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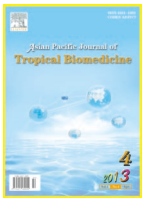

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Antioxidant, antibacterial and cytotoxic effects of the phytochemicals of whole *Leucas aspera* extract

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PEER REVIEW

Peer reviewer

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Comments

This is an interesting study in which the authors evaluated the antioxidative, antibacterial and cytotoxic effects of *L. aspera* extract. Materials and methods are well designed. Findings are interesting and interpreted scientifically in discussion section.

(Details on Page 278)

ABSTRACT

Objective: To investigate the antioxidant, antibacterial and cytotoxic activity of whole *Leucas aspera* (Labiatae) (*L. aspera*) alcoholic extract. **Methods:** Whole *L. aspera* powder was extracted by absolute ethanol (99.50%). The ethanolic extract was subjected to antioxidant, antibacterial and brine shrimp lethality assay. **Results:** The extract showed potent radical scavenging effect (antioxidant) with IC_{50} value of (99.58±1.22) μg/mL which was significant (P<0.01) in comparison to ascorbic acid with IC_{50} value of (1.25±0.95) μg/mL. In case of antibacterial screening, the extract showed notable antibacterial effect against the tested microbial strains. Significant (P<0.05) zone of inhibitions against Gram positive *Bacillus subtilis* [(12.00±1.32) mm] and *Bacillus megaterium* [(13.00±1.50) mm], *Staphylococcus aureus* [(8.00±0.50) mm] and Gram negative *Salmonella typhi* [(6.00±0.50) mm], *Salmonella paratyphi* [(8.00±1.00) mm], *Shigella dysenteriae* [(9.00±1.32) mm] and *Vibrio cholerae* [(9.00±0.66) mm] was observed. In brine shrimp lethality bioassay, the extract showed the LC_{50} value as (181.68±2.15) μg/mL which was statistically significant (P<0.01) compared to positive control vincristine sulfate [LC_{50}=(0.76±0.04) μg/mL]. **Conclusions:** The results demonstrate that the ethanolic extract of *L. aspera* could be used as antibacterial, pesticidal and various pharmacologic actives.

KEYWORDS

Leucas aspera, Radical scavenging, Antibacterial, Cytotoxic, Probit

1. Introduction

The investigation of medicinal properties of various plants attracted an increasing interest since last couple of decades because of their potent pharmacological activities, convenience to users, economic viability and low toxicity[1]. This regained interest to plant-derived medicines is basically due to the multidrug resistance of many antibiotics as well as current widespread perception that green medicine is safe and dependable than the expensive synthetic drugs most of which have adverse effects[2]. This belief and perception could lead to the exploration of new indigenous herbal medicines.

Since ancient times, the medicinal properties of plants have been investigated for scientific advancement throughout the world due to their potent radical scavenging activities. As antioxidants have been reported to prevent oxidative stress and damage caused by free radical, they can interfere with the oxidation process by reacting with free radicals, chelating agents, catalytic metals and also by acting as oxygen scavengers[3,4]. As a result, a recent upsurge of interest has been made in the therapeutic potentials of plants as antioxidants in reducing free radical induced tissue injury. Although several synthetic

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antioxidants, such as ascorbic acid, butylated hydroxyanisole and butylated hydroxytoluene, are commercially available, they are quite unsafe and their toxicity is a problem of concern[5].

