Free Radicals and Antioxidants, 2017; 7(2): 166-171
A multifaceted peer reviewed journal in the field of Free Radicals and Antioxidants
www.antiox.org | www.phcog.net

Original Article

Phytochemical Evaluation and *In vitro* Antioxidant Activity of Various Solvent Extracts of *Leucas aspera* (Willd.) Link Leaves

Vellaichamy Muthupandi Annapandian*, Shanmuga Sundaram Rajagopal
Department of Pharmacology, JKK Nattraja College of Pharmacy, Komarapalayam, Namakkal 638183, Tamil Nadu, INDIA.

ABSTRACT

Background: Free radicals initiate the oxidative stress and damage the healthy cells. These damages contribute ageing, cancer, cardiovascular and inflammatory diseases. Phenolic compounds, flavonoids and tannins are directly contributed to antioxidant activity. **Objective:** The present study was attempted to evaluate the phytochemicals present in various solvent extracts obtained from *Leucas aspera* (Willd) Link leaves (*L. aspera*) and its antioxidant activity using different *in-vitro* models. **Materials and Methods:** The free radical scavenging and antioxidant activity of petroleum ether, ethanol, isopropyl alcohol, ethyl acetate and chloroform extracts of *L. aspera* leaves were assessed by different *in-vitro* models include DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity, total antioxidant activity, ABTS (2,2,-azinobis (3-ethylbenzoline-6-sulfonic acid) radical scavenging activity and nitric oxide radical inhibition assay, in order to ensure the pharmacological effects of the plant. **Results:** Petroleum ether extract (non-polar solvent) showed better antioxidant activity among the solvents with IC_{50} values 18.96 µg/mL (DPPH assay), 17.22 µg/mL (total antioxidant assay), 16.00 µg/mL (ABTS assay) and 11.87 µg/mL (nitric oxide scavenging). Whereas, ethanol extract (polar solvent) exhibited better DPPH scavenging, ABTS assay and nitric oxide scavenging activity (IC_{50}=19.90, 11.60 and 13.47 µg/mL respectively) compared to chloroform, ethyl-acetate and isopropyl alcohol extracts. **Conclusion:** The results of our current study showed, *L. aspera* leaf is a significant source of phytochemicals that possess antioxidant and scavenging properties. Our study findings warrants for various pharmacological activities and further research on isolation and characterization of active principle responsible for the pharmacological activity.

Key words: *In vitro* antioxidant, Leucas aspera, Non-polar solvent, Polar solvent, Various solvents.

Key Meassage: Our study highlights the antioxidant activity of *L. aspera* leaves under various *in vitro* models and how the activity differs with the polarity of solvents.

Correspondence:

Vellaichamy Muthupandi Annapandian, M.Pharm, PGD. Clin.Pharm,

Department of Pharmacology, JKK Nattraja College of Pharmacy Komarapalayam, Namakkal District - 638183, Tamil Nadu, INDIA.
Phone no: +91-9994909190 / +91-9945211900

E-mail: vmannapandian@gmail.com
DOI: 10.5530/fra.2017.2.25

INTRODUCTION

A free radical is a molecule containing oxygen and one or more unpaired electrons which making it highly reactive with other molecules. Antioxidants are molecules which present in the cells that prevent these reactions by donating an electron to free radicals without becoming destabilized themselves.

Free radicals initiate the oxidative stress and damage the healthy cells and DNA along with lipid peroxidation. This damages can contribute to ageing, atherosclerosis, cancer, cardiovascular diseases, and inflammatory diseases.[1,2] Consumption of certain plant materials can promote health benefits and reduce the risk of stress related chronic diseases.[3] Phytochemicals such as flavonoids,[4] phenolic acids, alkaloids, lignins, stilbenes, and tannins are well known free radical scavengers and possessing multiple biological activities including anti-oxidant activity.[5-7] Supplementation of antioxidants can reduces the oxidative stress in experimental diabetes which include vitamin E, vitamin C, non nutrient antioxidant and plant derived natural antioxidants.[8]

Leucas aspera belongs to Lamiaceae family and commonly found throughout India and Philippines as well as the plains of Mauritius and Java. It is a very common weed in India and it is an annual plant grows up to 15-60 cm height.[9] The juice of *Leucas aspera* leaves are used as remedy for chronic skin eruptions, chronic rheumatism and psoriasis.[10] In villages *L.aspera* leaves are used as insecticides and mosquito repellent.

