Asian J Biol Life Sci., 2025; 14(2):375-381.
https://ajbls.com

Research Article

In vitro Antioxidant, Anti-Inflammatory, Anti-venom and Anti-cancer Effect of *Leucas aspera* Plant Extract

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ABSTRACT

In India, *Leucas aspera* is commonly utilized as an ethnomedicinal herb to treat numerous ailments. The current investigations assessed the anti-inflammatory, antioxidant, anticancer, apoptotic, and antivenom properties of an aqueous extract of *Leucas aspera*. Some *in vitro* models were used to estimate antioxidant activity, such as the DPPH photometric assay, OH radical scavenging capacity, anti-inflammatory activity, Cyclooxygenase (COX), and Lipoxygenase (LOX), anticancer, apoptotic, and antivenom activity. The study found that plant extracts' antioxidant and anti-inflammatory effects might be attributed to inhibiting DPPH and OH, COX, and LOX enzymes, respectively, supporting the traditional usage of the aforementioned plants in inflammatory illnesses. The MTT experiment showed that the aqueous extract of *Leucas aspera* was highly selective ($p<0.005$) against HepG2 cells, causing morphological alterations. Based on the current data, it was determined that the aqueous extract of *Leucas aspera* exhibits a more potent and safer anti-inflammatory, antioxidant capabilities, anticancer and anti-venom agent because of its significant PLA2 inhibition.

Keywords: *Leucas aspera*, Inflammation, Cyclooxygenase, Lipoxygenase, DPPH, Hydroxyl.

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Received: 17-01-2025;
Revised: 28-04-2025;
Accepted: 04-06-2025.

INTRODUCTION

Plant-derived bioactive chemicals have developed over millions of years due to plant adaption mechanisms.[1] These substances are essential to plant survival, reproduction, and interactions with the environment, even though they are not necessary for fundamental plant metabolism.[2] A complex and dynamic phytochemical profile is produced within each species as a result of the production of these secondary metabolites, which is often driven by particular developmental stages or environmental challenges.[3] A vast reservoir of potentially beneficial compounds for human use is provided by this natural diversity.[4] Research on the biosynthetic pathways, genetic regulation, and ecological activities of plant bioactive compounds is part of the study of these compounds in addition to their identification and characterization.[5]

By comprehending these characteristics, improved breeding programs, agricultural techniques, and biotechnology technologies can be developed to optimize the production of desired compounds.[6] Additionally, by fusing ethnobotanical knowledge with contemporary scientific research, studies of traditional medicinal plants continue to produce novel bioactive compounds.[7] Phytochemicals, which are chemical compounds with several health benefits, have traditionally been primarily found in plants.[8] A wide range of illnesses can be prevented and treated with the use of plant-based compounds. Their characteristics were first examined organoleptically through traditional medicine research.[9]

As science developed over time, it was able to research certain components included in things like essential oils and plant extracts. The expansion of chemical knowledge also made it possible to classify important chemicals. Among the most significant chemical groups present in plants are terpenoids, carotenoids, glucosinolates, saponins, phytosterols, and polyphenols. Each of these groups possesses different qualities that contribute to the improvement of human health. Three-quarters of all primary

OPEN ACCESS
ScienScript

DOI: 10.5530/ajbls.20251454

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and secondary liver malignancies in the US are hepatocellular carcinomas.[10]

The regional distribution of cirrhosis or chronic liver disease is increasing along with the expected number of new cases and fatalities. *Leucas aspera* is widely used as an ethnomedic herb in India to cure a variety of illnesses. The current studies were designed to assess the anti-inflammatory, antioxidant, anti-cancer, and anti-venom activity of the aqueous extract of *Leucas aspera*.

MATERIALS AND METHODS

Sequential extraction of plant samples

Using Soxhlet's apparatus, 10 g of the shed-dried powdered plant samples of *Leucas aspera* were extracted successively in hexane, ethanol, and water for up to 8 hr. At room temperature and with less pressure, the extracted samples were evaporated. For later analysis, the dehydrated extracts were kept in a refrigerator at 4°C.

DPPH radical scavenging test

With a few minor adjustments, the procedure provided was used to perform the DPPH radical scavenging experiment.[11] In short, 0.1 mM DPPH solution in ethanol was mixed with 1 mL of *Leucas aspera* (1 mg/mL). The absorbance at 517 nm was used to measure the DPPH decrease following 20 min of room temperature incubation. The reference chemical used was ascorbic acid (1 mM).