In recent years, multidrug resistance in human pathogenic microorganisms has been developed due to indiscriminate prescription and malpractice of commercially available antimicrobial drugs, which are mainly used in treatment of infectious diseases. This situation forced scientists to search for new antimicrobial agents from various sources like medicinal plants which are good sources of novel antimicrobial agents[6]. *Leucas aspera* (*L. aspera*), belonging to the family of Labiatae, is a common aromatic herb known as Darkolos or Dandokolos in Bangladesh and found as weed in Asia-temperate, Africa and Asia-tropical countries. Different parts of this plant (root, flower, leaf, stem) have been found to have antioxidant, antibacterial and cytotoxic effect[7]. Ethyl acetate extract of this plant has been evaluated for *in-vitro* activity against *Plasmodium falciparum* and assessed for cytotoxicity against HeLa cell line[8]. The efficacy of whole plant extracts of *L. aspera* has been proven on larvicidal and pupicidal activities against the malarial vector *Anopheles stephensi*[9]. Leaves of *L. aspera* are useful in chronic rheumatism, psoriasis, scabies, chronic skin eruptions and their juice is used as antibacterial agent. Its chloroform and ether extracts possess antifungal activity[10]. Traditionally, the whole plant is taken orally for analgesic, antipyretic, antirheumatic, anti-inflammatory and antibacterial treatment and its paste is applied topically to inflamed areas[11]. Apart from this, the entire plant is also used as an insecticide and indicated in traditional medicine for coughs, colds, painful swelling and chronic skin eruption[12]. Its anti-inflammatory activity has been shown in animal models[13,14] through prostaglandin inhibition[15,16]. The plant possesses wound healing property and is used in cobra venom poisoning[17]. Chemical components like diterpenes, tannins, saponins, sterols, oleic, linoleic, palmitic, stearic, oleanolic and alkaloids have been isolated from this plant[18,19].

This study aimed to evaluate the antioxidant effect of the whole plant extract in comparison with commercial standard antioxidant ascorbic acid. The study also investigated the antibacterial activity of the extract using reference antibiotic tetracycline. Cytotoxicity was also compared with the standard agent vincristine sulfate.

2. Materials and methods

2.1. Plant materials

Whole plants were collected from the abandoned land of Chittagong University Campus. The plants were taxonomically classified and identified scientifically by Dr. Saikh Bokhtear Uddin, Associate Professor and Taxonomist, Department of Botany, University of Chittagong, Bangladesh. A voucher specimen was preserved in Bangladesh National Herbarium with the accession No. 36070.

2.2. Chemicals and reagents

Absolute ethanol (99.50% v/v) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich, Munich, Germany. Ascorbic acid (BDH, England) and tetracycline disc (50 μg/disc, Oxoid, England) were used as reference standard as well as positive control for free radical scavenging and antibacterial screening assay, respectively. Vincristine sulfate (Merck, Germany) was used as reference cytotoxic agent in brine shrimp lethality test.

2.3. Extract preparation

Whole plants were ground into powdered form with a grinder (Moulinex Blender AK-241, Moulinex, France). Collected powder (40-80 mesh, 900 g) was then soaked in 2.5 L ethanol in a conical flask and let to soak for 7 d at room temperature (23 ±0.5) °C. Removal of whole dry plants was done by filtration through cheesecloth and Whatman filter paper No. 1. The filtrate was then further concentrated under reduced pressure at the temperature below 50 °C using rotary evaporator (RE 200, Sterling, UK). The extracts were placed in glass Petri dishes (90 mm×15 mm, Pyrex, Germany). Total 72 g of dried crude extract (blackish-green, yield 5.5% w/w) was found which was then re-dissolved in ethanol to obtain a solution containing 2.0 mg/mL of extract to be used for further assays.

2.4. Qualitative phytochemical group tests

The extract was subjected to qualitative screening for the detection of phytochemical groups by established methods[20]. In each test 10% (w/v) solution of the extract was taken unless otherwise mentioned in the individual test.

2.5. Antioxidant activity (DPPH assay)

The free radical scavenging effect of *L. aspera* extract and ascorbic acid was assessed with the stable scavenger DPPH with slight modifications of the method described by Silva *et al*[21]. Briefly, the concentrations (25, 50, 100, 200, 400 and 800 μg/mL) of *L. aspera* extract were prepared in ethanol. Positive control ascorbic acid solution was made with the concentration between 1-100 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. The degree of DPPH-purple decolorization to DPPH-yellow indicated the scavenging efficiency of the extract. The absorbance of the mixture was taken at 517 nm using UV-Visible spectrophotometer (UV-VIS 1 200, Shimadzu Corporation, Japan). Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. The scavenging activity against DPPH was calculated using the following equation: Scavenging activity (%)=[(A-B)/A]×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). The percentage of scavenging of the extract was compared with positive control.

