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Research Article

Bioactive Potential of *Salicornia europaea* Extracts Antioxidant, Anti-Inflammatory, Antivenom, and Anticancer Assay on HepG2 Apoptosis Induction

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ABSTRACT

Background: As an ethnomedical herb, *Salicornia europaea* is widely used in India to cure a variety of illnesses. **Objectives:** The present studies evaluated an aqueous extract of *Salicornia europaea* for its anti-inflammatory, antioxidant, anticancer, apoptotic, and anti-venom qualities. **Materials and Methods:** The DPPH photometric assay, OH radical scavenging capacity, anti-inflammatory activity, Cyclooxygenase (COX), Lipoxygenase (LOX), anticancer, apoptotic, and anti-venom action are a few examples of in vitro models that were used to measure antioxidant activity. **Results:** The study supported the traditional use of the aforementioned plants in inflammatory disorders by finding that the antioxidant and anti-inflammatory effects of plant extracts may be due to the inhibition of DPPH and OH, COX, and LOX enzymes, respectively. *Salicornia europaea*'s aqueous extract was extremely selective ($p<0.005$) against HepG2 cells, generating morphological changes, according to the MTT experiment. **Conclusion:** Because of its strong PLA2 inhibition, the aqueous extract of *Salicornia europaea* was found to have the strongest and safest anti-inflammatory, antioxidant, anticancer, and anti-venom properties based on the available data.

Keywords: *Salicornia europaea*, Inflammation, Cyclooxygenase, Lipoxygenase, DPPH, Hydroxyl.

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INTRODUCTION

Over millions of years, plant adaptation mechanisms have led to the development of bioactive compounds produced from plants.[1] Although these compounds are not required for basic plant metabolism, they are vital for plant survival, reproduction, and interactions with the environment.[2] The formation of these secondary metabolites, which is frequently triggered by certain developmental stages or environmental stressors, results in a complex and dynamic phytochemical profile within each species.[3] This natural diversity offers a huge store of potentially useful chemicals for human use. The study of plant bioactive chemicals includes not only their identification and characterization but also research on their biosynthesis processes, genetic regulation, and ecological activities.[4]

In order to maximize the production of desired compounds, better breeding programs, agricultural practices, and biotechnology technologies can be developed by understanding these features.[5] Additionally, studies of traditional medicinal plants continue to yield new bioactive chemicals by combining ethnobotanical knowledge with modern scientific study.[6] Traditionally, plants have been the main source of phytochemicals, which are chemical substances with a variety of health advantages. Plant-based substances can be used to prevent and treat a wide range of ailments.[7]

Traditional medicine study was the first to organoleptically investigate their properties.

As science advanced over time, it was able to study specific ingredients found in products like plant extracts and essential oils.[8] Important substances could now be categorized thanks to the growth of chemical knowledge. Terpenoids, carotenoids, glucosinolates, saponins, phytosterols, and polyphenols are some of the most important chemical groups found in plants. Every one of these groupings has unique characteristics that help to enhance human health.[9] Hepatocellular carcinomas account for three-quarters of all primary and secondary liver cancers in the United States.[10]

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Salty or brackish tidal water periodically floods places where the halophyte species European Salicornia (*Salicornia europaea* L.) flourishes. As one of the world's most salt-tolerant plant species, *S. europaea* can withstand more than 1000 mM NaCl, and after being treated with 200-400 mM NaCl, the plant has shown optimal growth and photosynthetic rates.[11] You can eat fresh or cooked European *Salicornia*. Furthermore, it has promise for usage in medicines and cosmetics, as well as a biofuel and animal pasture. Additionally, its seeds have a high fatty acid content, and these qualities make them a highly desirable nutritional source.[12]

According to recent research conducted at the United Arab Emirates' (UAE) International Centre for Biosaline Agriculture (ICBA), certain *S. europaea* cultivars that follow sound agronomic techniques can thrive on marginal soils and even turn a profit.[13] As the anticipated number of new cases and deaths from cirrhosis or chronic liver disease rises, so does the illness's regional distribution. In India, *Salicornia europaea* is used extensively as an ethnomedical herb to treat a range of ailments.[14] The purpose of the present investigation was to evaluate the aqueous extract of *Salicornia europaea*'s as anti-inflammatory, antioxidant, anti-cancer, and anti-venom properties.

