Research Article

Antioxidant Studies, *in vitro* Cytotoxic and Cell Viability Assay of Flavonoids and Alkaloids of *Leucas aspera* (Wild.) Linn Leaves

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ABSTRACT

Emerging drug advancements for anticancer research is need of the hour using the natural product and their bioactive molecules, among these some are highly potential to fight against cancers and exhibiting as biological agent. The current study was aimed to evaluate the 2, 2-diphenyl-1-picrylhydrazyl, hydroxyl radical, nitric oxide scavenging antioxidant properties, *in vitro* cytotoxic and cell viability assay of ethanol extracts of the Indian medicinal plant *Leucas aspera* leaves compounds. The antioxidant property of the ethanol extract was determined two compounds flavonoids and alkaloids and studied for their antioxidant potential by 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide radical and nitric oxide free radical scavenging assay and *in vitro* cytotoxic activity by 3-(4, 5-dimetylthiazol-2-yl)-2 and 5-diphenyltetrazolium bromide (MTT) assay using MCF-7 cell lines. The flavonoid compound exhibited maximum antioxidant activity than the alkaloid by comparing the standard gallic acid and also their IC_{50} concentration. The ethanol extracts of *L. aspera* leaves exhibited maximum DPPH scavenging property in flavonoid (90%) at 100 µg/mL concentration with an IC_{50} value 9.25 µg/mL. In cytotoxic assay to determine the % cell viability in MCF-7 cell line also exhibited maximum flavonoids compare to alkaloids, and it has been noticed that dose dependent activity of the compounds. MTT assay IC_{50} value 247.56 ug/ml was obtained for MCF-7 for flavonoids and 236.45 ug/ml was obtained for alkaloids compound with optimum effect on % cell viability. The results of this study conclude that *Leucas aspera* leaves possess the strong antioxidant and anticancer potential which are considered as active therapeutic agents.

Key words: *Leucas aspera*, Antioxidants, Flavonoids, Alkaloids, Gallic acid, Cell lines.

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INTRODUCTION

Plant leaves with varied medicinal potential have known to remedial for human beings. These leaves of plants were selected as Indian traditional medicinal plants they are known to possess useful biological properties like antioxidants, antiinflammatory, anticytotoxic, anticancer, hepatoprotective effects, etc.[1,2]

Indian traditional medicinal plant *Leucas aspera* (Wild.) Linn. belongs to the family of Labiatae is common and most popular all over the India. This medicinal herb grows to an altitude of 15-60 cm with stout and hispid acutely quadrangular branches with stems.[3] Plant leaves are found therapeutic application in psoriasis, chronic rheumatism and various skin diseases. *L. aspera* plant is found to be beneficial for respiratory tract disorders, gastrointestinal disorders, edema and used as an antidote to poison to cure it.[4] Chew *et al.* reported

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extensively evaluation of the antioxidant, antimicrobial and cytotoxic activity of different parts (root, flower, leaf and stem of methanol extract of *L. aspera*.[5]

Das *et al.* reported for phytochemical screening and antioxidant activity of different extracts of *L. aspera* whole plant and found potential for ethanol extract only.[6] Fractions of methanol extract of *L. aspera* flowers reported for significant antibacterial property, extracted flower juice and their alkaloidal residue also possess the antibacterial activity.[7] The ethanolic extract root of *L. aspera* was inhibited acetic acid induced writhing in mice at the dose level of 250 and 500 mg/kg body weight. In other findings extract exhibited the free radical scavenging activity with IC_{50} 8 µg/ml.[3] In our previous screening studies of medicinal plants and found biological activity moderately in *L. aspera* leaves extract compounds.[1] Due to over exciting previous findings, to explore it potential properties in detailed antioxidants of ethanol extract compounds of *L. aspera* leaves and to know their cytotoxicity using MCF-7 cell lines.

MATERIALS AND METHODS

Plants materials

Indian traditional medicinal plant, *Leucas aspera* leaves were collected from Devarayana Durga Hills, Tumkur District. The plant samples were authenticated by Dr. Shiddamallayya Mathapathi Research Officer (Botany), at Regional Ayurveda Research Institute, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH and Govt. of India.