Assay for scavenging Hydroxyl (OH) radicals

The Fenton reaction demonstrated how OH radicals can scavenge.[12] The reaction mixture contained 1.5 mL of individual *Leucas aspera* (1 mg/mL), 60 mL of FeCl2 (1 mM), 90 mL of 1e10 phenanthroline (1 mM), 2.4 mL of phosphate buffer (0.2 M, pH 7.8), and 150 mL of H2O2 (0.17 M). H_2O_2 was introduced to start the reaction. At room temperature, the absorbance was measured at 560 nm after 5 min of incubation. The reference chemical used was ascorbic acid (1 mM).

COX inhibition assay

The test was performed using the Colorimetric COX (human ovine) inhibitor Screening assay kit.[13] In short, 150 mL of assay buffer, 10 mL of heme, 10 mL of enzyme (COX-1 or COX-2), and 10 mL of plant material (1 mg/mL) make up the reaction combination. The COX catalytic domain's peroxidase component is used in the experiment. The appearance of oxidized N, N, N'-Tetramethyl-Phenylenediamine (TMPD) at 590 nm was used to calorimetrically measure the peroxidase activity. A common drug is aspirin (acetylsalicylic acid, 1 mM). Where T ¼ at 590 nm, the inhibitor absorbs heavily. C ¼ 100% initial activity absorbance at 590 nm in the absence of an inhibitor.

Cell lines and culture preparation

NCCS Pune provided the HepG2 cell lines. Roswell Park Memorial Institute (RPMI) media supplemented with 10% FBS, 1% L-Glutamine, 0.1 mM (millimolar) nonessential amino acid, and 100 u/mL penicillin/streptomycin, as well as Dulbecco's Modified Eagle's Media (DMEM), were used to cultivate HepG2 cells. The cells were cultivated at 37°C with 5% CO_2 in a humidified incubator.

MTT assay

A concentration of 1×10^5 cells per well was used to seed the cells in 96-well plates. Following 24 hr, cells were starved for an hour at 37°C after being twice rinsed with 100 µL of serum-free medium

Leucas aspera plant (Family - Lamiaceae).

and 2.5-25 µL of *Leucas aspera* extract. Following fasting, cells were cultivated for 24 hr at 37°C in a CO_2 incubator.[14] Following the treatment period, the medium was aspirated, serum-free media containing 0.5 mg/mL of MTT was added, and the mixture was incubated in a CO_2 incubator for 4 hr at 37°C. After discarding the MTT-containing media, 200 µL of PBS was used to rinse the cells. Use a pipette to thoroughly stir in 100 µL of DMSO to dissolve the crystals. A microplate reader (BioRad 680) was used to measure the absorbance of the blue and purple formazan dyes at 570 nm. The percentage of stable cells relative to the control is how the data are presented. The ideal dosages were identified throughout a range of periods, and the half-maximal Inhibitory Concentration (IC_{50}) values were computed. The number of samples that can reach 50% of cell growth is known as the medium effective dose, or IC_{50}. The proliferation curve for each well was visually determined.

Dual AO/EB fluorescent staining

To detect changes in morphological patterns caused by *Leucas aspera* inhibitory effect on HepG2 cells, dual AO/EB labeling was used.[15] The cancer cells were kept at room temperature for nearly an hour in a 3:1 combination of glacial acetic acid and methanol. HepG2 cells were exposed to several concentrations of *Leucas aspera* (15 µg/mL) for one day following incubation. The cancer cells were labeled with 20 µL of AO/EB in PBS buffer for 5 min at 37°C. Each sample contained at least 300 cells. The percentage of apoptotic cells were determined using a fluorescence microscope (those with bright orange-red nuclei).

In vitro inhibitory secretory phospholipase A2 (sPLA2) activity

The anti-venom potential of *Leucas aspera* on sPLA2 of *Leucas aspera* was assessed using a commercially available sPLA2 targeting assay obtained from Abcam® (Cambridge, United Kingdom).[16] The assay kit's reagents were prepared by the instructions on the accompanying information page.

RESULTS

Effect of the antioxidant action of *Leucas aspera* plant extract

The extractives had the highest level of DPPH activity. *Leucas aspera* exhibited a scavenging DPPH activity of 90.2±4.66 µg/mL at a concentration of 100 µg/mL (Figure 1A). *Leucas aspera* aqueous extracts have an IC_{50} of 54.77±4.66 µg/mL. It is advised that these extracts be used further because of their strong free radical scavenging properties. The extractives exhibited the most activity of OH. At a dose of 100 µg/mL, *Leucas aspera* demonstrated a scavenging OH activity of 62.2±3.06 µg/mL (Figure 1B). The IC_{50} of aqueous extracts of *Leucas aspera* is 42.02±1.96 µg/mL. Because of their potent ability to scavenge free radicals, it is recommended that these extracts be used further.