MATERIALS AND METHODS

Sequential extraction of plant samples

10 g of the shed-dried powdered plant samples of *Salicornia europaea* were extracted one after the other for up to 8 hr in hexane, ethanol, and water using Soxhlet's apparatus. The extracted samples were evaporated at reduced pressure and room temperature. The dehydrated extracts were stored at 4°C in a refrigerator for subsequent analysis.

DPPH radical scavenging test

A 0.1 mM DPPH solution in ethanol was combined with 1 mL of *Salicornia europaea* (1 mg/mL) to conduct the DPPH radical scavenging experiment, with a few minor modifications. After 20 min of room temperature incubation, the DPPH reduction was measured using the absorbance at 517 nm. Ascorbic acid (1 mM) served as the reference chemical.

Assay for scavenging Hydroxyl (OH) radicals

The Fenton reaction illustrated the scavenging ability of OH radicals. 40 150 mL of H_2O_2 (0.17 M), 60 mL of $FeCl_2$ (1 mM), 90 mL of 1e10 phenanthroline (1 mM), 2.4 mL of phosphate buffer (0.2 M, pH 7.8), and 1.5 mL of individual *Salicornia europaea* (1 mg/mL) were all included in the reaction mixture. The reaction was initiated by adding H_2O_2. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured. Ascorbic acid (1 mM) served as the reference chemical.

COX inhibition assay

Using the Colorimetric COX (human ovine) inhibitor Screening assay kit, the test was conducted. The reaction mixture is, in brief, 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). The experiment takes advantage of the peroxidase component of the COX catalytic domain. The peroxidase activity was colorimetrically measured by looking for oxidized N, N, N'-Tetramethyl-Phenylenediamine (TMPD) at 590 nm. Aspirin (acetylsalicylic acid, 1 mM) is a common medication. The inhabitants have a high absorption at 590 nm. C ¼ 100% initial activity absorbance without an inhibitor at 590 nm.

Cell lines and culture preparation

The HepG2 cell lines were supplied by NCCS Pune. HepG2 cells were cultivated using Dulbecco's Modified Eagle's Media (DMEM) and 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. The cells were grown in a humidified incubator at 37°C with 5% CO_2.

MTT assay

The cells were seeded in 96-well plates at a concentration of 1×105 cells per well. After a 24-hr interval, cells were washed twice with 2.5-25 µL of *Salicornia europaea* extract and 100 µL of serum-free media before starving for an hour at 37°C. Cells were cultured for 24 hr at 37°C in a CO_2 incubator after fasting. The medium was aspirated after the treatment period, serum-free media containing 0.5 mg/mL of MTT was added, and the combination was then incubated for 4 hr at 37°C in a CO_2 incubator. The cells were rinsed with 200 µL of PBS after the MTT-containing media was disposed of. To dissolve the crystals, add 100 µL of DMSO and stir well with a pipette. The absorbance of the blue and purple formazan dyes at 570 nm was measured using a microplate reader (BioRad 680). The statistics are displayed as a percentage of stable cells in comparison to the control. The half-maximal Inhibitory Concentration (IC_{50}) values were calculated, and the optimal dosages were determined throughout a range of periods. The proliferation curve for each well was visually assessed, and the medium effective dose, or IC_{50}, is the number of samples that can 50% inhibit cell growth.

Dual AO/EB fluorescent staining

HepG2 cells were treated to numerous doses of *Salicornia europaea* (15 µg/mL) for one day following incubation. For 5 min at 37°C, 20 µL of AO/EB was added to PBS buffer to label the cancer cells. There were at least 300 cells in each sample. A fluorescence microscope was used to measure the proportion of apoptotic cells, or cells with bright orange-red nuclei.

In vitro inhibitory secretory Phospholipase A2 (sPLA2) activity

A commercially available sPLA2 targeting assay purchased from Abcam® (Cambridge, United Kingdom) was used to evaluate the anti-venom potential of *Salicornia europaea* on sPLA2. The reagents in the assay kit were prepared in compliance with the guidelines on the information page that goes with the kit.