Preparation of plant extracts

L. aspera of plants were collected, separated healthy leaves, washed cleanly in distilled water and shade dried for complete removal of moisture. Then leaves were coarsely powdered and used for Soxhlet extraction in solvent as ethanol 60°C for 24 hr to separate the constituents. Then for the dryness, rotary vacuum evaporator was employed to concentrate the extract and refrigerated until use for the experiment.

Determination of flavonoids

Flavonoid determination was by the method reported by Ejikeme *et al.*[8] and Boham and Kocipai.[9] Exactly 50 cm^3 of 80% aqueous methanol added was added to 2.50 g of ethanol extract of *L. aspera* leaves in a 250 cm^3 beaker, covered, and allowed to stand for 24 hr at room temperature. After discarding the supernatant, the residue was re-extracted (three times) with the same volume of ethanol. Whatman filter paper number 42 (125 mm) was used to filter whole solution of sample. Each sample filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desicator and weighed until constant weight was obtained. The percentage of flavonoid was calculated as the following formula:

% Flavonoid = Weight of Flavonoid/Weight of Sample ×100

Determination of alkaloids

Quantitative determination of alkaloid was followed according to the methodology by Harborne.[10] Exactly 200 cm^3 of 10% acetic acid in ethanol was added to ethanol extract of *L. aspera* leaves (2.50 g) in a 250 cm^3 beaker and allowed to stand for 4 hr. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide drop wise to the extract until the precipitation was complete immediately after filtration. After 3 hr of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 20 cm^3 of 0.1 M of ammonium hydroxide and then filtered using Gem filter paper (12.5 cm). The content in the crucible was cooled in a desicator and weighed until constant weight was obtained. The percentage of alkaloid is expressed mathematically as the following formula:

% Alkaloid = Weight of Alkaloid/Weight of Sample ×100

Antioxidant analysis

Both the extracted compound used for the antioxidant properties of the plant extracts of *Leucas aspera* were determined by conducting DPPH assay, hydrogen peroxide scavenging and nitric oxide radical scavenging assay as given below:

DPPH free radical scavenging assay

DPPH assay was performed as per Blois[11] and Haleshappa *et al.*[12] method. 1.3 mg/ml DPPH was prepared in HPLC (High Pressure Liquid Chromatography) grade methanol of which 75 µl of DPPH solution was utilized, and various concentrations (6.25, 12.5, 50, and 100 µg/ml) of test solutions were prepared, and volume was made up to 3 ml with HPLC grade methanol. Plant extract compounds were compared with the gallic acid which was used as the reference standard. The reaction mixture was well mixed and incubated at room temperature for 30 min, and the absorbance was recorded at 517 nm. Control was prepared by adding 1ml of methanol and 2 ml of DPPH solution.

% Scavenged [DPPH] = [(AC−AS)/AC] × 100

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample of extracts or standard.

Hydroxyl radical scavenging assay

The deoxyribose assay was performed as described by Halliwell *et al.*[13] Here, the reaction volume of 1.0 ml contained of 5.6 mM deoxyribose, 2.8 mM H_2O_2, 40 mM $FeCl_3$, 100 mM EDTA, and varying dilutions of the compounds in 2.5 mM phosphate buffer (PBS) solution, pH 7.4. In the beginning, the reaction is triggered by adding of 0.1 mM ascorbic acid followed by the incubation for 90 min at 37°C. After incubation, 1 mL of TBA (Thiobarbituric acid) (0.7 % in 0.05 N KOH) and 1 mL of 2.5 % TCA (Trichloroacetic acid) was added to it and was heated at 100°C for 8 min. This mixture was cooled and the pink coloration formed was measured at 532 nm. Controls were devoid of the sample extracts. Gallic acid was used as the reference standard. The percentage inhibition of hydroxyl radicals (% scavenged) is calculated as the following formula:

% Scavenged = [(AC−AS)/AC] ×100

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample of extracts or standards.

Nitric oxide radical scavenging assay

Sodium nitropruside at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions which react with Greiss reagent to form purple azo dye. The degree of decrease in the formation of azo dye will reflect the extent of scavenging.