2.6. IC_{50} value of the extract

Based on the screening results of the triplicate measurement of the extract, 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 DPPH radical concentration by 50%. Lower IC_{50} value indicates the higher radical scavenging effect.

2.7. Antimicrobial activity of the plant extracts

2.7.1. Microorganisms

Gram positive *Bacillus cereus* (*B. cereus*), *Bacillus subtilis* (*B. subtilis*), *Bacillus Megaterium* (*B. megaterium*), *Staphylococcus aureus* (*S. aureus*) and Gram negative *Escherichia coli* (*E. coli*), *Salmonella typhi* (*S. typhi*), *Salmonella paratyphi* (*S. paratyphi*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Vibrio cholerae* (*V. cholera*), and *Shigella dysenteriae* (*S. dysenteriae*) were used for antimicrobial screening. All the stock cultures were collected from the Industrial Microbiology Division, Bangladesh Council of Scientific and industrial Research, Chittagong.

2.7.2. Media preparation and maintenance of bacteria

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[22].

2.7.3. Antimicrobial screening by disk diffusion technique

The antimicrobial activity of the extract was determined by disk diffusion technique[23]. The test microbes were taken from the broth culture with inoculating loop and transferred to test tubes containing 5.0 mL 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 plate was allowed to dry. Sterilized Whatman paper disks (6 mm in diameter) were treated with desired concentration of previously prepared ethanolic solution of extract using a micropipette and dried in air under aseptic condition and placed at equidistance in a circle on the seeded plate. The concentration of the extract used was 2 mg/disc. These plates were kept for 4-6 h at low temperature and the test materials diffuse from disc to the surrounding medium by this time. The same was done for ethanol (negative control) as well as tetracycline (positive control). The experiment was conducted in triplicates. The plates were incubated at 37 °C for 24 h. At the end of the period, the inhibition zone against each microorganism by plant extract was measured and analyzed by using one way ANOVA followed by Tukey's *post hoc* test for multiple comparisons using descriptive statistic in SPSS 18.0 (*post hoc* test).

2.8. Brine shrimp lethality bioassay of the extract

Brine shrimp lethality bioassay was carried out according to Meyer *et al*[24] 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 from 20 μg/mL to 1 000 μg/mL of the extract. Five milliliter of artificial sea water was added into all the 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 in 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 of (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[25] as well as linear regression equation using the software "BioStat-2009".

2.9. Statistical analysis

All data are presented as mean±standard deviation (SD) and were analyzed by One-way analysis of variance (ANOVA) (Tukey, Post hoc, SPSS for windows, version 18.0, IBM Corporation, NY, USA). The values were considered significantly different at $P<0.05$.

3. Results

3.1. Qualitative phytochemical screening of L. aspera extract

The phytochemical screening showed the presence of alkaloids, flavonoids, terpenoids, tannins, phlobatannins, saponins and glycosides. The results and observations were summarized in Table 1.

3.2. Antioxidant activity of L. aspera extract

The results of the free radical scavenging effect of the plant extract and ascorbic acid was presented in Figure 1. The results of one way ANOVA test and *post hoc* test indicated that there was a significant ($P<0.001$) difference of mean percentage scavenging effect between all the tested concentrations of the extract and commercial antioxidant ascorbic acid. The results showed that the extract exhibited the greatest free radical scavenging activity with a mean percentage of (86.62±0.49)% at the concentration of 800 μg/mL whereas the same concentration of ascorbic acid exhibited the mean radical scavenging activity of (97.34±1.22)%. The inhibition concentration of the extract was determined by plotting

a graph of percentage scavenging activity against the log concentration of the extract (Figure 2). The IC_{50} value of the extract and ascorbic acid was (99.58±1.62) μg/mL and (1.25±0.21) μg/mL, respectively. This value suggested that the radical scavenging activity of *L. aspera* whole extract was very high because the cutoff value was 1 000 μg/mL. The value higher than this indicated that the extract or other synthetic antioxidant was not effective as radical scavenger.

