



ANTIOXIDANT, ANTIBACTERIAL AND TOXICITY STUDIES OF AERIAL PARTS OF *Ludwigia adscendens* L.

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Abstract: The objective of this study was to figure out and scientifically establish the possible antioxidant, antibacterial, and toxic potentials of the plant *Ludwigia adscendens* through various established *in vitro* and *in vivo* test models. Dried and pulverized aerial part of this plant was extracted in cold with petroleum ether, chloroform and methanol and these crude extracts were subjected as such against some test to evaluate their antioxidant, toxicity and antibacterial properties. The crude methanolic extract showed more potent activity than other extracts in 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical and Nitric oxide (NO) scavenging assay methods with IC₅₀ values of 11.5 µg/mL and 28.7 µg/mL respectively. The reducing power of all the fractions were found to be concentration dependent. The methanol extract also showed the highest total phenolic content (TPC), total tannin content (TTC) and total flavonoids content (TFC) and were 105.6 mg gallic acid equivalent per g (GAE/g), 24.2 mg GAE/g, and 74.1 mg quercetin equivalent per g (QE/g) of dried plant extract, respectively. The methanol extract exhibited moderate antibacterial activity against all selected bacterial strains except *Staphylococcus aureus* at two different concentrations of 250 µg/disc and 500 µg/disc. In toxicity study, using brine shrimp nauplii the LD₅₀ value of methanol extract was found to be 25.1 µg/mL. This study demonstrates the traditionally used plant *L. adscendens* as a possible source of natural antioxidants and antimicrobial drugs.

Keywords: Antioxidant, antibacterial, cytotoxic, DPPH, *Ludwigia adscendens*, Nitric Oxide

Introduction

Life, diseases and decay are inseparable. Since ancient time, to manage the life threatening diseases caused by oxidative stress or microorganism or physical injury, different medicinal plants were used traditionally as local healers especially in developing countries. In Asia and Africa, over 80% of population's health care system depends on the herbal medicine due to the higher cost of modern medicine (Oyebode O *et al.* 2016). Various traditional health care systems are available in India and its subcontinent namely Ayurveda, Hamdard, Siddha, and Unani. Nature provides various medicinal plants which are usually considered to have fewer side effects. Approximately, 25% of modern medicine are from natural sources, and are

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administered against ailments as they were taken traditionally (Saha *et al.* 2013, Tapsell *et al.* 2006). Based on folklore uses, the researchers and pharmaceutical industries are consistently interested to search the effective pharmacological activities from natural products as well as isolation of chemical constituents which might be economic as well as effective for developing novel drugs with less side effects.

The plant *Ludwigia adscendens* (Local name: Kasardam) is also known as water primrose under the family of Onagraceae that consists of at least 82 species, available in East Asia, North Australia, South America and familiar as a weed in tropical Africa. *L. adscendens* is an aquatic ascending herb being 60 cm in length attached with branched and floating obovate stems which are 4 m tall. Leaves are thick and 1.25-7.6 cm long. Flowers are white in nature with very slightly yellow in base and obovated petals are about 1.25 cm long (Chen J *et al.* 1953). Traditionally, the whole plant is used as to treat ulcers, a variety of skin infections, and also as an antidiysenteric, emetic, astringent, antihelmintic and diuretic (Ahmed *et al.* 2005). Flowers are used as an anti-inflammatory agent and the leaves and stems possess antiseptic property (Ahmed *et al.* 2005). Previous literature study reported diverse phytoconstitents such as flavonoids (quercitrin, quercetin 3-*O*-rhamnoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-rutinoside, and myricetin 3-*O*-galactoside, kaempferol 3-*O*-glucoside, myricetin 3-*O*-rhamnoside ormyricitrin) (Glasby 1991, Shilpi *et al.* 2010) and several alkanes (Barik *et al.* 2004). Shilpi *et al.* also reported some chemical constituents from *L. adscendens* like: betulonic acid, betulin, and betulinic acid, squalene, phytosterols (24R)-6b-hydroxy-stigmasta-4-en-3-one, (22E,24R)-6b-hydroxy-stigmasta-4,22-dien-3-one, ellagic acid derivatives (pteleoellagic acid and 3,3,4-tri-*O*-methyl ellagic acid), some flavonoids [dihydroquercetin and afzelin (kaempferol 3-*O*-rhamnoside)], protocatechuic acid and methyl gallate (Shilpi *et al.* 2010).