Effect of *Leucas aspera* on Anti-inflammatory marker action of LOX and COX-2

Leucas aspera aqueous extracts demonstrated an IC_{50} value of 39.5 µg/mL and a peak LOX inhibitory efficacy of 60.19% at 100 µg/mL. *Leucas aspera* showed that LOX was suppressed in a dose-dependent manner. At dosages ranging from 25 to 150 µg/mL, *Leucas aspera* aqueous extracts demonstrated percentage inhibition, with IC_{50} values of 50.2 µg/mL (Figure 2A). *Leucas aspera* aqueous extracts demonstrated an IC_{50} value of 52.59 µg/mL and a peak COX-2 inhibitory efficacy of 83.46 % at 100 µg/mL. Extracts showed that COX-2 was suppressed in a dose-dependent manner. At dosages ranging from 25 to 150 µg/mL, *Leucas aspera* aqueous extracts demonstrated percentage inhibition, with IC_{50} values of 50.2 µg/mL (Figure 2B).

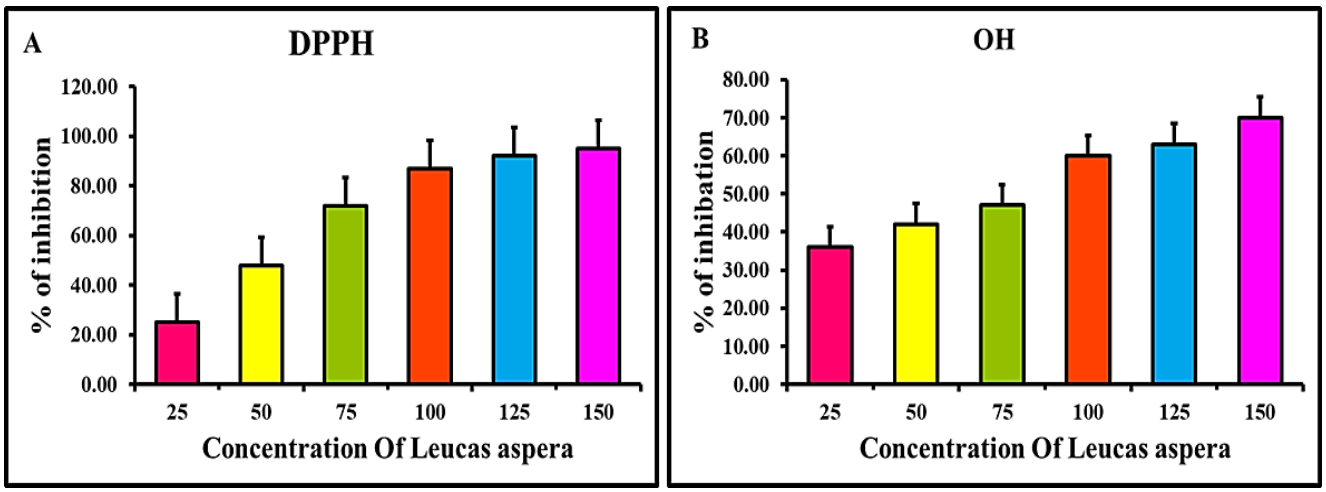

Figure 1: Antioxidant Scavenging Assays. A) *Effect of Leucas aspera on DPPH radical scavenging assay.* The image illustrates how the aqueous extracts of *Leucas aspera* can scavenge free radicals. B) Effect of *Leucas aspera* on Hydroxyl (OH) radical scavenging assay. Each value in the table is represented as Mean±SD Values in the same column followed by different letters are significantly different ($p<0.05$).