In our present study, we attempted to evaluate the phytochemicals present in various solvent extracts obtained from leaves of *Leucas aspera* (Willd.) Link (*L. aspera*) and its antioxidant properties using different *in vitro* models include ABTS (2,2,-azinobis (3-ethylbenzoline-6-sulfonic acid) radical scavenging activity, DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity, Nitric oxide radical inhibition assay and total antioxidant capacity in order to ensure the pharmacological effects of the plant.

MATERIALS AND METHOD

Plant collection and authentication

Well grown plants of *L. aspera* had been collected from Kandappakottai Village (Dindigul, Tamilnadu, India) between Nov-2014 and Jan-2015. The plant was authenticated (PARC/2014/2272) by Prof. P Jayaraman, Director, Institute of Herbal Botany and Anatomy Research Centre, Tambaram, Chennai. Then leaves were isolated, cleaned and dried at sun shadows. Dried leaves were crushed to powder and passed through pharmaceutical sieve no 40 and 80. Any materials which passed through sieve-40 and retained at sieve-80 were collected and used for extraction.

Preparation of crude extracts

Five different solvents were selected with increasing polarity, petroleum ether (60-80^{0}C), ethyl acetate, chloroform, ethanol (70%) and isopropyl alcohol for the extraction process. Soxhlet apparatus was used for petroleum ether extraction. Hundred grams of powdered leaf was extracted with 1200 mL of petroleum ether (60-80^0 C). Excess solvent was the removed by distillation method. Crude extract was dried at vaccum desiccators and stored in a glass bottle at -20^{0}C for further studies. All other solvent extracts were prepared by cold maceration technique. Weighed powder was soaked in solvents ethyl acetate (100 g + 900 mL), chloroform (100 g + 900 mL) isopropyl alcohol (100 g + 900 mL) and ethanol 70% (100 g + 1000 mL) in a separate round bottom flask for 72 hours. After soaking the mixture was heated at 30-40^0 C for 60 minutes. Heated

solution was cooled to room temperature and filtered through muslin cloth and whatman filter paper. Excess solvent was removed by distillation method and allowed to evaporate at room temperature. Dried crude extracts were collected and stored in an individual glass bottles at -20^{0}C for further studies.

Qualitative screening of phytochemicals presents in *L. aspera* leaves

All the five crude extracts were screened for the presence of phytochemicals as described in textbook by Harborne JB (eds.).[11] Freshly prepared extracts were tested for flavonoids (Shinoda's test, Alkaline reagent test, Lead acetate test and Sulphuric acid test), alkaloids (Dragendorff's test, Mayer's test, Hager's test, Wagner's test and Tannic acid test), steroids (Libermann-Burchard's test and Salkowsky's test), glycosides (Legal's test, Baljet test, Keller- killiani test and Borntrager's test), saponins (Foam test and Lead acetate test), tannins (Gelatin test, Ferric chloride test) Vanillin hydrochloride test, Lead acetate test, Ammonia test and Potassium dichromate test.), phenolic components, fixed oils (Spot test and Saponification test.), carbohydrates (Molisch's test, Fehling's test, Benedict's test, Tollen's test, Seliwanoff's test and Bromine water test.), proteins (Biuret test, Ninhydrin test, Xanthoproteic test and Millon's test) and terpenoids (Knoller's test).

Quantitative estimation of phyto-ingredients present in *L. aspera* leaves

Estimation of total flavonoid compounds

Total flavonoids were estimated by using spectrophotometric technique. Flavonoids give yellow colour when treated with aluminium chloride reagent (1% w/v) and this principle was used in flavonoid estimation. Rutin was used as the standard solution (100 mg/mL) with methanol. Test sample was prepared with methanol to the final concentration of 100 mg/mL. One mL of test and standard solutions were taken in a separate 10 mL volumetric flask and 1.0 mL of aluminium chloride reagent was added to both the test and standard solutions. Volumes were made up to 10 mL with ethanol and optical density was measured at 410 nm against blank solution exactly at 15 min after adding aluminium chloride reagent.