The present research was undertaken to evaluate *in vitro* free radical scavenging activity, antibacterial, and general toxicity potentials of the crude extracts of *L. adscendens* (with three different solvents) with a view to establish the scientific basis of its traditional uses.

Materials and Methods

Plant collection: The fresh plant was collected from the pond of the Khulna University, Khulna, Bangladesh, during the month of April, 2015 and authenticated by the National Herbarium of Mirpur, Dhaka, Bangladesh (sample no. DACB-37524) and a voucher specimen was stored in Pharmacy Discipline, Khulna University, Bangladesh for further reference.

Extract preparation: The aerial parts were collected from the plant and shade dried for 14 days. The plant materials were then pulverized into coarse powder using grinding machine. Cold extraction was carried out using three different solvents system, successively with petroleum ether, chloroform and methanol in an air tight glass container for 5 days with occasional shaking. Solvent was evaporated off under reduced pressure using a rotary evaporator at 45°C and after workup, the crude extracts were labeled as petroleum ether extract (LAPE), chloroform extract (LACF), and methanol extract (LAME) and stored at 4°C in a refrigerator.

Test microorganisms: Both Gram-positive and Gram-negative bacterial strains were collected from Microbiology Laboratory of International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) and stored in Microbiology Laboratory of Pharmacy Discipline, Khulna University. The following bacterial strains were used in this study: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Shigella dysenteriae*, *Escherichia coli*, *Shigella flexneri*, *Shigella sonnei*, *Shigella boydii*.

Chemicals and reagents: Ascorbic acid, aluminum chloride, sodium hydroxide, monosodium phosphate, disodium phosphate, sodium carbonate, sodium nitroprusside, trichloroacetic acid, potassium ferricyanide, ferric chloride, acetic acid, were purchased from Merck, Germany. Folin-Ciocalteu's reagent and 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin, griess reagent were purchased from Sigma Chemical Co. Ltd., (St. Louis, MO, USA).

Determination of total phenolics: Total phenol content of the extracts of *L. adscendens* was determined using Folin Ciocalteu's (FC) reagent (Marinova *et al.* 2005). Gallic acid was used as standard at different concentrations (400, 200, 100, 50, 25, 12.5, 6.25 mg/L) in methanol for making a standard calibration curve. For analysis, extracts were dissolved in methanol using ultrasonic sound bath for 15 min. Then the solution (2 mL) was centrifuged at 14000 rpm for 5 min. Supernatant (1 mL) was taken into 25 mL volumetric flask and 9 mL distilled water was added. Diluted FC solution (1 mL) was added to all the flasks and mixed properly with shaking. After 5 min, 10 mL Na₂CO₃ (7%) was added to individual flask and adjusted the volume up to 25 mL with distilled water. The flasks were then incubated at room temperature for 60 min and absorbances were recorded at 750 nm against blank. The blank solution was made similarly without addition of extract and standard. Using calibration curve, the amount of gallic acid was calculated and the result was expressed in terms of mg gallic acid equivalent per g of dried plant extract.

Determination of total flavonoids: The determination of flavonoids content of the extracts of *L. adscendens* was conducted by aluminium colorimetric method (Chang *et al.* 2002) using quercetin as a reference compound. Each extract (1 mL) and standard quercetin at a concentration of 400, 200, 100, 50, 25, 12.5 and 6.25 µg/mL were taken into 10 mL volumetric flask. Then 0.3 mL NaNO₂ (5%) were added in each mixture followed by addition of 4 mL distilled water. After 5 min, sequentially 0.3 mL AlCl₃ (10%) and 2 mL of 1M NaOH were added to the mixture and made up the volume up to 10 mL using distilled water and shaken vigorously. The absorbance was taken at 510 nm using an UV spectrophotometer. Standard calibration curve for quercetin was developed and flavonoids content of the extract was determined expressed as mg quercetin equivalent per g of dried mass.