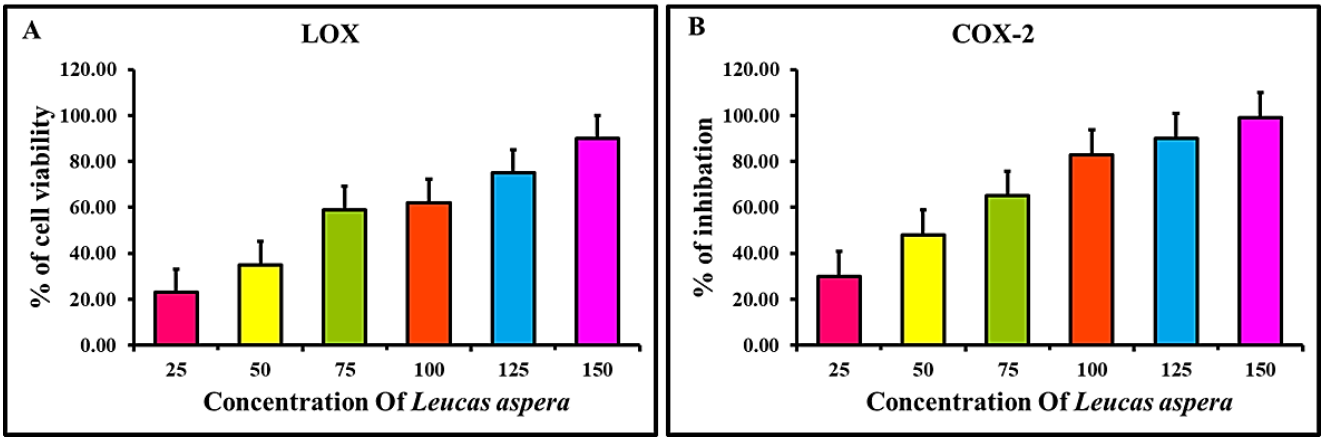

Figure 2: Anti-inflammatory activity. A) Effect of *Leucas aspera* on anti-inflammatory marker LOX assay. B) Effect of *Leucas aspera* on anti-inflammatory marker COX-2 assay. Each value in the table is represented as Mean±SD Values in the same column followed by different letters are significantly different (p<0.05).

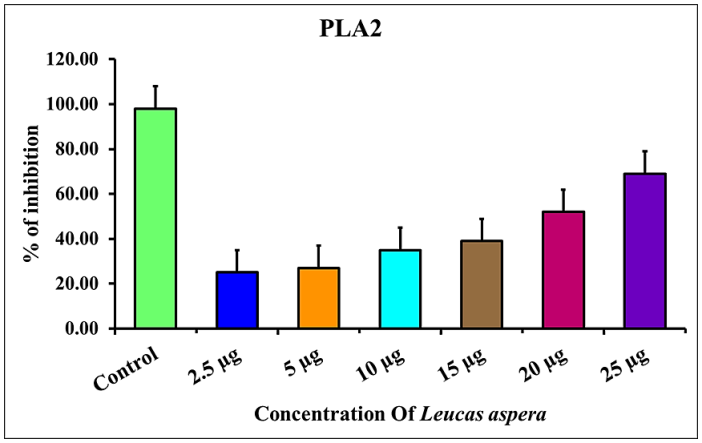

Figure 3: Anti-venom assay. This image illustrates the antivenom parameter of the sPLA2 activity of *Leucas aspera* plant extract. Each value in the table is represented as Mean±SD Values in the same column followed by different letters are significantly different (p<0.05).

Inhibitory sPLA2 activity of *Leucas aspera*

Figure 3 showed that *Leucas aspera*, the potential inhibitor, was present; the sPLA2 activity of 100 µg/mL *Leucas aspera* venom was measured. Plant extracts were tested against 100 µg/mL of *Leucas aspera* venom at doses ranging from 1 µg/mL to 1000 µg/mL. The actions of *Leucas aspera* venom without inhibitors were contrasted with those of sPLA2 in the presence of inhibitors. Phospholipase activity was considerably decreased by *Leucas aspera*, with an IC_{50} of only 20.2±1.82 µg/mL. It has been demonstrated that alkaloids inhibit the hyaluronidase and basic PLA2 enzymes.

Effect of *Leucas aspera* on MTT cytotoxicity assay

Hepatic cancer cell lines are specifically affected by the anti-cancer effects of *Leucas aspera* aqueous extract. The MTT test and morphological analyses were used to evaluate the anticancer effectiveness of *Leucas aspera* aqueous extract. The results of the MTT assay and morphological analysis are shown in the figures