Estimation of total phenolic compounds

Total phenolic content was calculated by calibration curve method using Gallic acid, Folin Ciocalteu reagent and sodium carbonate (20%) solution reagent. 100 mg of crude extract was added to 0.5 mL distilled water and 0.25 mL of the Folin-Ciocalteu reagent. Then 1.25 mL of sodium carbonate solution was added and vortexed. The absorbance of final solution was recorded at 725 nm after 40 min. Total phenols were calculated using the Gallic acid equivalent from the calibration curve equation.

Estimation of total alkaloid compounds

Total alkaloid compounds were estimated by using the method adopted from Anjanal *et.al*.,.[12] Small quantity of extract was dissolved in 2N hydrochloric acid and filtered. One mL of this solution washed with about 10 mL chloroform for three times in a separation funnel. The pH was adjusted to neutral with addition of 0.1N sodium hydroxide. Five mL of bromocresol green solution and 5 mL phosphate buffer were added to this solution and shaken well, then extracted with 1,2,3 and 4mL of chloroform by vigorous shaking and collected in a 10 mL volumetric flask then diluted with chloroform. The absorbance was measured at 470 nm and atropine was used as standard reference.

Estimation of total tannins

Total tannin compounds were estimated by spectrophotometric procedure using potassium ferricyanide reagent, ferric chloride reagent and tannic acid as standard. 100 mg of crude extract was refluxed with purified water in a water bath at 100^{0}C±2^{0}C for 60 min and cooled them volume made to 100 mL with purified water. Standard solution of tannic acid (0.01 mg/mL) was prepared with purified water and for working standard 1.0 mL of standard solution was added to 1.0 mL each potassium ferricyanide and ferric chloride in a 10 mL standard flask. The volume was made up to 10 mL with purified water. For working sample solution 0.2 mL of sample preparation was added to 1.0 mL of potassium ferricyanide and ferric chloride in a 10 mL standard flask. The volume was made up to 10 mL with purified water. Both standard and test samples optical density was measured at 720 nm exactly after 30 min from addition of reagents.

Assessment of *in vitro* antioxidant activity of crude extracts of *L. aspera* leaves

DPPH (Diphenyl-2-picrylhydrazyl) radical scavenging activity

DPPH free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability using stable DPPH radical, method described by Blois.[13,14] One mL of 0.1mM DPPH solution in ethanol was added to 3.0 mL of extract solution (or standard) and volume made up with water at different concentrations (10-100 µg/mL). Thirty minutes later absorbance was measured at 517 nm. Lower absorbance indicates the higher free radical scavenging activity. Capability to scavenge the DPPH radical was calculated using the following equation. Mean values were obtained from triplicate of experiment values.

% inhibition = [(Control OD – Sample OD) / Control OD)] × 100

OD – optical density.

Total antioxidant activity (Phosphomolybdic acid method)

Total antioxidant activity of the extract was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex.[15] An aliquot of 0.2 mL extract solution (10-100 µg/mL) was combined in a vial with 2.0 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95°C for 30 mins. Samples were cooled to room temperature and the absorbance was measured at 695 nm against blank solution. Total antioxidant activity was expressed relative to that of ascorbic acid.

Nitric oxide radical scavenging

Nitric oxide radical scavenging activity procedure was adopted from the method reported.[16] and rutin was used as standard. Nitric oxide was generated from sodium nitro prusside in aqueous solution at physiological pH, which interacts with oxygen to produce nitric ions, which may be determined by the Griess Ilosvay reaction. Two mL of 10 mM sodium nitro prusside and 0.5 mL of phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of sample at different concentrations (10-100 µg/mL) and the mixture incubated at 25°C for 2.5 h. Incubated mixture (1.5 mL) was added to 1.5 mL of griess reagent (1% sulphanilamide, 2% o-phosphoric acid, 0.1% napthyl ethylene diamine di hydrochloride) and again incubated at room temperature for 5 min. The absorbance of the mixture was read at 546 nm.