RESULTS

S. europaea on Antioxidant DPPH and OH radical scavenging Assays

The Figure 1A shows that *Salicornia europaea* aqueous extracts can scavenge free radicals. The extractives exhibited the most activity. At a dose of 100 µg/mL, *Salicornia europaea* demonstrated a scavenging activity of 82.38±6.31 µg/mL. The IC_{50} of aqueous extracts of *Salicornia europaea* is 41.30±3.16 µg/mL. Because of their potent ability to scavenge free radicals, it is recommended that these extracts be used further.

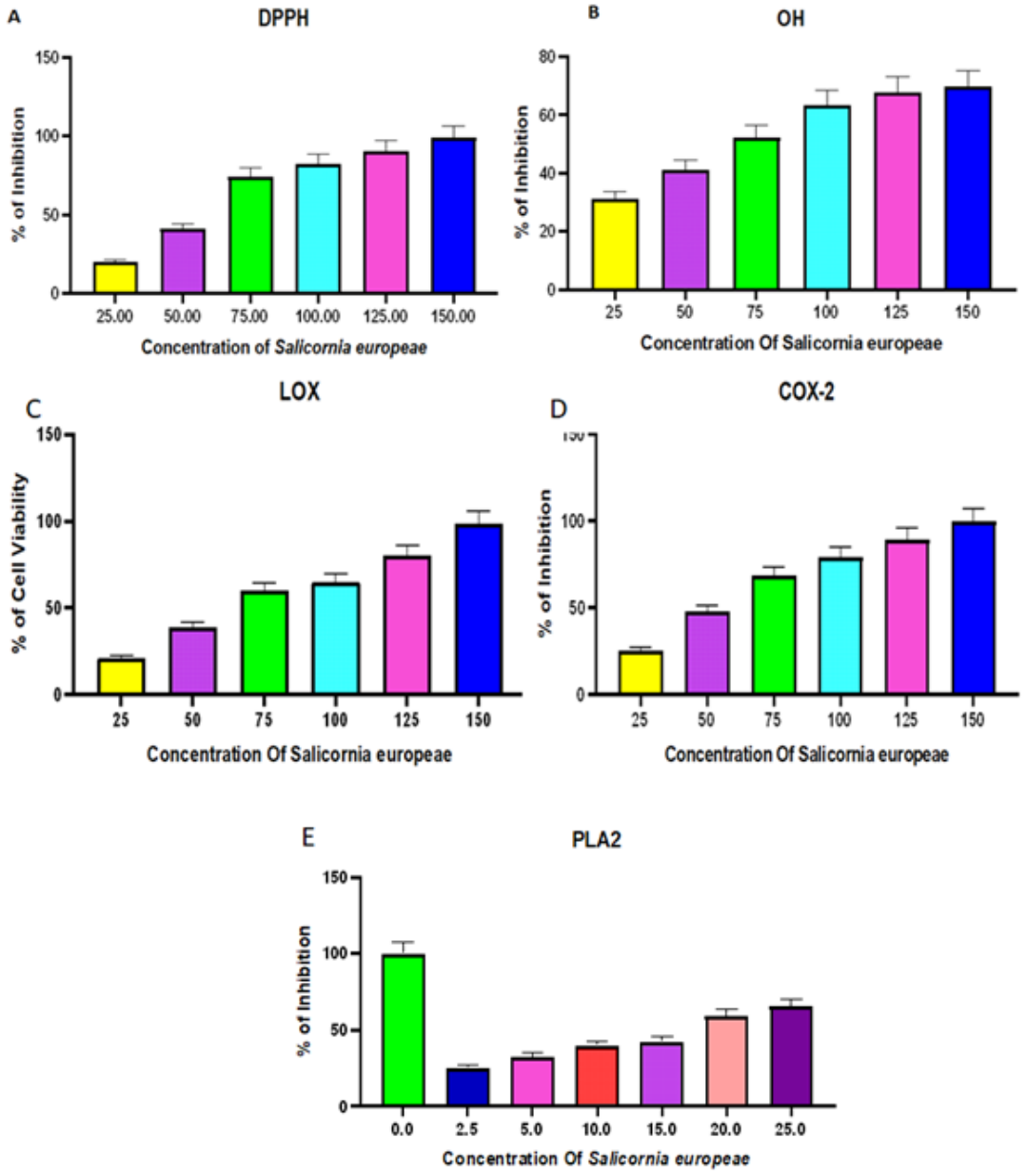

Figure 1: Effect of *S. europaea* on anti-oxidant, anti-inflammatory and anti-venom activity A) Shows that the Effect of *Salicornia europaea* on DPPH radical scavenging assay B) Shows that the Effect of *Salicornia europaea* on OH radical scavenging assay C) Shows that the Effect of anti-inflammatory action of *S. europaea* on LOX inhibitory effectiveness assay D) Shows that the Effect of anti-inflammatory action of *S. europaea* on COX-2 inhibitory effectiveness assay E).

In Figure 1B shows that the activity of hydrogen radicals for scavenging Aqueous extracts of *Salicornia europaea* showed hydroxyl radical scavenging activity that was dose-dependent manner. More activity was shown 69.93% of cell viability by *Salicornia europaea* aqueous extracts than at a concentration of 150 µg/mL. The levels in the figure corresponded to the scavenging activity of the aqueous extracts of *Salicornia europaea*.