0.5 ml of extract or standard of different concentrations, 0.75 ml of sodium nitroprusside (10mm) prepared in phosphate buffer was added. After incubation at room temperature for 60 min 1.25 ml of Griess reagent was added. The mixture was incubated in the dark and in room temperature for 5 min and absorbance was read at 520 nm.[14]

MTT cytotoxicity assay for *in vitro* anticancer study

The cytotoxicity assay was performed according to the microculture MTT method with slight modifications.[15] Raw cells of MCF-7 were dissociated with cell dissociating solution (0.2% trypsin, 0.02% EDTA, and 0.05 % glucose in PBS). The viability of the cells was checked and centrifuged. The cells were seeded at the density of 5 × 104 cells/well in 96-well culture plates and incubated for 24 h (at 37°C and 5% CO_2), 100 µl of different concentrations of compounds were added on to microtiter plates. The plates were then incubated at 37°C for 24 h in 5% CO_2 atmosphere. After incubation, the test solutions in the wells were discarded and 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37 °C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. Camptothecin was used as the reference standard for anticancer activity. The inhibition of cell viability was calculated using the following formula:

% Inhibition activity=1-T/C x 100

Where T= Absorbance of the test sample

C= Absorbance of the control sample.

RESULTS

Determination of flavonoid and alkaloid of ethanol extract of *L. aspera* leaves

The crude ethanol extract of *L. aspera* leaves further applied to determination of two novel metabolites, they are flavonoid and alkaloid for the biological studies. Total weight of the extract used for the separation of metabolites was 2.5gm in that flavonoid and alkaloid resulted in 1456gm and 1044gm respectively.

DPPH scavenging activity

The flavonoid and alkaloid of ethanol extract of *L. aspera* leaves exhibited a significant dose dependent inhibition of DPPH free radical scavenging activity. The concentration-dependent assays were carried out with these compounds and the results were presented in Figure 1. Among five different concentrations were used in the study (6.25 to 100 µg/ml), flavonoid compound showed scavenging activity of 50.00%, 55.60%, 61.80%, 72.70%, and 90.50% at the concentration of 6.25, 12.50, 25.50, 50.00, 100.00 µg/ml respectively and for alkaloid compound showed the scavenging activity of 40.40%, 43.10%, 46.80%, 50.70%, and 54.50% at the concentration of 6.25, 12.50, 25.50, 50.00, 100.00 µg/ml respectively.

On the other hand, gallic acid showed 29.80%, 34.79%, 50.82%, 72.57%, and 83.89% activity with the same concentration. The IC_{50} value of gallic acid, flavonoid and alkaloid of ethanol extract of *L. aspera* leaves compounds were found to be 26.00 µg/ml, 9.25 µg/ml and 60.05 µg/ml respectively (Table 1).

Hydroxyl radical scavenging activity

The flavonoid and alkaloid of ethanol extract of *L. aspera* leaves exhibited a significant dose dependent hydroxyl radical scavenging activity. The concentration-dependent assays were carried out with these compounds and the results were presented in Figure 2. Among five different concentrations used in the study (6.25 to 100 µg/ml), flavonoid compound showed scavenging activity of 40.57%, 51.60%, 62.50%, 71.80% and 87.50% at the coonentration of 6.25, 12.50, 25.50, 50.00 and 100.00 µg/ml respectively and for alkaloid 38.70%, 42.40%, 45.60%, 51.08%, and 57.80% at the concentration 6.25, 12.50, 25.50, 50.00, 100.00 µg/ml respectively.

On the other hand, gallic acid showed 42.45%, 53.36%, 64.65%, 73.57%, and 90.58% activity with the same concentration. The IC_{50} value of gallic acid, flavonoid and alkaloid of ethanol extract of *L. aspera* leaves compounds were found to be 16.00 µg/ml, 10.25 µg/ml and 65.05 µg/ml respectively (Table 2).

Nitric oxide radical scavenging assay

The flavonoid and alkaloid of ethanol extract of *L. aspera* leaves exhibited a significant dose dependent inhibition of Nitric oxide radical scavenging assay. The concentration-dependent assays were carried out with these compounds and the results are presented in Figure 3. Among five different concentrations used in the study (6.25 to 100 µg/ml), flavonoid compound showed scavenging activity of 40.60%, 47.50%, 62.50%, 70.60% and 78.60% at the concentration of 6.25, 12.50, 25.50, 50.00 and 100.00 µg/ml respectively and for alkaloid 32.70%, 42.30%, 44.50%, 50.10%, and 52.70% at the concentration of 6.25, 12.50, 25.50, 50.00, 100.00 µg/ml respectively.