Percentage inhibition (%) = [(Abs_{cont} - Abs_{test}) / Abs_{cont}] x 100

ABTS (2, 2,-azinobis (3-ethylbenzoline-6-sulfonic acid) radical scavenging activity

Scavenging activity of extract was tested by using $ABTS^+$ assay and quercetin was used as standard. This method was described with a slight modification.[17] The $ABTS^+$ radical solution was prepared by mixing 14 mM ABTS stock solution with 4 mM ammonium per sulphate and incubating for 16 h at room temperature in the dark until the reaction was stable. Absorbance of $ABTS^+$ solution was equilibrated to 0.70±0.02 by diluting with ethanol at room temperature. To 1.0mL of $ABTS^+$ solution various concentrations of test samples (10-100 µg/mL) was added. Six minutes later the absorbance was measured at 734 nm. Percentage inhibition of absorbance was calculated by using the following equation.

Percentage Inhibition (I %) = $\{(Abs_{control} - Abs_{sample}) / Abs_{control}\} \times 100$

RESULTS

Preliminary phytochemical studies

The present study was carried out on petroleum ether, ethyl acetate, chloroform, ethanol and isopropyl alcohol extracts of *L. aspera* leaves. The results have confirmed that *L. aspera* leaf contains flavonoids, alkaloids, glycosides, carbohydrates and less extent of steroids, terphenoids, tannins, phenolic compounds. Maximum number of phytochmicals were found in petroleum ether extract and ethanol extract. Complete details of presence of phytochemicals in each crude extracts were presented in Table 1.

Our current study demonstrated the amount of total flavonoid compounds, total phenolic compounds, total tannins and total alkaloids present in various solvent extract of *L. aspera* leaves. The complete details of phyto-ingredients present in each crude extract were given in Table 2. values are expressed in grams per 100 grams.

In vitro antioxidant studies DPPH scavenging activity

Petroleum ether, ethanol and isopropyl alcohol extracts of *L. aspera* leaves were exhibited a significant dose dependent DPPH scavenging activity (Figure 1). Amongst, petroleum ether extract exhibitted 66%, 68%, 69%, 70%, 71% and 72% of scavenging activity at 10, 20, 40, 60, 80 and 100 µg/mL concentrations respectively. Whereas ethanol extract exhibited 64%, 67%, 71%, 76%, 81% and 87% with the same concentrations. On the other hand, ascorbic acid standard showed 21%, 33%, 34%, 46%, 58% and 74% of activity with the same concentrations. Percentage of scavenging activity or percentage inhibition was calculated by linear regression method. The IC_{50} values (Table 3) of ascorbic acid, petroleum ether extract, ethanol extract and isopropyl alcohol extract was found to be 27.05µg/mL, 18.96µg/mL, 19.90µg/mL. and 24.74 µg/mL respectively.

Total antioxidant activity

Total antioxidant capacity of various solvent extracts of *L. aspera* leaves were calculated using the standard curve of ascorbic acid ($y = 0.029+0.021$; $R^2 = 0.813$) and expressed as number of equivalent of ascorbic acid per gram of extract. The IC_{50} values of total antioxidant capacity of ascorbic acid, petroleum ether extract, ethanol extract, isopropyl alcohol extract, chloroform extract and ethyl acetate extract was found to be 17.23 µg/mL, 17.22 µg/mL, 21.58 µg/mL, 10.40 µg/mL, 37.78 µg/mL and 23.61 µg/mL respectively (Figure 1). Amongst petroleum ether, ethanol and isopropyl alcohol exhibited significant dose dependent antioxidant activity (IC_{50} values are presented in Table 3).

Table 1: Phytochemicals present in *Leucas aspera* (Willd.) Link Leaves

Phytochemicals	Petroleum ether extract	Ethyl Acetate extract	Chloroform extract	Isopropyl alcohol extract	Ethanol extract
Alkaloids	++	+	+	++	++
Flavonoids	++	+	+	++	++
Carbohydrates	++	++	+	+	++
Tannins	+	+	+	+	+
Triterpenoids	+	+	+	+	-
Glycosides	+	+	+	+	++
Steroids	+	-	-	-	+
Phenolic Compounds	+	+	-	+	+
Fixed Oil & Fat	+	-	-	-	-
Proteins	-	+	+	+	+
Saponins	-	+	-	-	+

++ Strong positive, + Positive, – Negative/Absent.