S. europaea on Anti-inflammatory LOX and COX-2 Inhibitory Effectiveness Assays

In Figure 1C shows that the Aqueous extracts of *Salicornia europaea* showed a peak LOX inhibitory effectiveness of 61.23% at 100 µg/mL and an IC_{50} value of 39.10 µg/mL. Both extracts revealed dose-dependent suppression of LOX, with *Salicornia europaea* aqueous extracts exhibiting % inhibition with IC_{50} values of 21.20 µg/mL at dosages ranging from 25 to 150 µg/mL. In Figure 1D shows that the Aqueous extracts of *Salicornia europaea* showed a peak COX-2 inhibitory effectiveness of 79.24% at 100 µg/mL and an IC_{50} value of 47.92±3.67 µg/mL.

Inhibitory sPLA2 activity of *Salicornia europaea*

In Figure 1E shows that the Phospholipase Activity (sPLA2) was considerably reduced by *Salicornia europaea*, with an IC_{50} concentration of 20 µg/mL. *Salicornia europaea* significantly reduced phospholipase activity, with an IC_{50} of only 15-20 µg/mL. Alkaloids have been shown to inhibit the basic PLA2 and hyaluronidase enzymes.

Anticancer effect of *S. europaea* on HepG2 cells by morphological and cytotoxicity changes

The anti-cancer properties of the aqueous extract of *Salicornia europaea* specifically affect hepatic cancer cell lines. The anticancer