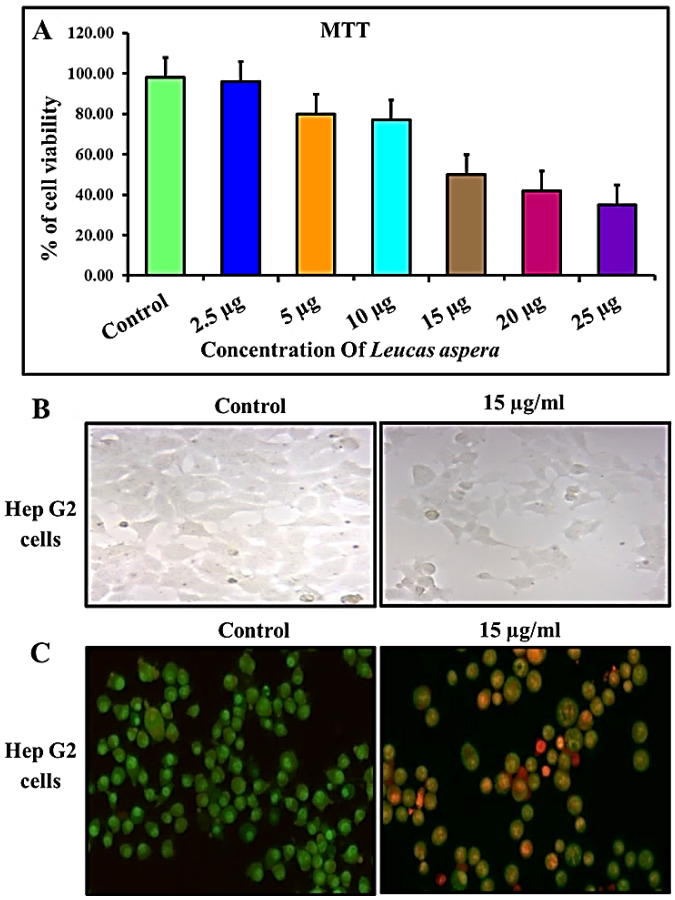

Figure 4: Cytotoxicity and Apoptotic effect of *Leucas aspera* on HepG2 cells. A) Effect of *Leucas aspera* on MTT cytotoxicity assay. B) Effect of *Leucas aspera* on Hep G2 cell morphology assay. Each value in the graph is represented as Mean±SD Values in the same column, followed by different letters are significantly different (p<0.05). C) Effect of *Leucus aspera* on HepG2 apoptotic morphological changes.

4A. They showed that, in comparison to untreated cells, the extract exhibited specific anticancer activity against the colon cancer cell line HepG2. In a dose-dependent manner, the plant extract demonstrated significant (p<0.05) cytotoxicity against

HepG2 cells; however, untreated tumors exhibited significantly lesser sensitivity. For HepG2 cells, the IC_{50} was found to be 15±0.81 µg/mL ($p<0.05$). Normal cells were shown to be far less harmed by the extract. The extract was tested morphologically at dosages of 15±0.81 µg/mL. According to the morphological analysis, HepG2 cells treated with the extract had a notable change in morphology when compared to cells treated with a vehicle; the cells were smaller and abnormally shrunken in comparison to untreated cancer cell lines, but their vehicle counterparts showed no change. We assessed the impact of *Leucas aspera* on apoptotic morphological alterations in HepG2 cells and looked into whether *Leucas aspera*'s reduction in growth rate caused apoptosis (Figures 4A and 4B).

Effect of *Leucas aspera* on apoptotic induction on HepG2 cells

We discovered that hepatic cancer cells underwent apoptosis when exposed to 15 µ doses of *Leucas aspera*. Following *Leucas aspera* treatment, HepG2 cells' apoptotic nuclear structure and shape changed, as shown by the AO/EB dual assay (Figure 4C). In comparison to normal cells, cancer cells treated with insufficient *Leucas aspera* (15 µg/mL) showed apoptosis and fragmented chromatin. The Figure 4B shows a quantitative analysis of apoptosis in hepatic cancer cells that were not treated and those that were treated with *Leucas aspera*. In HepG2 cells, *Leucas aspera* changed nuclear morphology. After consuming Acridine Orange/Ethidium Bromide (AO/EB) labelling, cells with apoptosis were visible in a 15 µg/mL. The green, orange, yellow, and red colour responses of AO/EB staining are used to distinguish between living, early, late, and necrotic cells.

DISCUSSION

The DPPH free radical creates a similar hydrazine when it reacts with hydrogen donors. When the purple DPPH radical comes into contact with a hydrogen donor, it changes color to yellow.[17] It was discovered that when the concentration of the *Leucas aspera* extract increased, so did its DPPH radical scavenging capacity. The basis for this experiment was 1,1-Diphenyl-2-Picrylhydrazyl's (DPPH) capacity to decolorize when antioxidants are present.