On the other hand, gallic acid showed 28.80%, 34.50%, 50.10%, 69.50%, and 78.70% activity with the same concentration. The IC_{50} value of gallic acid, flavonoid and alkaloid of ethanol extract of *L. aspera* leaves compounds were found to be 26.00 µg/ml, 13.10 µg/ml and 61.20 µg/ml respectively (Table 3).

MTT cytotoxicity assay for *in vitro* study

The cytotoxicity study was carried out for plant ethanolic extract of the compound flavonoid and alkaloid leaves part of *L. aspera* on MCF-7 cell lines at different concentrations to determine the IC_{50} by MTT assay. Cytotoxicity of ethanolic extract of the compound flavonoid leaves part of *L. aspera* against MCF-7 cell lines and found to be 95.0%, 84.0%, 77.0%, 70.0%, and 65.0% toxic at a concentration of 100, 200, 300, 400, and 500 ug/ml; and cytotoxicity of alkaloid compound leaves part of *L. aspera* against

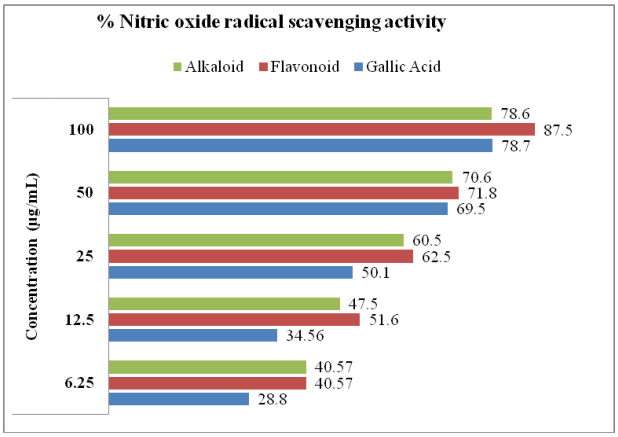

Figure 3: Nitric oxide radical scavenging assay of leaves extracts of *Leucas aspera* compound flavonoid and alkaloid against standard gallic acid.

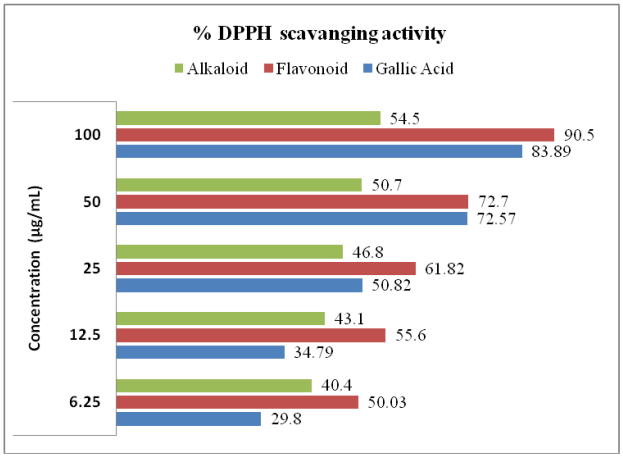

Figure 1: Diphenyl-1-picrylhydrazyl radical scavenging activity of leaves extracts of *Leucas aspera* compound flavonoid and alkaloid against standard gallic acid.

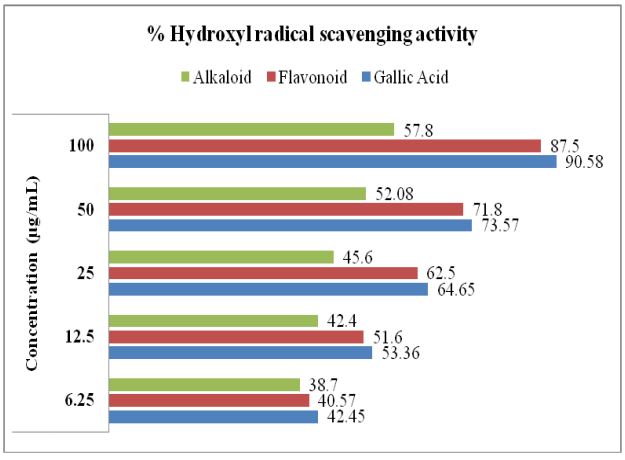

Figure 2: Hydroxyl radical scavenging activity of leaves extracts of *Leucas aspera* compound flavonoid and alkaloid against standard gallic acid.

Table 1: Table showing the IC_{50} concentrations of the leaves of *L. aspera* compounds against standard.