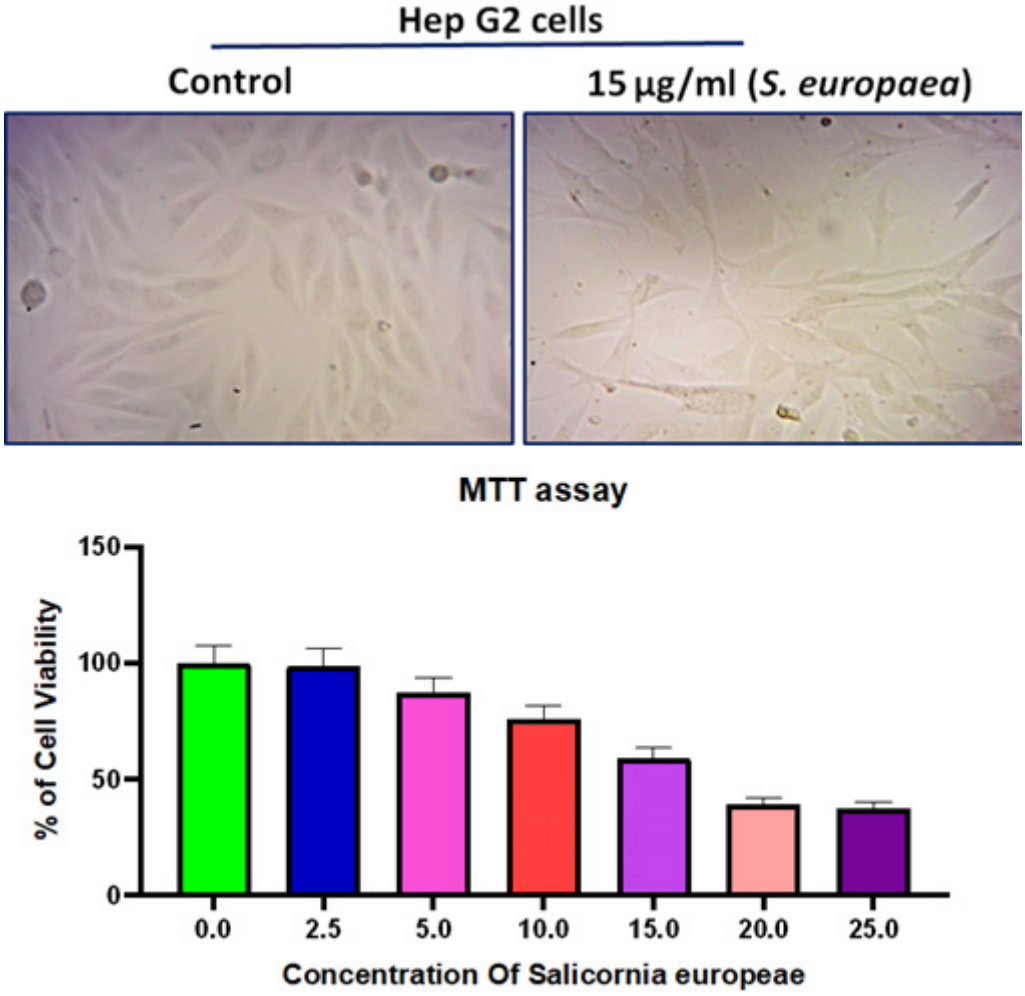

Figure 2: Effect of *S. europaea* on morphological characteristics and cell viability of Hepatic cacner HepG2 cells was assessed by MTT assay. Morphological changes in control and *S. europaea* treated HepG2 cells for 24 hr. B) show *S. europaea* treated with HepG2 cells at different concentration (2.5, 5.0, 10.0, 15.0, 20.0, 25.0 µg) respectively for 24 hr. Values were presented as mean±SD of three independent experiments (one way Analysis of Variance [ANOVA]) followed by Duncan's Multiple Range Test (DMRT).

efficacy of *Salicornia europaea* aqueous extract was assessed using morphological analysis and the MTT test. Figures 2A exhibit the outcomes of the morphological analysis and MTT assay. They demonstrated that the extract has particular anticancer action against the colon cancer cell line HepG2 when compared to untreated cells. In Figure 2B showed that 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. HepG2 cells treated with the extract exhibited a significant morphological change in comparison to cells treated with a vehicle, as indicated by the morphological analysis; the cells were smaller and abnormally shrunk when compared to untreated cancer cell lines, but their vehicle counterparts showed no change. We examined whether *Salicornia europaea's* decrease in growth rate resulted in apoptosis and evaluated its effect on apoptotic morphological changes in HepG2 cells. We found that 15 µ dosages of *Salicornia europaea* caused apoptosis in hepatic cancer cells. The AO/EB dual assay revealed a change in the apoptotic nuclear structure and shape of HepG2 cells after treatment with *Salicornia europaea*. Cancer cells treated with inadequate *Salicornia europaea* (15 µg/mL) displayed broken chromatin and apoptosis in contrast to normal cells. A quantitative comparison of apoptosis in hepatic cancer cells treated with *Salicornia europaea* and those that were not depicted in the image.

S. europaea on apoptotic induction of HepG2 cells via morphological changes

In Figures 3A, 3B Apoptotic changes were seen in human HepG2 cells using AO/EB dual labeling. HepG2 cells that had not been treated appeared as uniformly pigmented living green cells. *S. europaea* (15 µg) treated with HepG2 cells exhibited greater apoptotic alterations than control in a concentration-dependent manner. Human HepG2 cells treated with *S. europaea* (15 µg) showed early apoptotic cells, compacted chromatin, and vivid greenish-yellow areas caused by membrane blebbing, and latterly exhibited late apoptotic changes such as chromatin condensation, fragmented nuclei, and membrane blebbing. HepG2 cells were orange-red in color and had lost membrane integrity.

