, O.J., Alli, J.A., Soyemi, E.T., Olaonipekun, T.O., 2011. Phytochemical properties and in vitro antifungal activity of *Senna alata* Linn. crude stem bark extract. J. Med. Plants. Res. 5, 176–183.

Suresh, P.K., Sucheta, S., Sudarshana, V.D., Selvamani, P., Latha, S., 2008. Antioxidant activity in some selected Indian medicinal plants. Afr. J. Biotechnol. 7, 1826–1828.

Trease, G.E., Evans, W.C., 1989. Pharmacognosy, 11th ed. Brailliar Tiridel and Macmillian Publishers, London, UK.

Vinay, R.P., Prakash, R.P., Sushil, S.K., 2010. Antioxidant activity of some selected medicinal plants in western region of India. Adv. Biol. Res. 4, 23–26.

Yusuf, M., Wahab, M.A., Yousuf, M.D., Chowdhury, J.U., Begum, J., 2008. Some tribal medicinal plants of Chittagong Hill Tracts, Bangladesh. Bangladesh J. Plant. Tax. 14, 117–128.

Wong, Y.H., Abdul Kadir, H., 2012. Induction of mitochondria-mediated apoptosis in Ca Ski human cervical cancer cells triggered by Mollic Acid Arabinoside isolated from *Leea indica*. eCAM, 684740.

Wong, Y.H., Abdul Kadir, H., 2011. *Leea indica* Ethyl acetate fraction induces growth-inhibitory effect in various cancer cell lines and apoptosis in Ca Ski human cervical epidermoid carcinoma cells. eCAM, 293060.

Zuraini, Z., Yoga Latha, L., Suryani, S., Sasidharan, S., 2007. *Anticandida albicans* activity of crude extract of the local plant, winged beans leaf. Plant Kuala Lumpur 80, 653–657.