Determination of total tannins content: Total tannins content of the extracts were measured using Folin Ciocalteu's (FC) reagent (Murshid *et al.* 2015). 7.5 mL distilled water and 0.5 mL Folin Ciocalteu's reagent was added to 0.1 mL extract. Then, 1 mL sodium carbonate (35%) was added to the test tubes and the final volume was adjusted to 10 mL with distilled water. The mixture was then shaken well and incubated at room temperature for 30 min. Absorbances were measured at 725 nm against blank. The calibration curve was constructed by plotting the absorbance of different concentrations of standard gallic acid

(400, 200, 100, 50, 25, 12.5, and 6.5 µg/mL). The result of total tannins content of extracts were expressed in terms of mg GAE per g of plant extract.

DPPH scavenging activity assay. Antioxidant activity of different extracts of *L. adscendens* was measured using stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method (Saha *et al.* 2013). For analysis, crude extracts were dissolved in methanol to obtain the desired concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, 1 µg/mL, and applying serial dilution method. Then, solution (1 mL) from each concentration was taken and mixed with 3 mL of 0.004% DPPH solution and incubated in dark place for 30 min at 25 °C. The reduction of the DPPH radical was assessed by measuring the absorbance at 517 nm. Ascorbic acid was used as a standard antioxidant.

The radical scavenging activity of the extract was calculated using formula:

$$\text{Scavenging activity} = \left(1 - \frac{A_1}{A_0}\right) \times 100\%$$

Where A_0 is the absorbance of control and A_1 is the absorbance of sample or standard.

Reducing power assay. The reducing power was determined according to the method of Oyaizu (Oyaizu 1986). Ascorbic acid was used as a referenced compound and reducing power of the plant extracts were compared with that. Various concentrations (6.25, 12.50, 25, 50, 100, 200, 400 µg/mL) of the sample and standard were prepared. 1 mL of mixture of each concentration was mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide with shaking. After that, the mixture was incubated at 50 °C for 30 min and added 2 mL 10% trichloroacetic acid in each tube. The mixture was centrifuged for 10 min at 3000 rpm. Supernatant layer (2.5 mL) was collected and mixed with 2.5 mL distilled water and 0.5 mL of 0.1% (w/v) fresh ferric chloride. The absorbance was measured at 700 nm against the blank (without standard and extract).

Nitric oxide scavenging assay. Nitric oxide scavenging assay was carried out to determine the free radical, NO neutralizing capacity of plant extracts spectrophotometrically (Green *et al.* 1982). For analysis, plant extract (0.3 mL) was mixed with sodium nitroprusside (5mM) in standard phosphate buffer solution. The reaction was incubated at 25 °C for 1 h. Then, 0.5 mL incubated solution was mixed with griess reagent (1 mL) and the absorbance was measured at 546 nm against blank solution. Ascorbic acid was taken in this analysis as a standard.

The NO free radical scavenging activity of the plant extract was calculated using this formula:

$$\text{Scavenging activity} = \left(1 - \frac{A_1}{A_0}\right) \times 100\%$$

Where A_0 is the absorbance of control and A_1 is the absorbance of sample or standard.

Determination of antibacterial activity. Disc diffusion assay (Bauer *et al.* 1966, Islam *et al.* 2013) was used to evaluate the antibacterial activity. Antibacterial activity of different extracts was tested against selected Gram positive and Gram negative bacterial strains. Second generation subculture was prepared using each lab stock bacterial strain. Sterile loop was used to transfer a small portion of subculture into vials having nutrient broth medium. The medium was incubated at 37 °C for 16 h to maintain log phase of bacterial growth. The standard inoculum suspension was taken in nutrient agar medium and mixed properly. Then the seeded nutrient agar medium was poured into Petri-dishes, and allowed to solid formation at 25 °C maintaining aseptic environment. Standard antibiotic discs (kanamycin, 30 µg/disc, Oxoid Ltd, UK), blank discs as a control (infused with ethanol) and discs impregnated with the test extracts (250 and 500 µg/disk) were placed on the Petri-dishes using sterile forceps. Then Petri-dishes were incubated at 37 °C for 16 h. After incubation, zone of inhibition was measured using digital slide calipers.