DISCUSSION

Similar hydrazine is produced by the reaction of the DPPH free radical with hydrogen donors. The purple DPPH radical turns yellow when it comes into touch with a hydrogen donor.[14] It was shown that the *Salicornia europaea* extract's ability to scavenge DPPH radicals increased with its concentration. The ability of 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) to decolorize

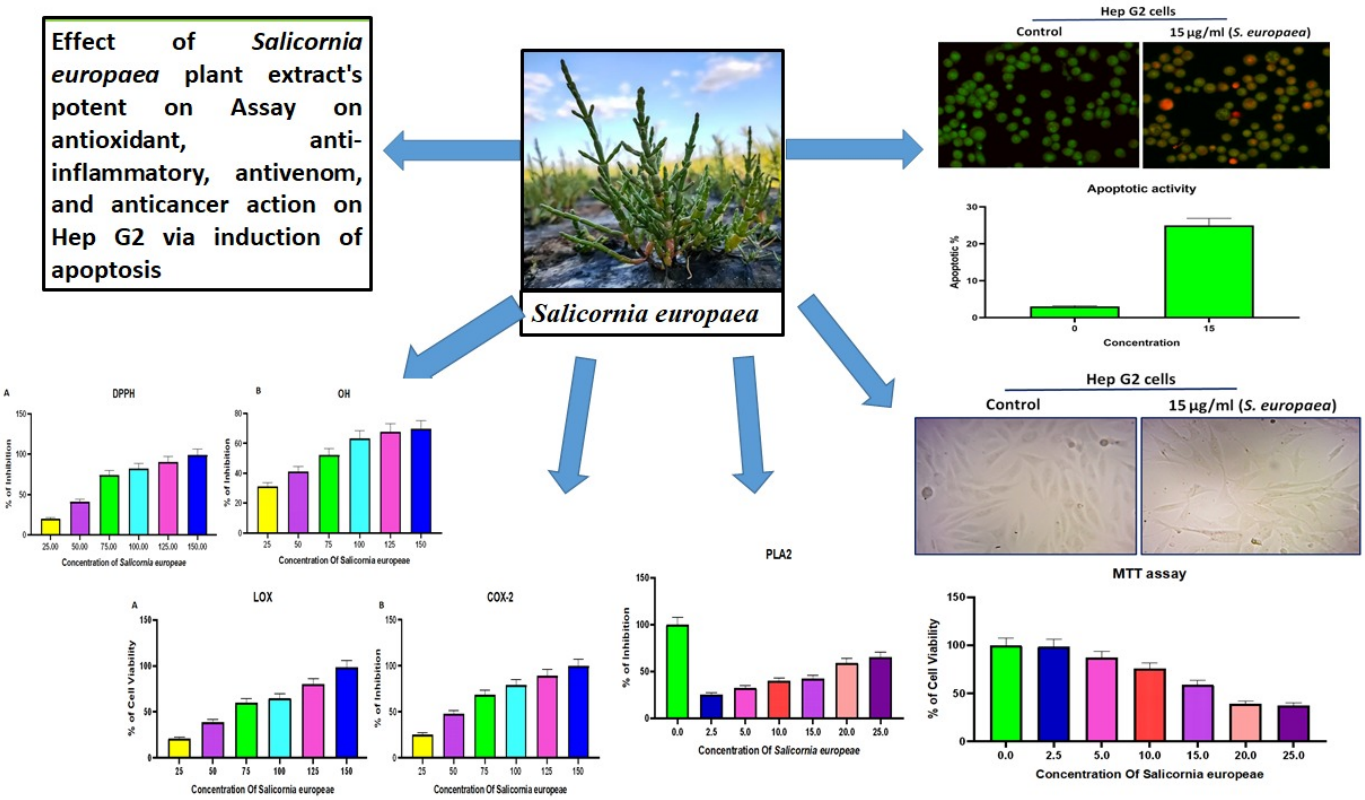

Figure 4: Schematic representation of *S. europaea* on antioxidant, anti-inflammatory, anti venom and anticancer mechanisms. This mechanism demonstrated that action of *S. eurpaea* on pharmacological activity through inhibition of cell proliferation, induction of apoptosis via antioxidant enzymes DPPH and OH activity levels increased, anti-iflammatory levels increased by LOX and COX-2 levels development, antivenom action PLA2 levels higher.