Sl.No.	Sample	IC_{50} (µG/mL)
1	Gallic Acid	28
2	Flavonoid	9.25
3	Alkaloid	60.05

Table 2: Table showing the IC_{50} concentrations of the leaves of *L. aspera* compounds against standard.

Sl.No.	Sample	IC_{50} (µG/mL)
1	Gallic Acid	16
2	Flavonoid	10.25
3	Alkaloid	65.00

Table 3: Table showing the IC_{50} concentrations of the leaves of *L. aspera* compounds against standard.

Sl.No.	Sample	IC_{50} (µG/mL)
1	Gallic Acid	26
2	Flavonoid	13.10
3	Alkaloid	61.20

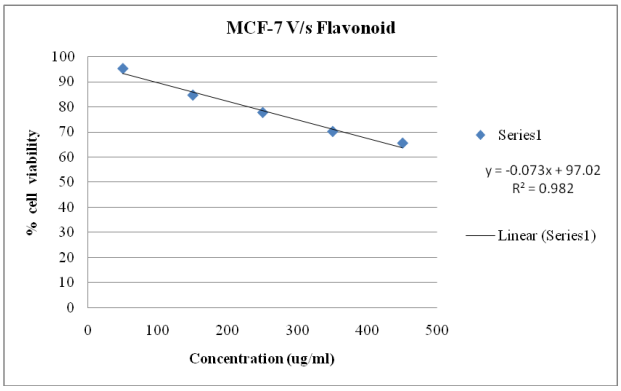

Figure 4: Effect of flavonoid compound of *L. aspera* leaves ethanol extract on MCF-7 cell viability.

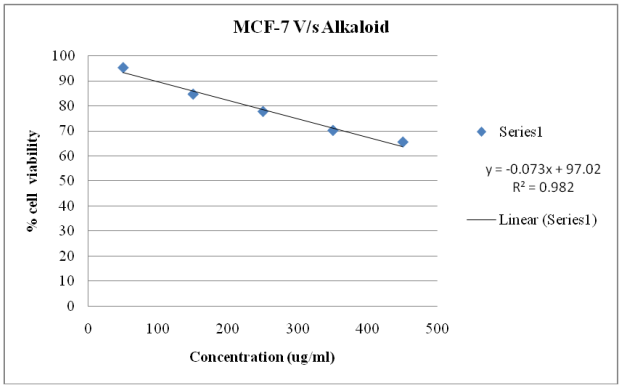

Figure 5: Effect of alkaloid compound of *L. aspera* leaves ethanol extract on MCF-7 cell viability.

MCF-7 cell lines and found to be 93.0%, 82.0%, 78.0%, 70.0% and 66 ug/ml toxic at a concentration of 100, 200, 300, 400, and 500 ug/ml respectively. IC_{50} value 247.56 ug/ml was obtained for MCF-7 for flavonoid and 236.45 ug/ml was obtained for alkaloid compound with optimum effect on % cell viability. Cytotoxicity of ethanol extract of the compound flavonoid leaves part of *L. aspera* toward MCF-7 was found to suppress the cell proliferation with concentration dependent and it was showed good cytotoxicity compared to alkaloid compound. The percentage of cell viability was found to be increasing with dose dependent concentration of the tested compounds and that have shown in Figures 4-5.

DISCUSSION

Flavonoids are large group of plant polyphenolic natural products named as flavones, flavonols, isoflavones, flavonones and chalcones, also called as nature's caring drugs, possess various types of biological and pharmacological properties. Previous findings of antiviral, antifungal, antioxidant, antiinflammatory, antiallergenic, antithrombic, anticarcinogenic, hepatoprotective, and cytotoxic activities of flavonoids have expressed significance in research of flavonoid rich plants and plant products. Flavonoids have established to apply valuable impact on acute and chronic diseases by interaction of lipid peroxidation. Higher capacity to interact with protein phosphorylation and antioxidant, metal-chelating and free radical scavenging activity deals with potential pharmacological outline of flavonoids.[16-21]