Brine shrimp lethality bioassay. *Artemia*, a genus of aquatic crustaceans known as brine shrimp was used as test organisms to measure the toxic activity of extracts of *L. adscendens* (Meyer *et al.* 1982). For artificial sea water, 18 g of table salt and 20 g of pure NaCl were dissolved in 1 L of distilled water and then filtered. It was kept in a rectangular tank of two compartments with perforated divider. Brine shrimp eggs (*Artemia salina* Leach) were taken into the larger dark compartment. Oxygen supply was maintained continuously by electric air pump and hatching was performed at constant temperature (25-30 °C) for 48 h. After hatching, matured nauplii were collected from the lightened compartment. Various concentrations (320, 160, 80, 40, 20, 10, 5 µg/mL) of extracts was prepared. DMSO was used to facilitate mixing ensuring the concentration of DMSO did not exceed 0.1%. Then 5 mL of each concentration was transferred into test tubes containing 10 nauplii each tube. Vincristine sulphate was used as positive control and treated at different concentrations of 5, 2.5, 1.25, 0.625 and 0.312 µg/mL. All the test tubes were kept at 25 °C for 24 h. Then each tube was observed with the help of a magnifying glass and the alive nauplii were counted.

Statistical analysis: All experimental data on antioxidant and antibacterial activity are the mean of triplicates experiment and results are expressed as mean ± S.D (standard deviation). The IC₅₀ values were measured using Prism 6.0 software (GraphPad software Inc., San Diego, CA). Total phenolic, total flavonoid and total tannin were carried out using in the Microsoft Excel program.

Results

Total phenolics content: Absorbance of gallic acid was used to prepare calibration curve ($y = 0.037x - 0.015$, $R^2=0.975$) and it was found that the methanol extract of *L. adscendens* contained more phenolic compounds than other extracts which are presented in Table 1.

Total flavonoids content: The flavonoids content of plant extracts were calculated from the quercetin calibration curve ($y = 0.066x + 0.012$; $R^2=0.991$) and methanol extract revealed the highest content of flavonoids in comparison to other extracts (Table 1).

Total tannins content: Based on gallic acid calibration curve, the methanol extract contained more tannins and were presented in Table 1.

Table 1: Total phenolics, flavonoids, and tannins content of aerial extracts of *L. adscendens*

Plant extracts (<i>L. adscendens</i>)	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg GAE/g)
LAPE	8.5±0.155	7.6±0.300	5.7±0.288
LACE	29.4± 0.449	21.5±0.312	15.8±0.190
LAME	105.6±.240	74.1± 0.423	24.2±0.592

***In vitro* DPPH scavenging potential:** In DPPH scavenging assay, the plant extracts showed significant scavenging activity. The methanol extract exhibited more scavenging activity compared to other extracts. The observed IC₅₀ (µg/mL) values were presented in figure 1 and table 2.

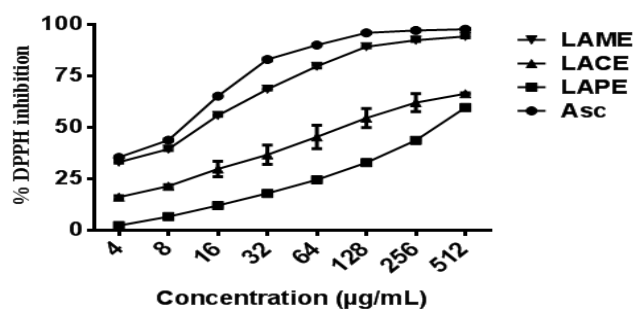


Fig. 1: DPPH scavenging activity of petroleum, chloroform, and methanol extract of *L. adscendens* and ascorbic acid (standard)