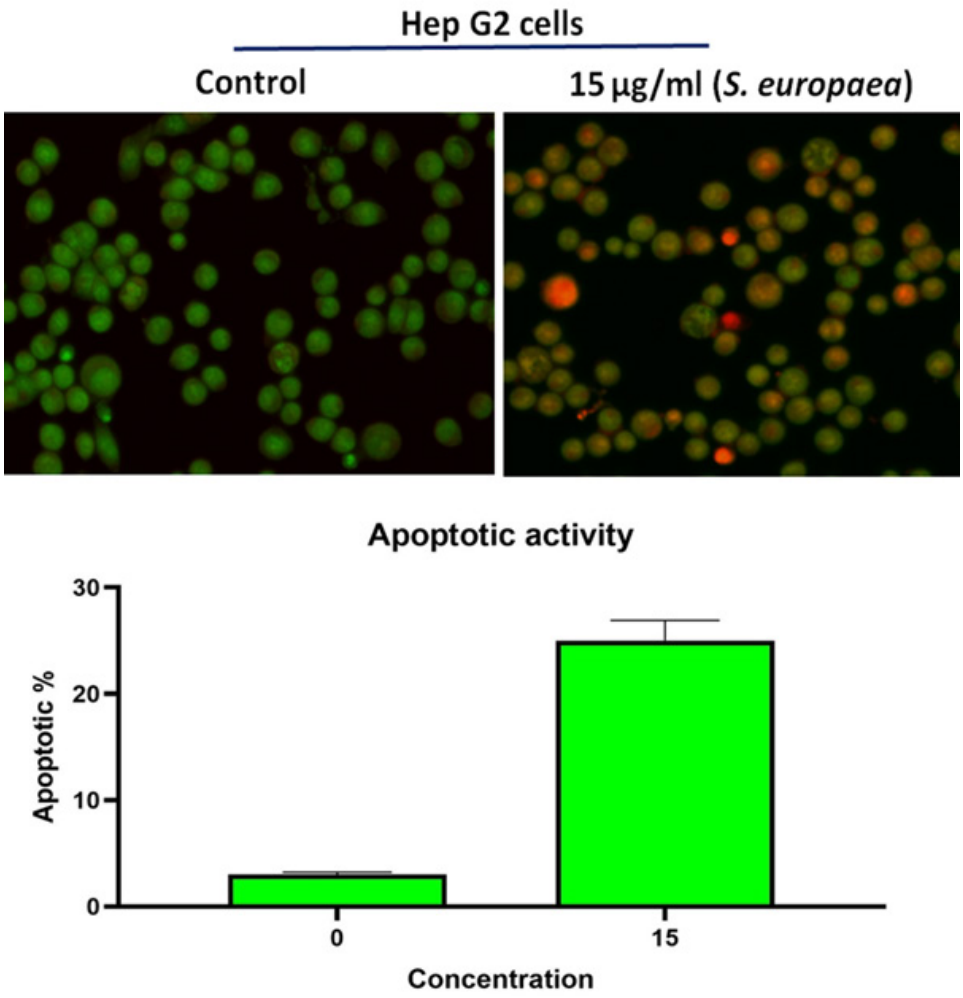

Figure 3: Effect of *S. europaea* induces apoptotic incidence. (A) HepG2 cells treated within control and *S. eurpaea* at concentrations (15 µg) at 24 hr, stained with dual dye EB/AO and then analyzed by fluorescence microscopy. (B) Percentage of apoptotic cells were calculated by scoring apoptotic and viable cells. The values are given as mean±SD of six experiments in each group ANOVA followed by DMRT. Asterisks indicate statically different from vehicle control: * $p<0.05$.