The antioxidant capacity of the extracts was evaluated utilizing a variety of *in vitro* systems, including DPPH•, ABTS•+, FRAP, OH•, β-carotene linoleic acid bleaching system, phosphomolybdenum reduction, and Fe^{2+} chelation. A recent study determined that *B. vahlii* leaf methanolic extract has high antioxidant properties. To isolate and characterize the active antioxidants, which could be a possible source of natural antioxidants, more research is required.[18] That was fulfilled. Our present study showed that the activity of hydrogen radical scavenging the aqueous extracts of *Leucas aspera* exhibited dose-dependent hydroxyl radical scavenging efficacy. *Leucas aspera* aqueous extracts showed more activity in the extracts than at a concentration of 150 µg/mL. The amounts shown in the Figure 1B correspond to the scavenging activity of *Leucas aspera* aqueous extracts.

Because of their low gastrointestinal side effects, anti-inflammatory medications that selectively inhibit COX-2 and have little to no effect on COX-1 activity are more commonly accepted as safe therapy.[19] Drug discovery and natural products, especially medicinal plants, have been shown to work very well together to promote the creation of novel therapeutic compounds. Performing COX activity-guided standardization of specific medicinal plants with a focus on antioxidant and cytotoxic profiles was the main objective of this investigation.[20]

Numerous phytochemicals have been demonstrated to possess significant anti-inflammatory qualities, such as flavonoids, terpenoids, alkaloids, and saponins. Numerous studies have demonstrated that naturally occurring flavonoids and coumarins[21] suppress the activities of 5-lipoxygenase and cyclooxygenase. Because it inhibits COX-1 and COX-2, curcumin (and its synthetic analogues) found in the Indian spice turmeric (*Curcuma longa* L.) has gained a reputation as an anti-inflammatory.[22] Prostaglandins, which are secondary messengers and involved in several immunologic reactions, are produced by the COX and lipoxygenase pathways and are inhibited by flavonoids.[23] Flavonoids reduce inflammatory disorders by inhibiting these enzymes.[24] Concern over creating novel anti-inflammatory medications that are both safer and more effective is growing on a global scale.[25] The results of the COX inhibition studies demonstrate the importance of certain plants as a useful tool for separating and identifying new COX-2 selective anti-inflammatory drugs.

Phospholipase activity was considerably reduced by *Leucas aspera*, with an IC_{50} concentration of 20±2.3 µg/mL. It has been demonstrated that alkaloids inhibit the hyaluronidase and basic PLA2 enzymes. This chemical family has been linked to local haemorrhages that facilitate the spread of the toxins and is widely distributed in the plant matrices under investigation. Alkaloids can lessen the myotoxicity and lethality of pit viper venom, according to other studies. The toxicity of snake venom is amplified by many signalling pathways that involve different proteins and chemicals. Among these are snake venom phospholipases (SPLA), which deactivate nicotinic receptors and raise intracellular Ca^{2+} and arachidonic acid when they are active.[26,27] More specifically, indole alkaloids, like AFM, were identified as promising candidates for screening against snake phospholipases and metalloproteases after being isolated from the latex of *Taberna Montana catharinensis*, which is also a member of the Alstonia plant family Apocynaceae.[28]

The anti-cancer properties of *Leucas aspera* aqueous extract specifically affect hepatic cancer cell lines. The anticancer efficacy of *Leucas aspera* aqueous extract was assessed using morphological analysis and the MTT test. The Figures 4A exhibit

the outcomes of the morphological analysis and MTT assay. They demonstrated that the extract has particular anticancer activity against the colon cancer cell line HepG2 when compared to untreated cells. The plant extract showed considerable ($p<0.05$) cytotoxicity against HepG2 cells in a dose-dependent manner, although untreated tumors showed significantly lower sensitivity. The IC_{50} for HepG2 cells was determined to be 15±0.81 µg/mL ($p<0.05$). The extract was found to cause far less damage to normal cells.

Morphological tests were performed on the extract at doses of 15±0.81 µg/mL. The morphological analysis revealed that HepG2 cells treated with the extract exhibited a significant change in morphology in contrast to cells treated with a vehicle; as compared to untreated cancer cell lines, the cells were smaller and abnormally shrunken, but their vehicle counterparts did not exhibit any change. We investigated whether *Leucas aspera's* decrease in growth rate induced apoptosis and evaluated its effect on apoptotic morphological changes in HepG2 cells.

We found that exposure to 15 µ doses of *Leucas aspera* caused apoptosis in hepatic cancer cells. The AO/EB dual assay revealed a change in the apoptotic nuclear structure and morphology of HepG2 cells after treatment with *Leucas aspera*. Cancer cells treated with inadequate *Leucas aspera* (15 µg/mL) displayed broken chromatin and apoptosis in contrast to normal cells. A quantitative comparison of apoptosis in untreated and *Leucas aspera*-treated hepatic cancer cells is depicted in the image.