Nitric oxide Inhibition capability. The inhibition capacity of all the three extracts were found to be concentration dependent and in figure 2, it has been shown that the inhibition of NO radical value was in following sequence: LAME>LACE>LAPE

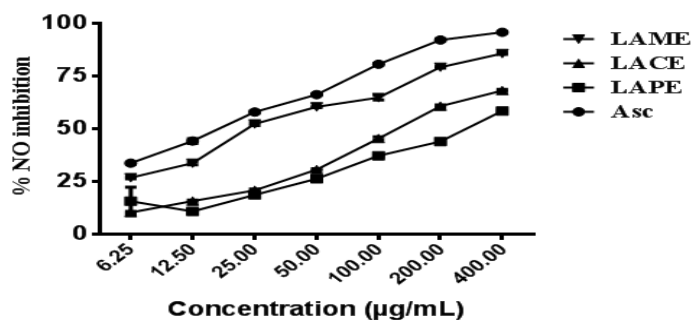


Fig. 2: NO radical scavenging activity of petroleum, chloroform, and methanol extract of *L. adscendens* and ascorbic acid

Table 2: Comparative study of free radical scavenging activity of different extracts of *L. adscendens* and standard (ascorbic acid)

IC ₅₀ (µg/mL) value of different extracts of <i>L. adscendens</i>		
Plant extracts/ standard	DPPH radical	NO radical
LAPE	326.8	269.26
LACE	118.3	131.3
LAME	11.5	28.7
Ascorbic acid	8.0	16.7

Reducing power. Fig. 3 reveals the concentration dependent significant reducing abilities for all extracts of *L. adscendens*.

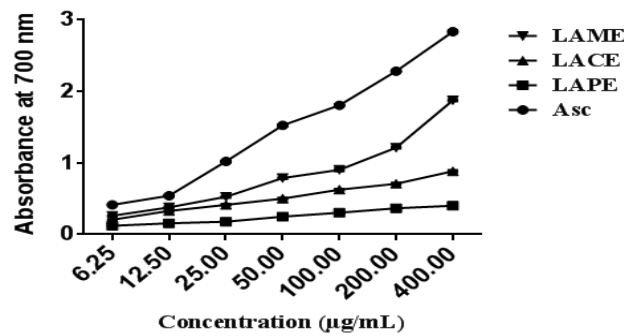


Fig. 3: Reducing power assay of petroleum, chloroform, and methanol extract of *L. adscendens* with ascorbic acid.

Antibacterial assay. The methanol extract exhibited inhibitory activity against all the tested bacterial strains except *Staphylococcus aureus*. The petroleum ether extract showed no activity in any tested bacterial strain. The diameters of observed zone of inhibition were 7 to 19 mm and represented in Table 3.

Table 3: Antibacterial activity of different extracts of *L. adscendens*

Bacterial strains	Zone of Inhibition in mm						
	LAPE		LACE		LAME		Kanamycin 30 µg / disc
	250 µg/ disc	500 µg/ disc	250 µg/ disc	500 µg/ disc	250 µg/ disc	500 µg/ disc	
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	27±0.5
<i>Staphylococcus epidermidis</i>	-	-	-	-	13±1	17±1	24±1.5
<i>Streptococcus pyogenes</i>	-	-	-	-	8±1	12±1.7	25±1
<i>Streptococcus agalactiae</i>	-	-	-	-	9±0.5	11±0.5	20±2
<i>Enterococcus faecalis</i>	-	-	7±2	9±2	7±1	10±1	24±1.7
<i>Shigella dysenteriae</i>	-	-	7±1	10±1	16±2	19±2	25±1.5
<i>Escherichia coli</i>	-	-	-	-	13±1	15±0.2	32±2
<i>Shigella flexneri</i>	-	-	8±1	8±1	10±1	11±1	29±1.5
<i>Shigella sonnei</i>	-	-	-	-	12±2	16±2	29±1
<i>Shigella boydii</i>	-	-	-	-	14±1.5	19±1	30±0.5

(-): No inhibition

Toxicity assay. The toxicity was concentration dependent and the highest lethality was shown by the methanol extract when compared with positive standard vincristine sulphate (Table 4).