Table 2: Amount of phyto-ingredients presents in *Leucas aspera* (Willd.) Link Leaves

Photochemicals	Petroleum ether extract	Ethyl Acetate extract	Chloroform extract	Isopropyl alcohol extract	Ethanol extract
Total Flavonoids	13.22	14.90	13.22	13.39	15.97
Total Phenolics	12.96	9.25	14.01	10.65	12.26
Total Tannins	0.17	0.53	0.83	0.55	0.79
Total Alkaloids	4.05	4.20	13.95	7.16	2.15

* all values are expressed in grams per 100 grams.

Table 3: Free radical scavenging and iron chelating activity (IC_{50} values) of various solvent extract of *Leucas aspera* (Willd.) Link Leaves (p value ≤ 0.05)

Antioxidant activity	Crude extract of *L. aspera* leaves IC_{50} values (µg/mL)					
	PET	EA	Et.OH	IPA	ChCl3	Std#
DPPH scavenging	18.96*	74.14	19.90*	24.74	86.79	27.05
Total antioxidant	17.22*	23.61	21.58	10.40*	37.68	17.23
ABTS assay	16.00*	52.93	11.60*	61.76	17.08	16.16
Nitric oxide scavenging	11.87*	20.75	13.47*	24.26	13.58*	-

PET-petroleum ether / EA-ethyl acetate / Et.OH-ethanol / IPA-isopropyl alcohol / ChCl3-chloroform / Std-standard used.
$all values are expressed in grams per 100 grams. Values are obtained from average of 3 determinations.
*significant antioxidant activity (p value ≤ 0.05).
Ascorbic acid used as standard for DPPH and total antioxidant activity. Quercetin used as standard for ABTS assay.

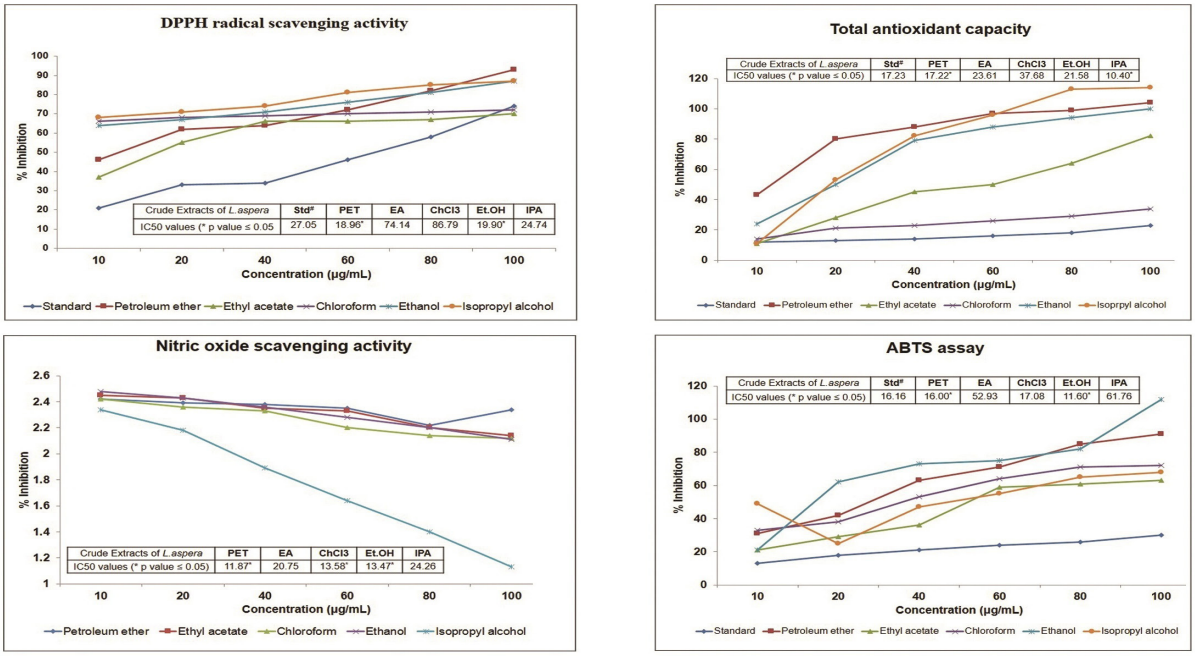


Figure 1: *In vitro* antioxidant activity of *L. aspera* leaves using various solvent extracts under different *in vitro* models.

