ISSN 2278- 4136

ZDB-Number: 2668735-5

IC Journal No: 8192

Volume 2 Issue 1

Online Available at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

In vitro Antioxidant and Cytotoxic Activities of Methanol Extract of *Leucas aspera* Leaves

Md. Sekendar Ali [1]*, Mohammed Abu Sayeed [1], Md. Mahamodun Nabi [2], Md. Aziz Abdur Rahman [3]

1. Department of Pharmacy, International Islamic University Chittagong, Chittagong-4203, Bangladesh. (Contact No. +8801717927804, E-mail: sa_pharm2000@yahoo.com)
2. Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh.
3. Department of Pharmacy, Faculty of Science, University of Rajshahi, Rajshahi-6205. Bangladesh.

This study was designed to investigate the antioxidant, cytotoxicity of methanol extract of the *Leucas aspera* leaves. The drug development programs in the pharmaceutical industry. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. In the continuation of this strategy of new drug discovery we have studied the fresh leaves of *Leucas aspera. In vitro* antioxidant activity of extract was studied using DPPH radical scavenging, reducing power, total phenol and total flavonoid content determination assays. The antioxidant activity of the extracts was found promising. The reducing power of this crude extract increase with the increase of concentration; IC_{50} values of DPPH scavenging activity was 150µg/ml; Total phenol and total flavonoid content was 131.15 and 135.85 mg/ml respectively. The cytotoxic activity of crude extract was determined using brine shrimp lethality bioassay and LC_{50} values of the sample was 30.00µg/ml whereas for standard vincristine sulphate was 10.44µg/ml as a positive control.

Keyword: *Leucas aspera,* Antioxidant, Free radical and Cytotoxicity

1. Introduction

Excessive amounts of these free radicals can lead to cell injury and death, which results in many diseases such as cancer, stroke, myocardial infarction, diabetes and major disorders. Many forms of cancer are thought to be the result of reactions between free radicals and DNA, resulting in mutations that can adversely affect the cell cycle and potentially lead to malignancy. Some of the symptoms of aging such as atherosclerosis are also attributed to free-radical induced oxidation of many of the chemicals making up the body. In addition free radicals contribute to alcohol-induced liver damage, perhaps more than alcohol itself. Radicals in cigarette smoke have been implicated in inactivation of alpha 1-antitrypsin in the lung. This process promotes the development of emphysema[1]. Free radicals may also be involved in Parkinson's disease, senile and drug-induced deafness, schizophrenia, and Alzheimer's. The classic free-radical syndrome, the iron-storage disease hemochromatosis, is typically associated with a constellation of free-radical-related symptoms including movement disorder, psychosis, skin pigmentary melanin abnormalities, deafness, arthritis, and diabetes mellitus. The free radical theory of aging proposes that free radicals underlie the aging process itself, whereas the process of mitohormesis suggests that repeated exposure to free radicals may extend life span[2,3]. In 2004 the SU.VI.MAX study found providing low

dose, combination supplementation in doses easily achieved in an everyday diet decreased incidence of cancer by 31% and reduced all-cause mortality by 37%. [4] Most edible herbs' active ingredients are often concentrated to make drugs. One quarter of all drugs come from plants. However, the "inactive" ingredients seem to work together with the "active" ones in whole plants, making them safer. The entire complex of substances evolved together, so it makes sense that they interact chemically with one another. This is why one should prefer herbs to drugs whenever possible. Thus, continued research in indigenous medicinal herbs to modernize the indigenous systems and to discover new remedies from the vast treasure of indigenous plants is an absolute necessity for the benefit of suffering humanity[5].

Leucus aspera (family-Labiatae or Lamiaceae) locally known as Darunaphula is distributed in India, Bangladesh and Philippines[6]. It is typically found in dry, open, sandy soil, A diffusely branched, annual herb, 15-45 cm high; branches quadrangular, hispid. Leaves sub sessile, 2.5-7.5 cm long, linear-oblong or oblong-lance late, obtuse entire or crenate, hairy. Flowers white, sessile or sub sessile in terminal and maxillary whorls, up to 2.5 cm diam., corolla 1 cm long. Leaves contain glucosides, tannins, saponins and sterols, α and β-sitosterol, aliphatic 28-hydroxy pentatriacon-7-one, 7-hydroxydotriacontan-2-one, 1-hydroxytetratriacontan-4-one, 32-methyl tetratriacontan-8-ol, 5-acetoxy-triacontane, dotriacontanol and β-sitosterol. Two sterols and galactose have been isolated from the plant. Plant also contains oleic, linoleic, palmitic, stearic, oleanolic and ursolic acids[7]. L*eusas aspera* is reported to have antifungal, prostaglandin inhibitory, antioxidant, antimicrobial, antinociceptive and cytotoxic activities[8]. It has also hepatoprotective activity [9].