Table 4: LD₅₀ value of LAME, LACF, LAPE and vincristine sulphate (standard)

Test sample	LD ₅₀ (µg/mL)
LAPE	171.3
LACE	52.7
LAME	25.1
Vincristine sulphate	0.7

Discussion

Plants have been taken as foods, and considered as a huge source of bioactive compounds against various ailments. Plants contain a wide range of secondary metabolites which are useful for improving the health care systems and are considered for medicinal purposes as a source of novel antioxidants. Natural antioxidants from medicinal plants, fruits, and vegetables, play an important role in neutralization of the free radicals by enhancing biological function (Gulcin 2012). These secondary metabolites from plant origin are of

great interest as they possess many promising therapeutic constituents. Naturally occurring antioxidant constituents are usually found with diverse polarities. The determination of free radical scavenging activity of the plant extracts and yield depend on the selected solvent (Shimada *et al.* 1992), And that's why various solvents were used for isolation of antioxidant phytoconstituents.

In recent past, researchers are deeply interested to find out the antioxidant activity using available methods (Chu *et al.* 2000). Hydroxyl radical (OH \cdot), hydrogen peroxide (H $_2$ O $_2$), peroxy radical (ROO \cdot), nitric oxide (NO) and peroxy nitrite anion (ONOO \cdot) are defined free radicals which have strong ability to generate major pathological manifestation in human body. Antioxidants play a potential role to block the activity of free radicals either by terminating the chain reaction or improving the cellular defense mechanism (Umamaheswari and Chatterjee 2008). Ingestion of daily rich antioxidants can significantly decrease the cell proliferation as well as metastasis by inhibition of oxidative stress or free radicals activity (Soler-Rivas *et al.* 2000, Ewan 2005). Researchers have found that various plant derived antioxidants, flavonoids can prevent the progression of cancer in animal model. In one study, naringenin, a well-known flavonoid displayed significant role in prevention of lung cancer and prolonged the life span in mice model with lower side effects (Qin *et al.* 2011).

In this study, different free radical scavenging methods were used and the results were compared to reference compound. Stable free radical, DPPH scavenging activity evaluation allow us to measure the intrinsic capability of chemicals to release an electron, to donate a hydrogen atom to reactive species, or convert them into stable form. In this analysis, the extract contains phytoconstituents (polyphenols, like flavonoids) turned the reactive DPPH radicals to nonreactive DPPH-H form (Paixão *et al.* 2007). When active DPPH gets reduced to DPPH-H, it changes its color from purple to yellow.

Several studies reported that secondary metabolites from plant origin has capability to strong inhibition against stable free radical that indicates rich antioxidant activity (Razali *et al.* 2008). The phenolics constituent found in vegetables have received considerable attention for being the main components of antioxidant activity. The antioxidant activity of phenolics has been attributed to its oxide-reduction activity, which play an important role in the adsorption or neutralization of free radicals (Karmakar *et al.* 2011). Phenolics compound exerts their scavenging activity following redox mechanism. It also helps to neutralize the lipid per-oxidation and inhibit the decomposition of reactive oxygen species (ROS) (Javanmardi *et al.* 2003, Li *et al.* 2009). The Folin Ciocalteu (FC), a mixture of Phosphomolybdate and Phosphotungstate, is widely used reagent for the determination of total phenolic components. The reaction forms a blue chromospheres established by a phosphotungstic-phosphomolybdenum complex. This analysis showed that the polar, methanol extract contained notable amounts of phenolics compound.