3.3. Antibacterial activity of L. aspera extract

Antibacterial activity results of *L. aspera* ethanolic extract were given in Table 2. In general, the mean zone of inhibition produced by the reference antibiotic, tetracycline, was between 15 to 25 mm and was larger than that produced by the extract which was between 9 to 13 mm. The extract showed the highest zone of inhibition against the Gram positive *B. subtilis* [(12.00±1.32) mm] and *B. megaterium* [(13.00±1.50) mm] and Gram negative *P. aeroginosa* [(13.00±1.00) mm], *V. choleriae* [(9.00±0.66) mm] and *S. dysenteriae* [(9.00±1.32) mm]. Gram positive strains were found more sensitive than Gram negative organisms to the extract on an average. However, the extract showed the lowest antibacterial activity to *S. typhi*.

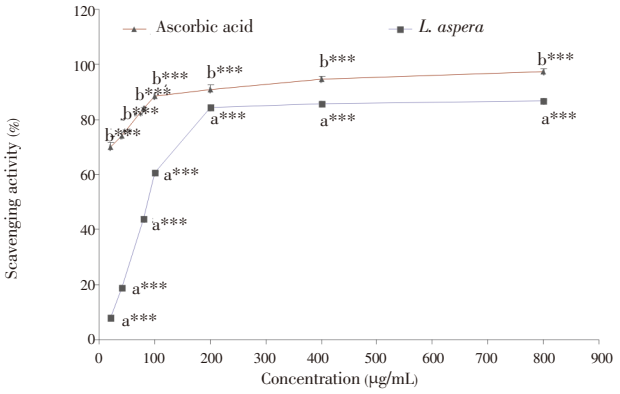

Figure 1. DPPH free radical scavenging action of *L. aspera* and ascorbic acid with the plotted concentration.
a and b: Subscript letters shown on the line graph indicate that the values are significantly different (Tukey multiple range, *post hoc* test, $P<0.05$) from each other (***$P<0.001$, SPSS for windows, version 18.0).

Table 1
Observation on phytochemical screening for *L. aspera* extract.

Phytochemical	Name of the test	Observed changes	Result
Alkaloids	Mayer's test	Creamy white precipitate	+
	Wagner's test	Brown or deep brown precipitate	+
	Hager's test	Yellow crystalline precipitate	+
Glycosides	General test	Yellow color	+
Cardiac glycosides	Legal's test	Pink to red color	+
	Baljet's test	Yellow orange color	+
Anthraquinone glycoside	Aglycones	No bright pink, red or violet color	−
	O-glycoside	Cherry red or violet color in the aqueous layer	+
	C-glycoside	No rose red color in aqueous layer	−
Terpenoids	Salkowski test	Red color	+
Flavonoids	General test	Red color	+
	Specific test	Red to crimson color	+
Steroids	Libermann-Burchard's test	Greenish color	+
Tannins	$FeCl_3$ test	Brownish green color	+
Phlobatannins	General test	Red precipitate formation	+
Saponins	Frothing test	Formation of stable foam	+

+: Presence, −: Absence.

Table 2
In-vitro antibacterial activity of *L. aspera*.

