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Antioxidant Activity and Phytochemical Analysis of Methanolic Leaf Extract of *Leucas aspera* (Wild) Link.

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Abstract
The present work has been conducted to evaluate the phytochemical screening and antioxidant activity of the methanolic leaf extract of *Leucas aspera*. The phytochemical screening result showed that the carbohydrate, protein, flavonoids, phenolic flavonoids, saponins and tannins were present in the plant sample. The antioxidant activity of leaf extract was tested by Phosphomolybdenum assay, reducing power activity, nitric oxide scavenging activity and hydrogen peroxide assay**.** The result suggested that the methanolic leaf extract of *Leucas aspera* could be a potential source for antioxidant activity and it increased with the increasing concentration. Hydrogen peroxide activity of methanolic leaf extract of *Leucas aspera* was 98.3%. The present observation suggested that the methanolic leaf extract of *Leucas aspera* has potent antioxidant activity in a dose dependent manner.

Keywords
Antioxidant, Phosphomolybdenum, Methanolic, Flavonoids, Tannins.

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INTRODUCTION
Antioxidants are the natural or man-made substances act against cell damage caused by destructive molecules called free radicals. Free radicals are the byproducts of oxygen metabolism and toxic in nature that can cause damage to cell membrane and cellular protein structures. In human, these free radicals cause many chronic health diseases called cataract, cardiovascular disease, Alzheimer's disease, Parkinson's disease, aging and cancer. Antioxidants reduce such free radicals induced cell damage. There is an inverse relationship between antioxidative status and incidence of human diseases such as cancer, ageing, neurodegenerative diseases and atherosclerosis[1]. The use of antioxidants in pharmacology is intensively studied as oxidative stress might be an important part of many human diseases particularly stroke and neurodegenerative incidents. Therefore, antioxidants are added to meals, oils, foodstuffs and other materials to prevent free radical damage[2]. Recently, interest has increased considerably in

finding naturally occurring antioxidant for the use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods[3]. Wild herbs have been investigated for their antioxidant properties[4]. Antioxidants are highly present in fruits and vegetables. Higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases particularly cardiovascular diseases and cancer[5]. Natural antioxidants from plant materials are mainly polyphenols (phenolic acids, flavonoids, anthocyanins, lignans and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C) [6]. Many plants have been reported for its antioxidant activity such as *Eclipta alba, Ocimum tenuiflorum, Solanum nigrum* and *Piper longum.*

Leucus aspera commonly known as 'Thumbai' in tamil is a common herb belongs to the family of Lamiaceae and is a well-known medicinal plant for its medicinal properties. It is found in throughout India. It is used as a traditional medicine of Philippines to treat scorpion bites. It has antibacterial, antioxidant, anti-diabetic and fever reducing activity. The leaves were used for the treatment of headache, asthma and skin disease. The smoke of *Leucas aspera* leaf was more toxic to filarial vector mosquitoes *Culex quinquefasciatus* than the synthetic mosquito mats[7]. The leaves were used for the treatment of psoriasis, rheumatisms, and other skin disease. The flowers are given with honey to treat cough and cold in children[8]. The present study was designed to investigate the phytochemical screening and antioxidant activity of methanolic leaf extract of *Leucus aspera* by using reducing power activity, nitric oxide assay, hydrogen peroxide activity and phosphomolybdenum assay.

MATERIALS AND METHODS

Sample collection and extraction

Fresh leaves of *Leucus aspera* were collected from the plains of Virudhunagar District, Tamil Nadu, India. The leaves were shade dried for the period of 15days. The shade dried leaves were ground into powder with the help of electronic blender. The powder sample was extracted with methanol by cold extraction method. 5 gm of plant powder was mixed with 30 ml of methanol to obtain a mixture that was placed in a shaker for 5 days and filtered through Whatman No1 filter paper. The filtrate was used for further studies.

Phytochemical screening

Secondary metabolites present in the plant sample were analyzed by standard phytochemical screening method[9].

Antioxidant activity

Free radical scavenging activity of plant sample was analyzed by the reducing power activity, phosphomolybdenum assay, nitric oxide scavenging activity and hydrogen peroxide activity.

Phosphomolybdenum assay

Various concentrations (100-1000µg/µl) of methanolic leaf extracts were added to each test tube individually containing 3ml of distilled water and 1ml of molybdate reagent solution. Molybdate reagent solution was prepared by 0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate were added in 20ml of distilled water and made up the final volume to 50ml by adding distilled water. The tubes were kept incubated at 95°C for 90 mins. After incubation, these tubes were kept at room temperature for 20-30 mins and the absorbance of the reaction mixture was measured at 695 nm. Ascorbic acid was used as a standard[10].