Numerous plant species include secondary metabolites called polyphenolic chemicals, which have been demonstrated to have significant antioxidant properties. The antioxidant effects of phytochemicals, especially flavonoids, have been extensively studied through the process of single electron delocalization of the radical.[29] Significant free radical scavenging potential was demonstrated by the plants examined in this study, which may improve the management of inflammatory responses. The most crucial concerns in botanical standardization are the safety and authenticity of plant medicines. To guarantee their satisfaction and the broad acceptance of plant-based drugs, these challenges must be discussed with end users. The non-toxic character of the chosen plants allays worries about the safety of the botanicals for human health.[29]

CONCLUSION

The results of the current study could improve the standardization process for botanicals that use the chosen plants as an ingredient. Many times, the molecules or compounds that are extracted from plants do not act as medications; instead, they help create potentially novel therapeutic agents. According to the current study, the methanolic extract of *Leucas aspera* aqueous extract shows potent, dose-dependent activity against HepG2 hepatic cancer cells. Because of this special mechanism, *Leucas aspera* aqueous extract might be a superior therapeutic option for people with hepatic cancer. Therapeutic drug discovery marathons are becoming increasingly dependent on the quick discovery of new compounds from plant resources that have potent anti-oxidant, anti-inflammatory, anti-cancer, induce apoptotic, and antivenom qualities.

ACKNOWLEDGEMENT

The authors are grateful to GM. Srinivasan, Managing Director, Galileovasan Offshore and Research and Development Pvt. Ltd., Nagapattinam, Tamil Nadu, INDIA. for providing laboratory facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of Interest.

ABBREVIATIONS

DPPH: 1,1-diphenyl-2-picrylhydrazil; **OH:** hydroxyl; **LOX:** lipoxygenase; **COX-2:** Cycloxygenase; **MTT-3:** (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **PLA2:** Phospholipases A2; **TMPD:** Tetramethyl-P-Phenylenediamine; **Rpmi:** Roswell Park Memorial Institute (Rpmi) 1640 Medium; **DMEM:** Dulbecco's Modified Eagle Medium; **AO/EB:** Acridine Orange Ethidium bromide.

AUTHOR CONTRIBUTIONS

SR, MA. Designed experiments, supervised, MP. Wrote the initial draft and edited the manuscript, SK. carried out an investigation, DB. Validation, characterization, and editing of the manuscript, SR. wrote the initial draft and edited the manuscript, MP. Funding acquisition, and MA: characterization and software analysis.

SUMMARY

The current study's findings may enhance the standardization procedure for botanicals that contain the selected plants. The molecules that are taken from plants frequently contribute to the development of potentially innovative therapeutic agents rather than functioning as pharmaceuticals. The current investigation indicates that the aqueous extract of *Leucas aspera* in methanol exhibits strong, dose-dependent action against HepG2 liver cancer cells. *Leucas aspera* aqueous extract may be a better treatment choice for patients with hepatic cancer due to its unique mechanism. The rapid identification of novel compounds from plant resources with strong anti-oxidant, anti-inflammatory, anti-cancer, induce apoptotic, and antivenom properties is becoming more and more important in therapeutic drug discovery efforts.

REFERENCES

1. Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. Nucleus (Calcutta); 2022; 65(3): 399-411.