2. Materials and Methods

2.1 Plant Materials

Adequate amounts fresh leaves of *Leucus aspera* for this study were collected from the local area of Chittagong, Bangladesh and were authenticated by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, and University of Chittagong, Bangladesh. The leaves were dried at room temperature in the shade and away from direct sunlight for 5 days and in hot air oven for 2 days.

2.2 Extraction

The dried leaves were coarsely powdered and extracted by dissolving 7 days with methanol. The sediments were filtered and the filtrates were dried at 40ºC in a water bath. The solvent was completely removed by filtering with Hartman filter paper and obtained dried crude extract which was used for experiment.

2.3 Antioxidant Activity

2.3.1 DPPH Radical Scavenging Activity

The free radical scavenging activity of the methanol extract of *Leucas aspera* leaves, based on the scavenging activity of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Braca *et al.,*(2001)[10]. Crude extract (0.1 ml) was added to 3 ml of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was taken after 30 min and the percentage inhibition activity was calculated by using the equation:

$$\% \text{ scavenging activity} = [(A_0-A_1)/A_0] \text{ X100.}$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the extract. The curves were prepared and the IC_{50} value was calculated from the graph.

2.4 Reducing Power

According to the method described by Srinivas *et al.,* (2007) the reducing power

was determined[11]. The different concentrations of extract (125, 250, 500 and 1000µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide- $K_3Fe(CN)_6$ (2.5 ml, 1% w/v). The obtained mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v) and the absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the reference and phosphate buffer (pH 6.6) was used as blank solution.

2.5 Total Phenol Content

Total phenol content of the extract was determined using Folin-Ciocalteu reagent using the method of Singelton *et al*.,(1999)[12]. The sample (200µg/ml) was mixed with 400 µl of the Folin-Ciocalteu reagent and 1.5 ml of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water and then allowed to stand for 2 hours. Then the absorbance was taken at 765 nm wavelength. The concentration of total phenol content in the extract (10-200µg/ml) was then determined as mg of Gallic acid equivalent by using an equation that was obtained from the standard Gallic acid graph.

2.6 Total Flavonoids Content

The total flavonoids content was determined using a method described by Kumaran *et al*.,(2007)[13]. where quercetin was used as a reference compound. 1 ml of the plant extract in methanol (200µg/ml) was mixed with 1 ml aluminium trichloride in methanol (20 mg/ ml) and a drop of acetic acid, and then diluted with methanol to 25 ml. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 1 ml of plant extract and a drop of acetic acid, and then diluted to 25 ml with ethanol. The total flavonoid content was determined using a standard curve of quercetin (12.5-100 µg/ml) and expressed as mg of quercetin equivalent (QE/mg of extract).

2.7 Cytotoxicity screening

Brine shrimp lethality bioassay was used to determine the cytotoxic compounds using simple zoological organism *Artemia salina* for convenient monitoring. The eggs of the brine shrimp were hatched in artificial seawater (3.8% NaCl solution) for 48 hours to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using the method of Meyer *et al*.,(1982)[14]. The test sample was prepared by dissolving them in DMSO (not more than 50 µl in 5 ml solution) with sea water (3.8% NaCl in water) to attain concentrations of 6.25, 12.5, 25, 50, 100, 200, 400 and 800 µg/ml. A vial containing 50µl DMSO diluted to 5 ml was used as a negative control. Vincristine sulphate was used as positive control. The matured nauplii were applied to each of all experimental vials and control vial. After 24 hours, the vials were inspected using a magnifying glass and the number of surviving nauplii in each vial was counted. The percent (%) of mortality of the brine shrimp was calculated for each concentration using the formula:

$$\% \text{ Mortality} = \frac{N_t}{N_0} \times 100.$$

Where, N_t = Number of killed nauplii after 24 hours of incubation, N_0 = Number of total nauplii transferred i.e. 20. The LC_{50} (Median lethal concentration) was then determined from the graph.

3. Results

The DPPH radical scavenging activity of *Leucas aspera* was found (Figure 1) to increase with increasing concentration of the

extract. This assay was based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) to decolorize in the presence of antioxidants. The sample extract displayed significant reducing power which was found (Figure 2) to increase with the increasing concentration. It was found that the amount (Table 1) of phenol and flavonoid were significant. The lethality of the crude extract of *Leucas aspera* leaves to brine shrimp was determined on *Artemia salina* after 24 hours of exposure the samples, the positive control and vincristine sulphate. This technique was applied for the determination of general toxic property of the plant extract. The LC_{50} value (figure 3) of the extract was 30 µg/ml and that for standard vincristine sulphate was 10.44µg/ml. No mortality was found in the control group, using DMSO and sea water.