Nitric oxide scavenging activity

Nitric oxide is a free radical and over production of nitric oxide radicals contributes to some inflammatory diseases. Ethanol extract and petroleum ether extract were exhibited the higher nitric oxide scavenging activity among the five extracts (Figure 1). The IC_{50} values of ethanol and petroleum ether extracts are 11.87µg/mL and 13.47µg/mL respectively (Table 3).

ABTS assay

The scavenging activity of 2,2,-azinobis (3-ethylbenzoline-6-sulfonic acid ($ABTS^+$) assay was measured with the comparison of quercetin standard reference. Among the extracts, petroleum ether, ethanol and chloroform extracts were exhibited better inhibition compared to quercetin standard (Figure 1). Petroleum ether extract exhibited 33%, 38%, 53, 64%, 71% and 72% of scavenging activity and ethanol extract had shown 3%, 8%, 15%, 23%, 34% and 77% at 10, 20, 40, 60, 80 and 100 µg/mL concentrations respectively. The IC_{50} values (Table 3) of quercetin petroleum ether, chloroform and ethanol was found to be 16.66 µg/mL, 16.0 µg/mL, 17.08 µg/mL and 11.6µg/mL respectively.

DISCUSSION

Constant generation of free radicals occurs in living system and it causes extensive damages to the cells, tissues and organs which may lead to various disease statuses, especially degenerative disorders and inflammatory diseases. Antioxidants offer resistance to cells and prevent the diseases through scavenging the free radical molecules, by inhibiting lipid peroxidations and many other mechanisms.[18] Numbers of plant constituents had been proven for free radical scavenging or antioxidant activities, among phenolic compounds are very important plant constituents. Total phenolic compounds of many plants and antioxidant activity has positive relationship due to the presence of hydroxyl group which has a scavenging ability.[19] By donating an electron a phenolic compound can also scavenge the hydrogen peroxide and neutralize it to water.[20]

Researchers continuously exploit the medicinal plants and their extracts in order to discover the potential drugs with reduced toxicity. In this context we have reported the significant *in vitro* antioxidant activity of various solvent extracts of *L. aspera* leaves. The various antioxidant activities of solvent extracts such as petroleum ether, ethyl acetate, ethanol,

chloroform and isopropyl alcohol extracts from the leaves of *L. aspera* were tested and the results are summarized in Table 3.

The DPPH scavenging activity of the extracts evaluated against the positive control ascorbic acid and the DPPH reduction is directly proportional to the antioxidant content in the extract. Higher the antioxidant contents produced higher DPPH reduction. Petroleum ether extract exhibited the maximum DPPH scavenging with the value of 92% at a concentration of 100 µg/ml with IC_{50} value of 18.96 µg/ml and it showed the antioxidant activity in a concentration dependent manner (20 to 92 %) in the DPPH assay. The scavenging activity of *L. aspera* leaf extracts are in the order of petroleum ether > ethanol > isopropyl alcohol > ethyl acetate > chloroform. *L. aspera* leaf extracts showed the potential scavenging of DPPH radicals similar to that of ascorbic acid, it's likely due to the proton-donating ability of *L. aspera* leaf and stabilizes the free radicals in association with a number of hydroxyl groups.[21] The result of this study suggests that these crude extracts of *L. aspera* leaves contains phytochemicals that are capable of donating hydrogen to a free radical.

The total antioxidant capacity of isopropyl alcohol extract was found to be higher (82% at 100 µg/mL) when compared to other solvents used. The total antioxidant capacity of different solvent extracts of leaves of *L. aspera* at various concentrations (10–100 µg/ml) was in the following order: isopropyl alcohol > petroleum ether > ethanol > ethyl acetate > chloroform.