Alkaloids significantly play a role as a natural product and acting as novel antioxidant called as isoquinoline alkaloids namely stylopine, protopine, fumaritine, fumaricine, fumarophycine, fumariline, fumarofine fromthe various part of plants and products to possess various types of biological and pharmacological properties. Previous findings of antimicrobial, antimalarial, cytotoxic, antiparasitic, antizoonotic for the communicable and non-communicable disease including human immunodeficiency virus (HIV) activities by the type of alkaloids extensively worked and reported. The possible chemotherapeutic action on tumor promoters are due to their antioxidant potential as a free radical scavenging activity against DPPH in various concentration.[22] Phenolic alkaloids

also act as another class of antioxidant molecules of the plants and plant products. Antioxidant properties by different phenolic alkaloid compounds reported previously named as oleracein A, oleracein B, oleracein E, they were also reported depends on the scavenging potential against DPPH radical, hydrogen peroxide-induced lipid peroxidation in rat brain homogenates as a inhibitory effect on neuronal cells.[23] Recently revealed the literature on some alkaloids can be acting as natural antioxidants for the medicinal remedies and commercial production.[24]

Antioxidants are very active in scavenging free radicals and to play it vital role in diseases of cardiovascular, aging, cancer, genetic, neurogenic and inflammatory disorders.[25] Most of the naturally occurring antioxidants play it as formulated to give touch up with nutraceuticals property; it can prevent and help to avoid oxidative stress without damaging cellular physiology in the body. Hence, it is easy to assess antioxidant activity by the use of stable free radical scavenging of DPPH.[26-28] In the present study findings show that the activities of the ethanol extract both the compound exhibited very good antioxidant activities and also possess moderate cytotoxic activity on MCF-7 cell line assay. All the studied antioxidant results were compared to the standard gallic acid and found to be effective maximum flavonoid and minimum effect by alkaloid compound and their IC_{50} concentration. This is known phenomenon used gallic acid is pure form, whereas the plant compounds still need to be processed in order to isolate the fractions responsible for their antioxidant activity. Co-relate to our findings, Sambandam *et al.*[29] reported on flavonoid quercetin from the leaves of *Trigonella foenum-graecum* on 250 μg/ml concentration for the % relative antioxidant activity. Previous reports have also mentioned the efficient antioxidant activity of the compound flavonoids from aquatic fern *Azolla microphylla*.[30] However, these assays may be used to guide to the further action for fractionation and isolation of potential antioxidant compounds from the plant *Leucas aspera* leaves by the spectroscopy studies.

Several plant constituents already explored for free radical scavenging and proven antioxidant properties in *in vitro* studies.[31,32] Phenolic compounds are very potential plant metabolites and rich in antioxidants like types of flavonoids and alkaloides. The relationship between total phenols and their antioxidant activity are highly positive with significant in biological action, due to their scavenging role in the compound functional groups.[33] The flavonoid content of the extract can also scavenge hydrogen peroxide, nitrogen oxide by donating electrons and thereby neutralizing it to water.[34] In the literature flavonoid compounds known to be as effective hydrogen donors to make them potential antioxidants compare to alkaloid compounds.[35] Antioxidant activity of any compounds is mainly due to their redox potential, which interacting in adsorbing and neutralizing DPPH radicals or hydroxyl radicals or nitric oxide radicals, quenching singlet and triplet oxygen or neutralizing peroxides.[36] Polyphenolic molecules materialize to function it as good electron and donor of hydrogen atom therefore, it can be terminate radical chain reaction which is involved in lipid peroxidation by the conversion of free radicals and stable reactive oxygen species level. Thus, the antioxidant activity of *L. aspera* extracts might be attributed to these modes of action due to their flavonoid and alkaloid polyphenolic molecules and also it is evidenced by the cell viability from the MCF-7 cytotoxic assay.

CONCLUSION

The findings of this study conclude that *Leucas aspera* leaves possess the strong antioxidant potential which are responsible for their active therapeutic potential. Antioxidant properties estimated using DPPH, hydroxyl radical and nitric oxide scavenging assays exerted the potent antioxidant property in flavonoid compound of leaves of *Leucas aspera*. It is also confirmed the anticancer activity on MCF-7 cell lines by MTT assay with moderate concentration. Hence, this plant is a potent source of antioxidant and anticancer properties, also supporting its medicinal use for other various ailments. The plant possesses pharmaceutically important phytochemicals and their free radical scavenging activities are attributed to the higher amount of flavonoid then the alkaloid. However, further research through *in vivo* studies and spectral analysis on the ability of the leaves to act as an antioxidant is needed.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

cm: centi meter; **DMSO**: Dimethyl sulfoxide; **DPPH:** 2, 2-diphenyl-1-picrylhydrazyl; **EDTA:**