Bacterial strains	Test organism	Zone of inhibition (mm)	
		L. aspera (2 mg /disc)	Tetracycline (50 μg/disc)
Gram positive	*B. cereus*	$7.00\pm1.01^{a***}$	$26.00\pm0.67^{b***}$
	B. subtilis	$12.00\pm1.32^{a**}$	$25.00\pm2.17^{b**}$
	B. megaterium	$13.00\pm1.50^{a**}$	$23.00\pm2.29^{b**}$
	S. aureus	$8.00\pm0.50^{a***}$	$13.00\pm2.29^{b***}$
Gram negative	*S. typhi*	$6.00\pm0.50^{a***}$	$16.00\pm3.54^{b***}$
	S. paratyphi	$8.00\pm1.00^{a***}$	$15.00\pm2.50^{b***}$
	S. dysenteriae	$9.00\pm1.32^{a***}$	$15.00\pm1.52^{b***}$
	E. coli	7.00 ± 0.75^{c}	26.00 ± 1.60^{d}
	V. cholerae	$9.00\pm0.66^{a***}$	$13.00\pm1.80^{b***}$
	P. aeroginosa	$13.00\pm1.00^{a***}$	$40.00\pm3.00^{b***}$

Data are shown as mean±SD for triplicate of concentration. Different superscript letters shown in the data indicate that the values are significantly different (Tukey's multiple range, *post hoc* test, $P<0.05$) from each other (***$P<0.001$, **$P<0.01$ SPSS for windows, version 18.0).

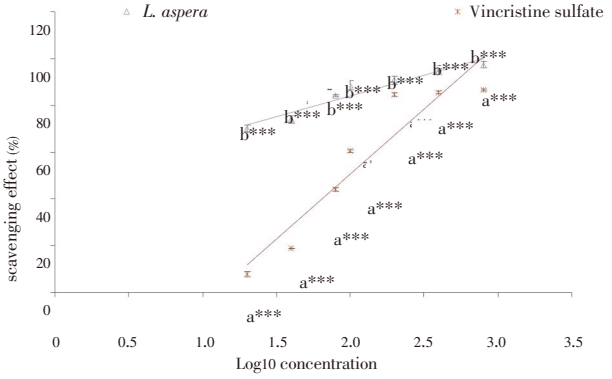

Figure 2. Determination of IC_{50} values of both the *L. aspera* extract and ascorbic acid through regression analysis.
a and b: Subscript letters shown on the regression line indicate that the values are significantly different (Tukey multiple range, post hoc test, $P<0.05$) from each other (***$P<0.001$, SPSS for windows, version 18.0).

3.4. Cytotoxic activity of L. aspera extract

The results of brine shrimp nauplii testing were presented in Figure 3. The LC_{50} value indicated the concentration by which 50% of the shrimps were killed. The effect of the extract was compared with vincristine sulfate (positive control). The LC_{50} values of the extract and vincristine sulfate were (181.68±2.15) μg/mL and (0.76±0.04) μg/mL, respectively. Probit analysis showed the "*Chi* square" value of the extract (0.76) was closed to that of vincristine sulfate (0.63). 95% confidence limit of *L. aspera* was 125.12-265.96 μg/mL, and 0.57-0.82 μg/mL for vincristine sulfate. The regression equation was Y=56.28X-76.74 and Y=3.16+2.98X, respectively.

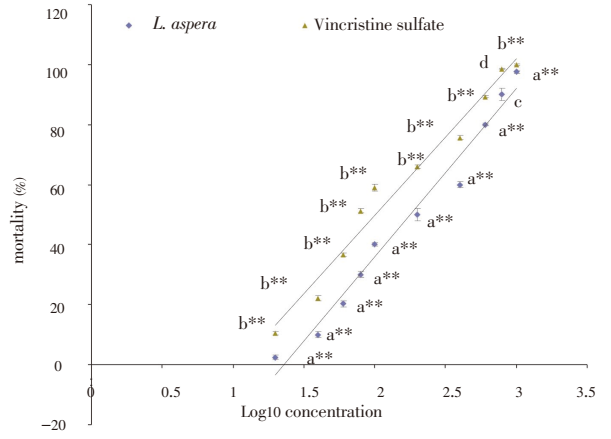

Figure 3. Determination of LC_{50} values of both the *L. aspera* extract and ascorbic acid through regression analysis.
a and b: Subscript letters shown on the regression line indicate that the values are significantly different (Tukey multiple range, post hoc test, $P<0.05$) from each other; c and d: depicts their insignificance to each other (**$P<0.01$, SPSS for windows, version 18.0).