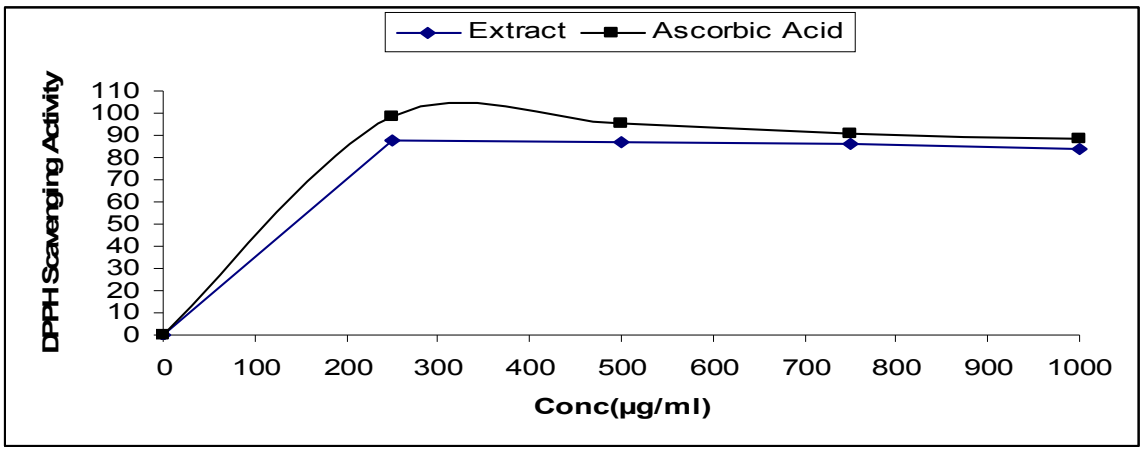

Fig: 1: %-scavenging activity of ascorbic acidand methanol extract of *Leucas aspera* at different concentration.

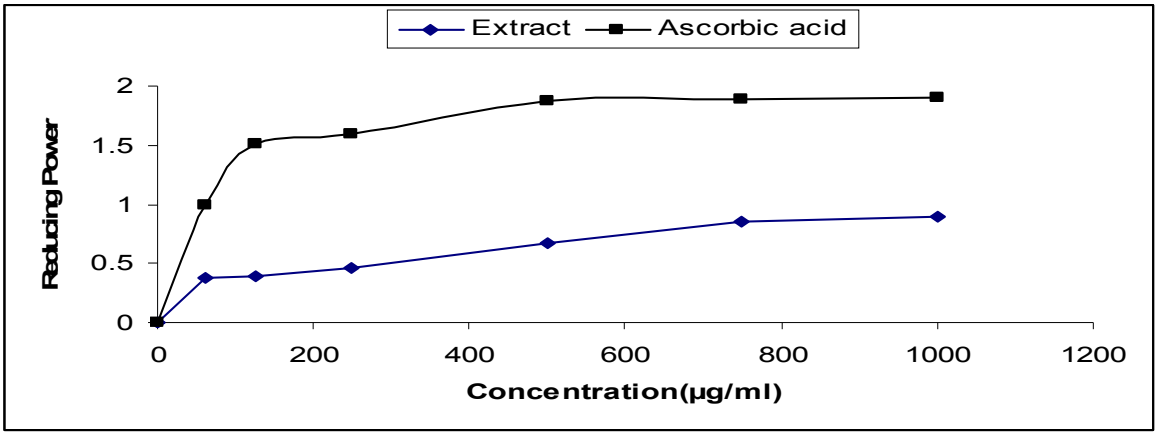

Figure 2: Reducing power of Ascorbic acid and Methanol extracts of Leucas aspera.

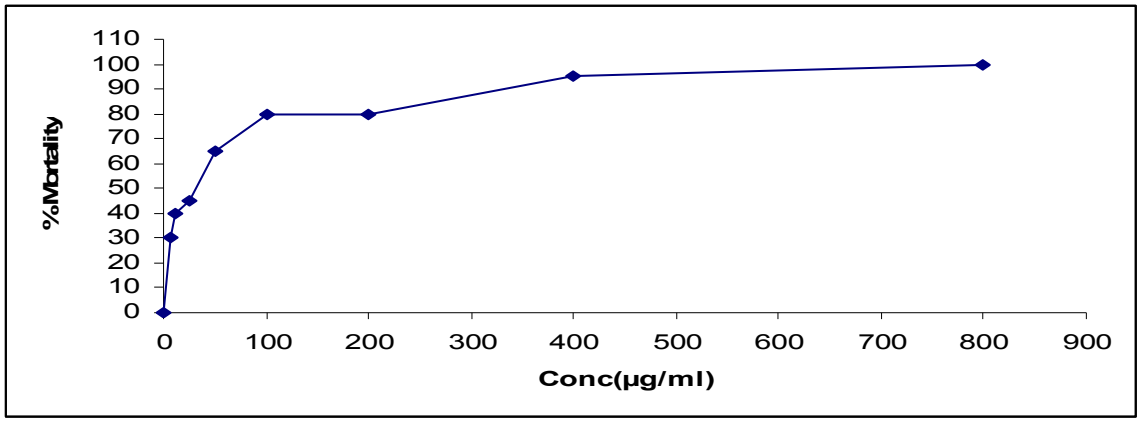

Figure: 3: Graphical representation of % mortality of nauplii versus concentration in of methanol extract of *Leucus aspera*.