Ethylenediaminetetraacetic acid; **$FeCl_3$:** Ferric Chloride; **g:** gram; **H_2O_2:** Hydrogen Peroxide; **HIV:** Human Immunodeficiency Virus; **HPLC:** High Pressure Liquid Chromatography; **IC_{50}:** Inhibitory Concentration; **KOH:** Potassium Hydroxide; **MCF-7:** Michigan Cancer Foundation-7; **mg:** mili gram; **min:** minute; **mL/min:** mili litre/ minute; **mL:** mili litre; **mM:** mili molar; **MTT:** 3-(4, 5-dimetylthiazol-2-yl)-2 and 5-diphenyltetrazolium bromide; **nm:** nano meter; **PBS:** Phosphate Buffer Solution; **TBA:** Thiobarbituric Acid; **TCA:** Trichloroacetic Acid; **µG:** micro gram.

REFERENCES

1. Kolgi RR, Shivakumar SL, Sajeeda N, Shivaraj Y, Karigar CS. Therapeutic Properties of Extracts of *Leucas aspera* and *Anisomeles malabarica*. Int J Biol Med Res. 2019;10(1):6631-4.
2. Goudgaon NM, Basavaraj NR, Vijayalaxmi A. Antiinflammatory Activity of Different Fractions of *Leucas aspera* Spreng. Ind J Pharmacol. 2003;35:397-8.
3. Rahman MS, Sadhu SK, Hasan CM. Preliminary Antinociceptive, Antioxidant and Cytotoxic Activities of *Leucas aspera* Root. Fitoterapia. 2007;78(7-8):552-5.
4. Lopez A, Hudson JB, Towers GHN. Antiviral and Antimicrobial Activities of Colombian Medicinal Paints. J Ethnopharmacol. 2001;77(2-3):189-96.
5. Chew AL, Jessica JJA, Sasidharan S. Antioxidant and Antibacterial Activity of Different Parts of *Leucas aspera*. As Pac J Trop Biomed. 2012;2(3):176-80.
6. Das BK, Das B, Arpita FK, Morshed MA, Uddin A, Bhattacherjee R, Hannan JMA. Phytochemical Screening and Antioxidant Activity of *Leucas aspera*. Int J Pharm Sci Res. 2011;2(7):1746-52.
7. Mangathayaru K, Lakshmikant J, Sundar SN, Swapna R, Grace FX, Vasantha J. Antimicrobial Activity of *Leucas aspera* Flowers. Fitoterapia. 2005;76(7-8):752-4.
8. Ejikeme CM, Ezeonu CS, Eboatu AN. Determination of Physical and Phytochemical Constituents of Some Tropical Timbers Indigenous to Niger Delta Area of Nigeria. Eur Scient J. 2014;10(18):2470-70.
9. Boham BA, Kocipai AR. Flavonoids and Condensed Tannins from Leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium*. Pacific Scien. 1994;48(4):458-63.
10. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, UK. 1973.
11. Blois MS. Antioxidant Determinations by the Use of a Stable Free Radical. Nature. 1958;181(4617):1199-250.
12. Haleshappa R, Keshamma E, Girija CR, Thanmayi M, Nagesh CG, Fahmeen LGH, *et al*. Phytochemical Study and Antioxidant Properties of Ethanolic Extracts of *Euphorbia milii*. Asian J Biolog Sci. 2020;13(1):77-82.
13. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Clarendon Press. 1989;3:617-783.
14. Ebrahimzadeh MA, Pourmorad F, Hafezi S. Antioxidant Activities of Iranian Corn Silk. Turkish J Biol. 2008;32(1):43-9.
15. Sylvester PW. Optimization of the Tetrazolium Dye (MTT) Colorimetric Assay for Cellular Growth and Viability. Drug Des Dis. 2011;716(9):157-68.
16. Saija A, Scalese M, Lanza M, Marzullo D, Bonina F. Flavonoids as Antioxidant Agents: Importance of their Interaction with Biomembranes. Free Rad Biol Med. 1995;19(4):481-6.
17. Moon YJ, Wang X, Morris ME. Dietary Flavonoids: Effects on Xenobiotic and Carcinogen Metabolism. Toxicol. 2006;20(2):187-210.
18. Veitch NC. Isoflavonoids of the Leguminosae. Nat Prod Rep. 2007;24(2):417-64.