4. Discussion

4.1. Phytochemical screening of L. aspera extract

The secondary metabolites existed in the plant extract play the key role in the pharmacological actions of the whole plant parts. This study was conducted to make a better logical approach in ascertaining the mentioned biological functions of *L. aspera* 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 shown the presence of diterpenes, tannins, saponins, sterols, oleic, linoleic, palmitic, stearic, oleanolic and alkaloids[18,19].

4.2. Antioxidant activity of L. aspera extract

To evaluate the scavenging effect of the extracts in this study, DPPH reduction was investigated against positive control ascorbic acid. The more antioxidants occurred in the extract, the more DPPH reduction will occur. High reduction of DPPH is related to the high scavenging activity performed by particular sample. At a higher concentration, the extract may exhibit more significant free radical scavenging activity. IC_{50} was calculated as amount of antioxidant present in the extracts. The IC_{50} value [(99.58±1.22) μg/mL], on the individual part of this plant, recorded in this study was varying from other reports[10,14]. This report showed the higher antioxidant effect of the whole extract than the parts of the plant. This difference in the results is probably due to the concentration of active phytochemicals responsible for antioxidant effect, and it is higher in whole extract than the plant part or parts. The solvent system used for extraction might be another reason. These results imply that the ethanolic extract of the plant shows higher radical scavenging activity which may be attributed to its stronger proton-donating abilities.

Indeed, DPPH-stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts[26]. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. 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 number of electrons captured. Ascorbic acid, the standard antioxidant in this study, acts as a chain breaking scavenging agent that impairs the formation of free radicals in the process of intracellular substances formation throughout the body, including collagen, bone matrix and tooth dentine[27].

The quantitative determination of antioxidants explored that high quantity of scavenging substances are found to be in *L. aspera* which plays the key role in showing free radical scavenging activity of this plant[28]. And this leads to the conclusion that the plants with such scavenging capacity of reducing free-radical induced tissue injury could be the source of therapeutic importance[29].

4.3. Antibacterial activity of L. aspera extract

Plant has long been a very important source of drug and many plants have been screened whether they contain compounds with therapeutic activity[30]. Therefore, it is vital to evaluate the antimicrobial activity of *L. aspera*. The bacterial strains were chosen to be studied as they are important pathogens and rapidly develop antibiotic resistance as antibiotic use increases. In disc diffusion technique, the mean zone of inhibition produced by the commercial antibiotic, tetracycline, was larger than those

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[31]. In classifying the antimicrobial activity it would be generally expected that much greater number would be active against Gram positive than Gram negative bacteria[32]. However, in this study, the plant extract was effective against both Gram positive and Gram negative bacteria suggesting the presence of broad spectrum of antibiotic compounds or simply general metabolic toxin in the plant extract[33].

In a research conducted by using the entire plant of *L. aspera*, Srinivasan *et al*[34] indicated that the aqueous extract of the whole plant exhibited a higher range of zone of inhibition against *S. aureus*, *E. coli* and *P. aeruginosa*. These results are consistent with those of our study although the solvent system was different. However, the aqueous extract did not exhibit any activity against *S. typhi* whereas this study showed that the organic extract showed mild activity against this strain, which implies that the secondary metabolites responsible for antibacterial activity are greatly dependent on solvent system for their release in extractive. Moreover, growth area also affects the chemical components of the plants and leads to the activity difference. Another fact that these organisms have intrinsic resistance from a restrictive outer membrane barrier and transenvelope multidrug resistance pumps (MDRs)[35].