Reducing power assay

The reducing power assay was determined by the method of Srinivasan[11]. Different concentration (100-1000µg/µl) of extract was mixed in 1ml of distilled water and 2.5ml of phosphate buffer (pH 6.6) and potassium ferricyanide (2.5ml, 1% w/v). The reaction mixture was incubated at 50ºC for 20 mins. 2.5ml of 20% Trichloroacetic acid was added after 20mins to stop the reaction. The mixture was centrifuged at 3000 rpm for 10mins. 2.5ml of distilled water and 0.5ml of ferric chloride (0.1%) were mixed with 2.5ml of the centrifuged sample and the absorbance was measured at 700nm. Increased absorbance of the mixture indicated increasing the reducing power. Ascorbic acid was used as a standard. Phosphate buffer (pH 6.6) was used as a blank.

Hydrogen peroxide reducing assay

20 mM of Hydrogen peroxide was prepared in Phosphate buffer saline (pH 7.4). 1ml of different concentration of extract (100-1000µg/ml) was prepared in methanol. The prepared methanol extract was mixed with 2ml of hydrogen peroxide solution. The reaction mixture was kept in room temperature for 10 mins incubation. The absorbance was measured 230nm. Hydrogen peroxide solution without extract was served as blank and Ascorbic acid is used as a positive control[12].

% Scavenged (H_2O_2) = (Ac – At / Ac) x 100

Where; Ac is the absorbance of control and at is the absorbance of test.

Nitric oxide scavenging activity

10mM sodium nitroprusside was prepared in Phosphate buffer (pH 7.4). 1ml of different concentration of leaf extract was mixed with 0.5ml sodium nitroprusside solution. The reaction mixture was incubated at 25ºC for 180mins. After, 180 mins 0.5ml of incubated solution was mixed with 0.5ml of Griess reagent. The reagent without extract was used as a control. The absorbance was measured at 546nm. The percentage inhibition was calculated using the formula[13].

% scavenging activity = $[(A_c - A_t) / A_c] \times 100$

Where A_c is the absorbance of control, A_t is the absorbance of test.

RESULTS AND DISCUSSION

Phytochemical Studies

Phytochemicals present in the methanolic leaf extract of *Leucas aspera* shown in Table1. Carbohydrate, Protein, Alkaloids, Saponins, Flavonoids, Terpenoids, Phlobatannins and Phenolic flavonoids are present in the methanolic leaf extract of *Leucas aspera.* Ilango et al. reported the presence of carbohydrate, alkaloids and tannins in methanol leaf extract of *Leucas aspera*[14]. Rahman reported the presence of Alkaloids, tannins, flavonoids, glycosides, Terpenoids, saponins, steroids and phlobatannins in the ethanol leaf extract of *Leucas aspera*[15].

Antioxidant activity

Reducing power activity of methanolic leaf extract of *Leucas aspera* is given in figure 1. The reducing ability of a compound generally depends on the presence of reductants. The reluctants break a free radical chain and donating a hydrogen atom. The reluctants present in the methanolic leaf extract of *Leucas aspera* cause the reduction of the Fe3+/ferricyanide complex to the ferrous form[16]. The reducing power activity was increased with the increasing concentration.

Phosphomolybdenum activity of methanolic leaf extract of *Leucas aspera* was shown in figure 2. Phospho molybdenum activity is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate/Mo (V) complex with the maximal absorption at 695 nm. In this study, the activity was found to be very strong. Ascorbic acid was used as standard. The activity of methanolic leaf extract increased with the increasing concentration of the sample from 100-1000 µg/µl. The highest activity was found in 1000 µg/µl of methanolic leaf extract. The phosphomolybdenum reduction assay showed maximum activity of 0.069 at 21µg/mL concentration in the methanol leaves extract of *Hypericum hookerianum*[17].

Hydrogen peroxide is a week oxidizing agent, it can inactivate a few enzymes directly by oxidation of essential thiol (-SH) group. It can cross cell membranes rapidly and inside the cell. H_2O_2 probably reacts with Fe2+ and possibly Cu2+ ions to form hydroxyl radical which may be the origin of many of its toxic effects[18]. Hydrogen peroxide activity of methanolic leaf extract of *Leucas aspera* was given in figure 3. In this study, the hydrogen peroxide activity of methanolic leaf extract of *Leucas aspera* was measured at 98.3%. Archana et al. reported that the hydrogen peroxide activity of methanol leaf extract of *Leucas aspera* was 5456.497µg/ml[19]

The nitric oxide scavenging activity of methanolic leaf extract was shown in figure 4. It is an important chemical mediator generated by epithelial cells. Excess concentration of nitric oxide causes various disorders like cancer, AIDS and arthritis. The activity is based on the inhibition of nitric oxide radical generated from sodium nitroprusside in buffered saline and measured by Griess's reagent. The activity is expressed as % reduction of nitric oxide. In this study the nitric oxide scavenging activity was found to be very strong. Moni et al. reported that the methanol leaf extract of *Leucas aspera* have caused a greater inhibition in nitric oxide (74.56%) than the ascorbic acid[20]. This scavenging activity may be due to the presence of secondary metabolites present in the methanolic leaf extract of *Leucas aspera*.