19. Jiang H, Zhan WQ, Liu X, Jiang SX. Antioxidant Activities of Extracts and Flavonoid Compounds from *Oxytropis falcate* Bunge. Nat Prod Res. 2008;22(18):1650-6.
20. Kim HP, Son KH, Chang HW, Kang SS. Anti-inflammatory Plant Flavonoids and Cellular Action Mechanisms. J Phamacol Sci. 2004;96:229-45.
21. Acker VSA, Berg VDDJ, Tromp MN, *et al*. Structural Aspects of Antioxidant Activity of Flavonoids. Free Rad Biol Med. 1996;20(3):331-42.
22. Cui WH, Iwasa K, Tokuda H, Kashihara A, Mitani Y, Hasegawa T, *et al*. Potential Cancer Chemopreventive Activity of simple Isoquinolines, I-benzylisoquinolinjes, and Protoberberines. Phytochemis. 2006;67(1):70-9.
23. Zijuan Y, Cejia L, Lan X, Yinan Z. Phenolic Alkaloids as a New Class of Antioxidants in *Portulaca oleracea*. Phytother Res. 2009;23(7):1032-5.
24. Fadila MB, Sabiha K, Khalida B, Mohamed C, Sandrine A, Yves C, *et al*. Antioxidant Activities of Alkaloid Extracts of Two Algerian Species of *Fumaria: Fumaria capreolata* and *Fumaria bastardii*. Rec Nat Prod. 2007;1(2-3):28-35.
25. Cioffi G, D'Auria M, Braca A, Mendez J, Castillo A, Morelli I, *et al*. Antioxidant and Free Radical Scavenging Activity of Constituents of the Leaves of *Tachigalia paniculata*. J Nat Prod. 2002;65(11):1526-9.
26. Molyneux P. The Use of the Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity. Songklanakarin J Sci Technol. 2003;26(2):211-9.
27. Brand-Williams W, Cuevelier ME, Berset C. Use of a Free Radical Method to Evaluate Antioxidant Activity. Lebensm Wiss U-Technol. 1995;28(1):25-30.
28. Moon JK, Shibamoto T. Antioxidant Assays for Plant and Food Components. J Agric Food Chem. 2009;57(5):1655-66.
29. Sambandam B, Thiyagarajan D, Ayyaswamy A, Raman P. Extraction and Isolation of Flavonoid Quercetin from the Leaves of *Trigonella foenum-graecum* and their Anti-Oxidant Activity. Int J Pharm Pharmaceut Sci. 2016;8(6):120-4.
30. Selvaraj K, Chowdhury R, Bhattacharjee C. Isolation and Structural Elucidation of Flavonoids from Aquatic Fern *Azolla microphylla* and Evaluation of Free Radical Scavenging Activity. Int J Pharm Pharm Sci. 2013;5:743-749.
31. Aruoma OI, Cuppett SL. Antioxidant Methodology *in vivo* and *in vitro* Concepts. AOCS Press, Champaign, Illinois. 1997;41-172.
32. Patil SJ, Sathishgoud S, Vishwanatha T, Sathish SV. *In vivo* Adrenal Gland Antioxidants Profile on Chromatographic Fractions of *Citrus medica* seeds in Female Albino Rats. Life Science Edge. 2017;03(03):21-31.
33. Haleshappa R, Patil SJ, Usha T, Murthy SM. Phytochemicals, Antioxidant Profile and GCMS Analysis of Ethanol Extract of *Simarouba glauca* Seeds. Asi J Biolog Life Sci. 2020;9(3):379-85.
34. Haleshappa R, Patil SJ, Murthy SM. Phytochemical Analysis, *in vitro* Evaluation of Antioxidant and Free Radical Scavenging Activity of *Simarouba glauca* Seeds. Adv Pharmacol Pharma. 2021;9(1):01-8.
35. Yen GC, Duh PD, Tsai CL. The Relationship between Antioxidant Activity and Maturity of Peanut Hulls. J Agric Food Chem. 1993;41(1):67-70.
36. Osawa T. Novel Natural Antioxidants for Utilization in Food and Biological Systems; Post Harvest Biochemistry of Plant Food Materials in the Tropics. Japan Scientific Societies Press, Tokyo, Japan. 1994;1:241-51.

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