Reducing power serves as an important and sensitive characteristics to evaluate antioxidant activity of plant extracts (Yen *et al.* 1993). In reducing power analysis, the test sample converts yellow color to green during the reaction time depending on the ability of the sample. The plant extracts showed strong reducing ability by donating an electron and convert reduced potassium ferricyanide (Fe $^{3+}$) into ferrocyanide (Fe $^{2+}$). After addition of ferric chloride (FeCl $_3$), ferric-ferrous complex is formed as Prussian's blue and exhibits

absorbance at 700 nm. It was noticed that reducing power increased with higher concentration of each extract. Higher absorbance of the test sample ensured the strong reducing ability.

Flavonoids and tannins are recognized as prime antioxidants or free radical scavengers and also suggested that these compounds have prevented activity against mutagenesis and carcinogenesis in cells (Tsao and Akhtar 2005). Aluminum chloride colorimetric assay was used to determine the total flavonoids contents in these studies. Flavonoids are effective scavengers by donating the hydrogen to the oxidized molecules (Sandhar *et al.* 2011). Tannins are readily soluble in more polar solvent and may also play a role in plant defense to counter the reactive oxygen species (ROS) (Murshid *et al.* 2015).

Nitric oxide (NO) was identified as a threat for human health since its invention (Ebrahimzadeh *et al.* 2006) and it is responsible for creating various pathological manifestations such as sepsis, muscle diseases, HIV-associated dementia, stroke and inflammatory bowel disease (Mouokeu *et al.* 2014). Moreover, NO initiates the production of neurotoxin that causes neuronal cell damage and has been recognized as a severe threat for neuronal diseases like Parkinson's disease and Alzheimer disease (Aliev *et al.* 2009, Zhang *et al.* 2006, Nath and Madri 2006). So, the extracts were evaluated to determine the NO scavenging activity. The extracts revealed the strong NO scavenging activity in this assay.

It has been reported that some antioxidants were isolated from *L. adscendens* which strongly support the free radical scavenging activity and its traditional uses. It is known that phenolics are strong chain breaking antioxidants due to its hydroxyl group (Shahidi and Wanasundara 1992). It was found that the phytoconstituents (flavonoids) are not only responsible for antioxidant activity (Hateno *et al.* 1987, Kumar *et al.* 2012) but also exhibited some beneficial activity like antimicrobial and toxic activity (Narayana *et al.* 2011, Cushnie and Lamb 2005). Tannins were previously reported that it has ability to prevent the molecular damages caused by herbivores, insects, and microorganisms (Tamilselvi *et al.* 2012). Moreover, it may be possible that all phytoconstituents and their interaction synergistically enhanced the observed activities.

Disk diffusion method is very simple, inexpensive and widely used test to determine antibacterial activity against selected bacterial strain. In disk diffusion assay, the methanol extract showed considerable amount of zone of inhibition against all tested bacterial strains compared to other extracts. This result indicates that the compounds, responsible for antibacterial activity, are mostly polar. Although this technique is very easy way to carry out the extracts or compounds antibacterial activity but it has some limitations because non-polar compounds are unable to disperse in water properly as the agar media is made of water which may be the reason of lower zone of inhibition for bacterial strains (Moreno *et al.* 2006).

Toxicity assessment is very important consideration before drug development. Brine shrimp lethality bioassay is not only useful tool for determination of the general toxicity, but also a primary process to screen various pharmacological activities involving

antimicrobial, antitumor and pesticidal of plant extracts (Siraj *et al.* 2012). It is widely employed bench-top method due to easy laboratory technique and small amount of sample is essential for performing the test (Anderson *et al.* 1991). The mechanism involved in the toxicity of the brine shrimp can be of pharmacological effects linked to enzyme inhibition, ion channel interference or cytotoxicity (Borowitz *et al.* 1992, Silva *et al.* 2007). In this study, the methanol extract exhibited potential toxic activity against brine shrimp nauplii. This result strongly support that, the methanol extract certainly contains pharmacologically active constituents.

Conclusion

The experimental results reported here reveal the traditionally used *L. adscendens* as a possible source of natural antioxidants and antimicrobial drugs. Moreover, it justifies the usage of this plant as alternative and/or complimentary medicine. Further research is required to isolate and identify the lead compounds which are specific for such activity. This may open a new window for research in discovering better antioxidants and antibacterial drugs.

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