4.4. Cytotoxic activity of L. aspera extract

Brine shrimp lethality is a general bioassay which is indicative of cytotoxicity, antibacterial activities, pesticidal effects and various pharmacologic actions[24]. 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. LC_{50} from the regression and probit analysis[25] in 24 h showed that LC_{50} value of *L. aspera* extract was (181.68±2.15) μg/mL (confidence limit 95%) where the lower and upper limits were 265.96 and 125.12 μg/mL. Comparison of this result with the standard vincristine sulfate [(0.76±0.04) μg/mL] indicated that the lethality of *L. aspera* extract is statistically significant (P<0.001) suggesting the notable clinical importance of the extract against tumor cells, pesticides *etc*., since the brine shrimp assay is considered as convenient probe for preliminary assessment of toxicity, detection of fungal toxins, pesticidal and anti-tumor effect and other pharmacological actions[24]. Recent studies on the ethyl acetate extract of whole *L. aspera* have already proved the larvicidal, pupicidal and cytotoxic effect against HeLa cell line[8,9]. 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 disappear any discrepancy between expected mortality rates and the actual mortality rates of freshly hatched *Artemia salina*. However, this result is consistent with the study conducted by Rahman *et al*[10] on the alcoholic root extract of *L. aspera*.

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[36]. This is also consistent with our observation because the phytochemical group analysis of the extract showed the presence of alkaloid, terpenoids, tannins, phlobatannins, steroids, flavonoids and saponins. Further toxicity studies could be conducted on individual cell line to confirm the toxic effect of phytochemical groups.

In conclusion, the study demonstrates that ethanolic extract of whole *L. aspera* has promising antioxidant and antibacterial effect to use for human health. The extract also has very prominent cytotoxic effect to be used in many pharmacological as well as biological actions. However, further pharmacological and toxicity studies on individual cell lines are also suggested to confirm this attribution.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Plant derived medicines attracted an increasing interest since last couple of decades because of their potent pharmacological activities, convenience to users, economic viability and low toxicity. Multi-drug resistance of human pathogenic organisms to synthetic medicines enforced to use phytomedicinal sources. A recent upsurge of interest has also been made in the therapeutic potentials of plants as antioxidants in reducing free radical induced tissue injury because antioxidants can fight against the oxidative stress and tissue damage while synthetic antioxidants (*e.g.* ascorbic acid, butylated hydroxyanisole, butylated hydroxytoluene) are commercially available, but quite unsafe and toxic. This situation forced scientists to search for new antimicrobial agents from various medicinal plants like *L. aspera* having no cytotoxic effect.

Research frontiers

Significant inhibitory effect of *L. aspera* against Bacillus cereus Bacillus subtilis, Bacillus Megaterium, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Salmonella paratyphi, Pseudomonas aeruginosa, Vibrio cholerae and Shigella dysenteriae have been explored with promising radical scavenging effect. The cytotoxicity of the plant extract has also been studied which could be used as anticancer therapy with further study.

Related reports

This study is quite different from the similar investigation reported by Chew *et al*. (2012) who searched on the individual parts of the *L. aspera* whereas present study is on the whole plant material extracted with different solvent system than that used by mentioned authors. Even in antimicrobial work, similar strains were not used which made the findings completely different.

Innovations and breakthroughs

This study has explored the possibility for the use of whole *L. aspera* as pharmaceutical formulation without toxic effect.

Use of this material against some bacterial strains which were not previously studied has been showed.

Applications

It is very significant to use this plant extract as natural medicine in bacterial infections and oxidative stress with appropriate doses and administration which could be studied further. This study can lead to investigate other pharmacological application of this plant.

Peer review

This is an interesting study in which the authors evaluated the antioxidative, antibacterial and cytotoxic effects of *L. aspera* extract. Materials and methods are well designed. Findings are interesting and interpreted scientifically in discussion section.

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