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

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PEER REVIEW

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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