Table 2. The total amount phenol and flavonoid which are obtained in separate experiment have been shown.

Extract	Total phenol (mg/gm, Gallic acid equivalent)	Total flavonoid (mg/gm, quercitn equivalent)
Leucus aspera.	131.15	135.85

4. Discussion

Free radicals play an important role in a number of biological processes, some of which are necessary for life, such as the intracellular killing of bacteria by phagocytes cells such as granulocytes and macrophages. Free radicals have also been implicated in certain cell signaling processes. Because free radicals are necessary for life, the body has a number of mechanisms to minimize free radical induced damage and to repair damage that occurs, such as the enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. In addition, antioxidants play a key role in these defense mechanisms. These are often the three vitamins, vitamin A, vitamin C and vitamin E and polyphenol antioxidants[15-17]. The methanol extract of the leaves of the plant *Leucas aspera* has been tested for the determination of antioxidant activity. The reducing activity of a compound depends on the presence of redactors, which has been exhibited antioxidative potential by breaking the free radical chain, donating a nitrogen atom. The presence of reductants in the fraction *Leucas aspera* extracts causes the reduction of the Fe^{2+} ferric cyanide complex to the ferrous form.

5. Conclusion

From through study of this experiment it may be concluded that methanol extract of the *Leucas aspera* has antioxidant and cytotoxic activities. However this is the primary study. So, further experiment like compound isolation may discover what compounds are responsible for this activities which ultimately it may lead the new drug discovery.

6. References

1. Bitterman H. Effects of hyperbaric oxygen in circulatory shock induced by Splanchnic artery occlusion and reperfusion in rats. Can J Physiol Pharm. 1989; 67:1033-1037.
2. Dirks RC, Faiman MD. Free radical formation and lipid peroxidation in rat and mouse cerebral cortex slices exposed to high oxygen pressure. Brain Res. 1982; 248: 355-60.
3. Grim PS, Nahum A, Gottlieb L. Lack of measurable oxidative stress during HBO

therapy in burn patients. Undersea Biomed Res. 1989; 16:22.
4. Hercberg S, Galan P, Preziosi P, Bertrais S, Mennen L, Malvy D, et al. The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Archives of Internal Medicine 2004; 164(21):2335-2342.
5. Ho PY, Lisowski FP. Concepts of Chinese science and traditional healing arts: A historical review. World Scientific Publishing Company Incorporated, 1993.
6. Srinivasan R. Leucus aspera-Medicinal Plant: A Review. International Journal of Pharma and Bio Sciences 2011; 2(1):153–159.
7. Ghani A. Medicinal Plants of Bangladesh with chemical constituents and uses. 2nd edition, Asiatic Society of Bangladesh, Dhaka, 2003.
8. Prajapati MS, Patel JB, Modi K, Shah MB. Leucas aspera: A review. Phcog Rev 2010; 4:85-87
9. Gupta, Nakul. Evaluation of Hepatoprotective Activity of the Plant Leucas aspera spreng. In Rats. 2005.
10. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from Bauhinia terapotensis. J Natl Prod 2001; 64: 892-95.
11. Srinivas K, Jimoh FO, Grierson DS, Afolayan AJ. Antioxidant activity of two steroid alkaloids extracted from Solanum aculeastrum. J Pharmacol Toxicol 2007; 2:160-61.
12. Singelton VR, Orthifer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 1999; 299:152-78.
13. Kumaran A, Karunakaran AJ. *In vitro* antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT 2007; 40: 344-52.
14. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen JE, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plants constituents. J Med Plant Res 1982; 45: 31-34.
15. Wagner BA. Free radical-mediated lipid peroxidation in cells: Oxidizability is a function of cell lipid bis-allylic hydrogen content. In Biochemistry 1994; 33:4449-4453.
16. Harabin AL, Braisted JC, Flynn ET. Response of antioxidant enzymes to intermittent and continuous hyperbaric oxygen. J Appl Physiol 1990; 69(1):328-335.
17. Bagchi D, Grag A, Krohn RL, Bagachi M, Tran MX, Stohs SJ. Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract in vitro. Research Communications in Molecular Pathology and Pharmacology 1997; 93:179-89.