CONCLUSION

From the present study, it is concluded that the methanolic leaf extract of *Leucas aspera* has a potent antioxidant activity. Phenols, flavanoids and other phytochemical constituents may be attributed for such antioxidant activity of *Leucas aspera.* This plant, therefore, can be a natural source of antioxidants with therapeutic qualities to combat oxidative stress related ailments.

Table 1: Phytochemical screening of Methanolic leaf extract of *Leucas aspera*

Test	Reagents/ chemicals used	Inference	Result
Carbohydrate	Benedict's reagent	Bluish green color	+
Protein	Millon's reagent	White precipitate turns red	+
Alkaloids	Dragendroff's reagent	Turbid orange color	+
Saponin	Distilled water	Foam formation	-
Flavonoids	Sodium hydroxide	Golden yellow color	+
Terpenoids	Chloroform Con. Sulphuric acid	Reddish brown color formation	-
Tannins	Ferric chloride	Dark green color	+
Phlobatannins	1% Aqueous Hcl	Red precipitation	-
Phenolic flavonoids	10% lead acetate	Brown precipitate	+

+ indicates Present - indicates Absent

Figure 1: Reducing power assay

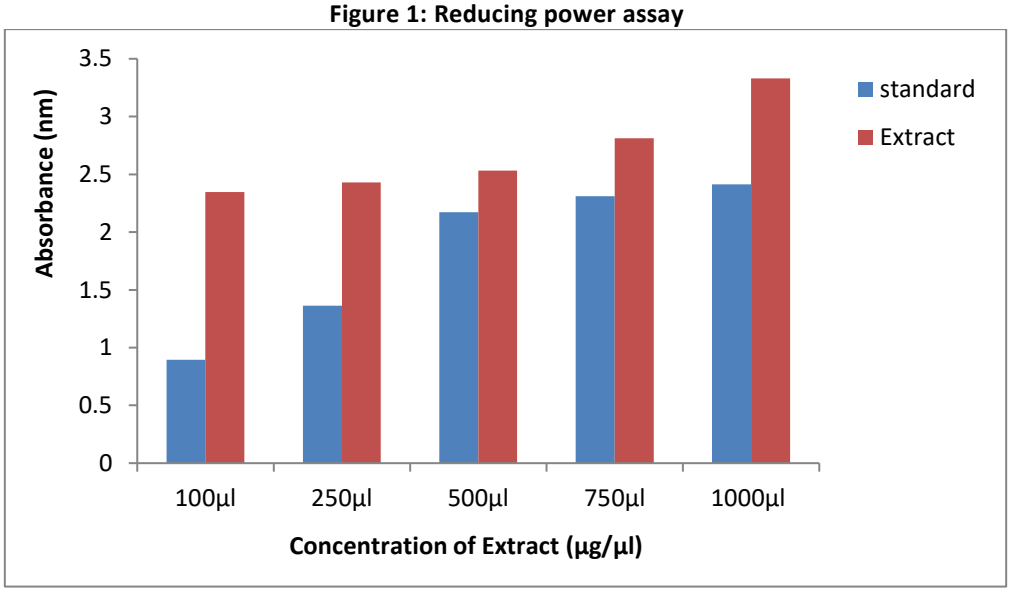


Figure 2: Phosphomolybdenum assay

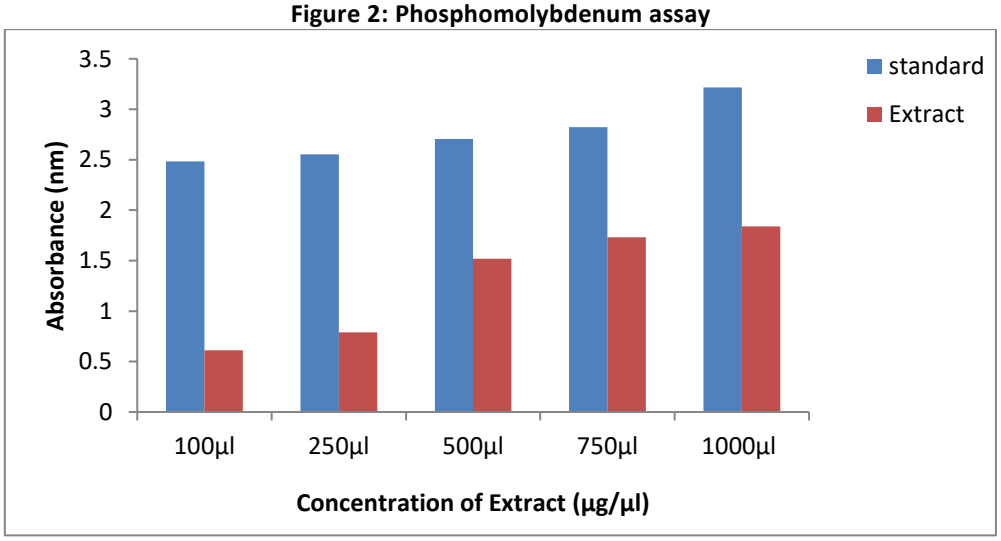


Figure 3: Hydrogen peroxide assay

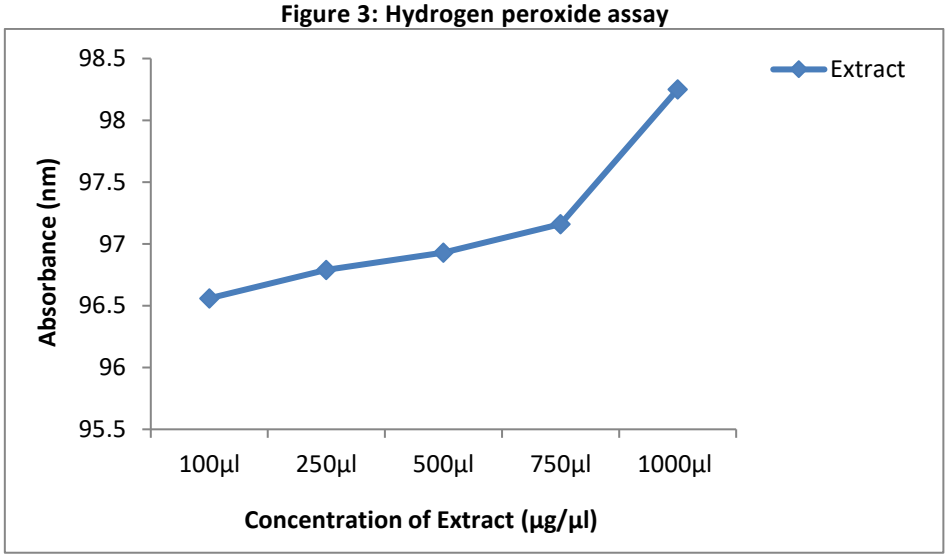
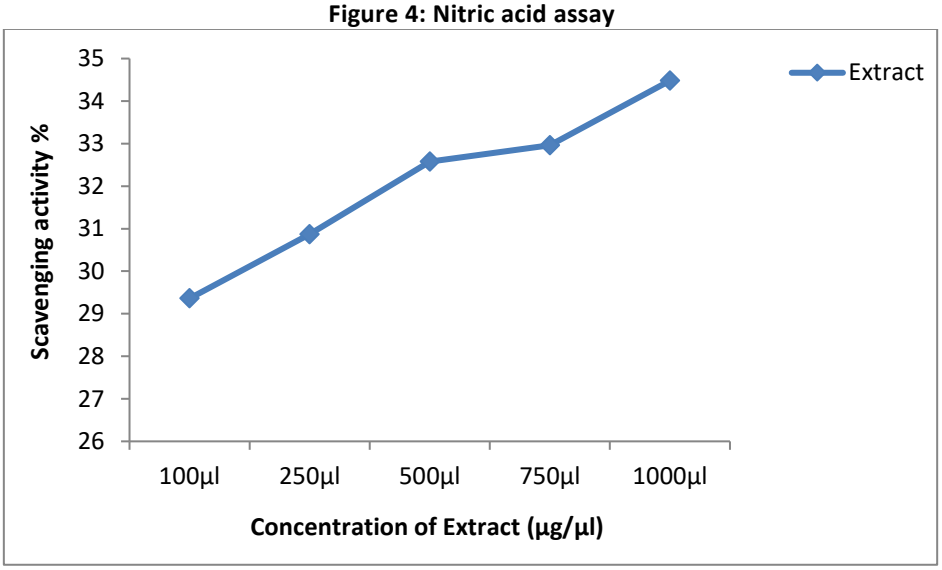


Figure 4: Nitric acid assay



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