Nitric oxide is a free radical produced from mammalian cells and it involved in various physiological processes. The excess production of nitric oxide is directly toxic to the cells and associated to septic shock, vascular collapse, arthritis and diabetes mellitus.[22] Nitric oxide is generated from sodium nitroprusside and it produces nitrite when reacts with oxygen. This crude extracts of *L. aspera* leaves scavenge the free nitric oxides in the medium and prevent the formation of nitrite. Crude extract of petroleum ether exhibited higher nitric oxide scavenging at the concentration of 100µg/mL (IC_{50} = 11.87) and the order of nitric oxide scavenging activity of various solvent extracts of *L. aspera* leaf was found to be petroleum ether > ethyl alcohol > chloroform > ethyl acetate > isopropyl alcohol.

ABTS assay is a decolourization method, the ABTS oxidant generated from 2,2,-azinobis (3-ethylbenzoline-6-sulfonic acid) by persulfate oxidation. The antioxidant activity of extracts of leaves of *L. aspera* in this method is due to either inhibiting or scavenging the radicals. Because both the inhibition and scavenging activity of $ABTS^{++}$ was reported earlier.[23] The order of antioxidant potency of *L. aspera* leaf extract in our study was found to be, ethanol > petroleum ether > chloroform > ethyl acetate > isopropyl alcohol.

CONCLUSION

The data of our present study revealed that *Leucas aspera* (Willd.) Link leaves have significant antioxidant potential and scavenging activity. Petroleum ether, ethanol and isopropyl alcohol extracts of *Leucas aspera* (Willd.) Link leaves showed better DPPH scavenging activity. These could be due the presence of flavonoids, phenolic compounds, tannins, saponins and alkaloids. Similarly isopropyl alcohol (polar solvent), ethanol (polar solvent) and petroleum ether (non-polar solvent) exhibited better ABTS scavenging activity and total antioxidant activity compared to chloroform and ethyl acetate extracts. From our study results we suggest, ethyl alcohol or isopropyl alcohol (polar solvent selection) and petroleum ether (non-polar solvent selection) for the better extraction of phytochemicals from plants. This *in vitro* antioxidant study indicates *Leucas aspera* (Willd.) Link is a significant natural source of antioxidants and may be helpful in preventing the oxidative stress and damages to cells. Our present study warrants further research to isolation, characterization and in vivo experiments on animal models to assess the potency and safety before the clinical use.

ACKNOWLEDGEMENT

The authors would like to acknowledge Mrs Gomathi S (Asst. Professor) for her help during reagent preparation and phytochemical screening procedures.

CONFLICT OF INTEREST

All authors have no conflict of interest.

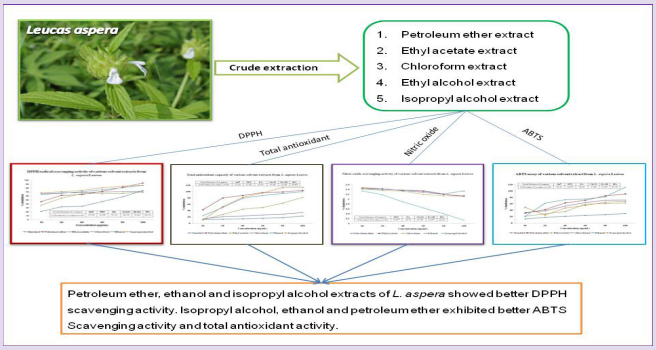
ABBREVIATION USED

Abs: Absorbance; **C:** Celsius; **DNA:** Deoxyribonucleic acid; **L:** Litre; **L. aspera:** Leucas aspera; **mg:** Milligram; **mL:** Millilitre; **mM:** Millimole.