2. Salam U, Ullah S, Tang ZH, Elateeq AA, Khan Y, Khan J, *et al.* Plant Metabolomics: An Overview of the Role of Primary and Secondary Metabolites against Different Environmental Stress Factors. Life (Basel), 2023; 13(3): 706.
3. Reshi ZA, Ahmad W, Lukatkin AS, Javed SB. From Nature to Lab: A Review of Secondary Metabolite Biosynthetic Pathways, Environmental Influences, and *in vitro* Approaches. Metabolites, 2023; 13(8): 895.
4. Mushtaq S, Abbasi BH, Uzair B, Abbasi R. Natural products as reservoirs of novel therapeutic agents. EXCLI J, 2018; 17: 420-51.
5. Lu N, Zhang L, Tian Y, Yang J, Zheng S, Wang L, *et al.* Biosynthetic pathways and related genes regulation of bioactive ingredients in mulberry leaves. Plant Signal Behav. 2023; 18(1): 2287881.
6. Singh RK, Prasad A, Muthamilarasan M, Parida SK, Prasad M. Breeding and biotechnological interventions for trait improvement: status and prospects. Planta. 2020; 252(4): 54.
7. Tran N, Pham B, Le L. Bioactive Compounds in Anti-Diabetic Plants: From Herbal Medicine to Modern Drug Discovery. Biology (Basel). 2020; 9(9): 252.
8. Sharifi-Rad J, Sureda A, Tenore GC, Daglia M, Sharifi-Rad M, Valussi M, *et al.* Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems. Molecules. 2017; 22(1): 70.
9. Álvarez-Martínez FJ, Barrajón-Catalán E, Herranz-López M, Micol V. Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. Phytomedicine. 2021; 90: 153626.
10. Barr E, Brannan GD. Quality Improvement Methods (LEAN, PDSA, SIX SIGMA) [Updated 2024 Jan 11] In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK599556/
11. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, *et al.* Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of*Ficus religiosa*. Molecules, 2022; 27(4): 1326.
12. Sasak K, Nowak M, Wlodarczyk A, Sarniak A, Nowak D. Effect of Selected Organic Solvents on Hydroxyl Radical-Dependent Light Emission in the Fe^{2+}-EGTA-H2O2 System.*Molecules*, 2024; 29(23): 5635.
13. Walker MC, Gierse JK. *In vitro* assays for cyclooxygenase activity and inhibitor characterization. Methods Mol Biol, 2010; 644: 131-44.
14. Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. Int J Mol Sci. 2021; 22(23): 12827.
15. Hidalgo I, Sorolla MA, Sorolla A, Salud A, Parisi E. Secreted Phospholipases A2: Drivers of Inflammation and Cancer. Int J Mol Sci, 2024; 25(22): 12408.
16. Hidalgo I, Sorolla MA, Sorolla A, Salud A, Parisi E. Secreted Phospholipases A2: Drivers of Inflammation and Cancer. Int J Mol Sci, 2024; 25(22): 12408.
17. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol, 2011; 48(4): 412-22.
18. Sowndhararajan K, Kang SC. Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii* Wight and Arn. Saudi J Biol Sci, 2013; 20(4): 319-25.
19. Sohail R, Mathew M, Patel KK, Reddy SA, Haider Z, Naria M, *et al.* Effects of Non-steroidal Anti-inflammatory Drugs (NSAIDs) and Gastroprotective NSAIDs on the Gastrointestinal Tract: A Narrative Review. Cureus. 2023; 15(4): e37080.
20. Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. Nucleus (Calcutta), 2022; 65(3): 399-411.
21. Kumar A, P N, Kumar M, Jose A, Tomer V, Oz E, Proestos C, *et al.* Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. Molecules, 2023; 28(2): 887.
22. Rao CV. Regulation of COX and LOX by curcumin. Adv Exp Med Biol, 2007; 595: 213-26.
23. Scher JU, Pillinger MH. The anti-inflammatory effects of prostaglandins. J Investig Med. 2009; 57(6): 703-8.
24. Al-Khayri JM, Sahana GR, Nagella P, Joseph BV, Alessa FM, Al-Mssallem MQ. Flavonoids as Potential Anti-Inflammatory Molecules: A Review. Molecules. 2022; 27(9): 2901.
25. Vonkeman HE, van de Laar MA. Nonsteroidal anti-inflammatory drugs: adverse effects and their prevention. Semin Arthritis Rheum, 2010; 39(4): 294-312.
26. Larréché S, Chippaux JP, Chevillard L, Mathé S, Résière D, Siguret V, *et al.* Bleeding and Thrombosis: Insights into Pathophysiology of*Bothrops* Venom-Related Hemostasis Disorders. Int J Mol Sci, 2021; 22(17): 9643.
27. Omar F, Tareq AM, Alqahtani AM, Dhama K, Sayeed MA, Emran TB, *et al.* Plant-Based Indole Alkaloids: A Comprehensive Overview from a Pharmacological Perspective. Molecules, 2021; 26(8): 2297.
28. Dias MC, Pinto DCGA, Silva AMS. Plant Flavonoids: Chemical Characteristics and Biological Activity. Molecules. 2021; 26(17): 5377.
29. Lalhminghlui K, Jagetia GC. Evaluation of the free-radical scavenging and antioxidant activities of Chilauni,*Schima wallichii* Korth*in vitro*. Future Sci OA. 2018; 4(2): FSO272.

Cite this article: Raguraman S, Arumugam M, Palanivel M, Kathiroli S, Bharathi D. *In vitro* Antioxidant, Anti-Inflammatory, Anti-venom and Anti-cancer Effect of *Leucas aspera* Plant Extract. Asian J Biol Life Sci. 2025;14(2):375-81.