REFERENCES

1. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from Licania licaniaeflora. J Ethnopharmacol. 2002;79(3):379-81.
2. Kumar V, Lemos M, Sharma M, Shriram V. Antioxidant and DNA damage protecting activities of Eulophia nuda Lindl. Free Radicals Antioxidants. 2013;3(2):55-60.
3. Halliwell B. Antioxidants and human disease: a general introduction. Nutr Rev. 1997;55(1):S44–49; discussion S49-52.
4. Bratkov VM, Shkondrov AM, Zdraveva PK, Krasteva IN. Flavonoids from the genus Astragalus: Phytochemistry and biological activity. Pharmacogn Rev. 2016;10(19):11.
5. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha J-P, Pihlaja K, Kujala TS, *et al.* Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. J Agric Food Chem. 1999;47(10):3954-62.
6. Yang Z, Liu C, Xiang L, Zheng Y. Phenolic alkaloids as a new class of antioxidants in Portulaca oleracea. Phytother Res. 2009;23(7):1032-5.
7. Chung HS, Woo WS. A quinolone alkaloid with antioxidant activity from the aleurone layer of anthocyanin-pigmented rice. J Nat Prod. 2001;64(12):1579-80.
8. Rao BK, Rao CH. Hypoglycemic and antihyperglycemic activity of Syzygium alternifolium (Wt.) Walp. seed extracts in normal and diabetic rats. Phytomedicine. 2001;8(2):88-93.
9. Prajapati MS, Patel JB, Modi K, Shah MB. Leucas aspera: A review. Pharmacogn Rev. 2010;4(7):85-7.
10. Kirtikar KR. Indian medicinal plants: Vol. III. 2nd edn. Allahabad: Lalit Mohan Basu; 1593.
11. Harborne AJ. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. 3rd ed. Springer Science and Business Media; 1998. 334.
12. Ajanal M, Gundkalle MB, Nayak SU. Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Anc Sci Life. 2012;31(4):198-201.
13. Blois M. Antioxidant determinations by the use of a stable free radical. Nature. 1958;26:1199-1200.
14. Blois S. A note on free radical formation in biologically occurring quinones. Biochim Biophys Acta. 1955;18(1):165.
15. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999;269(2):337-41.
16. Garratt D. The Quantitative Analysis of Drugs. Chapman and Hall Ltd: Tokyo, Japan. 1964;3:456–8.
17. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9-10):1231-7.
18. Pari L, Satheesh MA. Antidiabetic activity of Boerhaavia diffusa L.: effect on hepatic key enzymes in experimental diabetes. J Ethnopharmacol. 2004;91(1):109-13.
19. Vinson JA, Hao Y, Su X, Zubik L. Phenol antioxidant quantity and quality in foods: vegetables. J Agric Food Chem. 1998;46(9):3630-4.
20. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. *In vitro* Antioxidant and Free Radical Scavenging Activity of Diospyros lotus and Pyrus boissieriana growing in Iran. Phcog Mag. 2009;5:122-6.
21. Brand-Williams W, Cuvelier M-E, Berset C. Use of a free radical method to evaluate antioxidant activity. Lwt-Food Sci Technol. 1995;28(1):25-30.
22. Sunil C, Latha PG, Suja SR, Shine VJ, Shyamal S, Anuja GI, *et al.* Effect of ethanolic extract of Pisonia alba Span. leaves on blood glucose levels and histological changes in tissues of alloxan-induced diabetic rats. Int J Appl Res Nat Prod. 2009;2(2):4-11.
23. Miller NJ, Rice-Evans CA. Factors Influencing the Antioxidant Activity Determined by the ABTS++ Radical Cation Assay. Free Radic Res. 1997;26(3):195-9.

PICTORIAL ABSTRACT



SUMMARY

- Petroleum ether, ethanol and isopropyl alcohol extracts of *L. aspera* leaves showed better DPPH scavenging activity.
- Isopropyl alcohol, ethanol and petroleum ether exhibited better ABTS scavenging activity and total antioxidant activity.
- This *in vitro* antioxidant study indicates *Leucas aspera* (Willd.) Link is a significant natural source of antioxidants and may be helpful in preventing the oxidative stress and damages to cells.

AUTHOR PROFILE

Mr. V.M. Annapandian: Has completed M.Pharm and PG Diploma in Clinical Pharmacy. Having 10 years of cross functional experience in Clinical Pharmacy, Hospital & Healthcare and Pharmaceutical Industries.

Dr. R. Shanmuga Sundaram: Has completed M.Pharm and Ph.D. Currently working as Professor and Vice Principal, JKK Nattraja College of Pharmacy, Komarapalayam. Having 21 years of experience in teaching profession.