in the presence of antioxidants served as the foundation for this investigation.[15] Anti-inflammatory drugs that selectively inhibit COX-2 and have negligible to no effect on COX-1 activity are more widely recognized as safe treatments due to their low gastrointestinal adverse effects.[16] It has been demonstrated that the development of new therapeutic molecules can be greatly aided by the combination of drug discovery with natural products, particularly medicinal plants. This investigation's primary goal was to standardize particular medicinal plants based on COX activity, with an emphasis on their cytotoxic and antioxidant characteristics.[17]

Many phytochemicals, including flavonoids, terpenoids, alkaloids, and saponins, have been shown to have strong anti-inflammatory properties. Several investigations have shown that coumarins and naturally occurring flavonoids inhibit the activities of cyclooxygenase and 5-lipoxygenase. The Indian spice turmeric (*Curcuma longa* L.) contains curcumin (and its synthetic analogs), which has been shown to have anti-inflammatory properties since it inhibits COX-1 and COX-2.[9] Flavonoids block the COX and lipoxygenase pathways, which create prostaglandins, secondary messengers involved in several immune reactions. By blocking these enzymes, flavonoids lessen inflammatory conditions.[18] Celecoxib, Rofecoxib, and Valdecoxib are a class of novel anti-inflammatory drugs that were introduced to the market a few years ago. However, due to serious negative effects on cardiovascular function, particularly in older patients, pregnant women, and newborns, the majority of these drugs were removed off the market.[19] This chemical family is extensively present in the plant matrices being studied and has been connected to local haemorrhages that aid in the propagation of toxins. Other investigations have shown that alkaloids can reduce pit viper venom's myotoxicity and lethality. In particular, after being isolated from the latex of *Taberna Montana catharinensis*, a member of the Alstonia plant family Apocynaceae, indole alkaloids such as AFM were shown to be

potential candidates for screening against snake phospholipases and metalloproteases.[19]

Globally, there is increasing concern about developing new anti-inflammatory drugs that are safer and more efficient. The COX inhibition experiments' findings highlight the significance of certain plants as a practical means of separating and identifying novel COX-2 selective anti-inflammatory medications.[20]

Alkaloids have been shown to block the hyaluronidase and basic PLA2 enzymes, a chemical family that is widely dispersed in the plant matrices studied and has been associated with locals that aid in the propagation of the poisons. Other investigations have shown that alkaloid haemorrhages can reduce pit viper venom's myotoxicity and lethality. Numerous signaling pathways involving various proteins and substances increase the venom's toxicity. These include snake venom Phospholipases (SPLA), which, when active, increase intracellular Ca^{2+} and arachidonic acid while deactivating nicotinic receptors. The sPLA2 activity of 100 µg/mL of *Salicornia europaea* venom was evaluated in the presence of the putative inhibitor. The effects of sPLA2 in the presence of inhibitors were compared to those of *Salicornia europaea* venom without inhibitors.

Acridine Orange/Ethidium Bromide (AO/EB) labeling demonstrated apoptotic cells at a concentration of 15 µg/mL. Living, early, late, and necrotic cells can be distinguished using the green, orange, yellow, and red color responses of AO/EB staining. Secondary metabolites known as polyphenolic compounds are found in many plant species and have been shown to possess strong antioxidant qualities.[21] The process of single electron delocalization of the radical has been used extensively to study the antioxidant properties of phytochemicals, particularly flavonoids.[22] Significant free radical scavenging potential was demonstrated by the plants examined in this study, which may improve the management of inflammatory responses. The safety and legitimacy of plant medicines are the two most important issues in botanical standardization.[23] These issues need to be explored with end users in order to ensure their satisfaction and the widespread acceptance of plant-based medications. Concerns regarding the botanicals' safety for human health are allayed by their non-toxic nature.[24] Dual AO/EB labeling was employed to identify morphological alterations brought on by *Salicornia europaea* 's inhibitory action on HepG2 cells. Over all findings were represented as mechanism of action of *S. europaea* on Antioxidant, Anti-Inflammatory, Antivenom and Anticancer in Figure 4.

CONCLUSION

The current study's findings may enhance the standardization procedure for botanicals that contain the selected plants. The molecules or molecules that are taken from plants frequently contribute to the development of potentially innovative therapeutic agents rather than functioning as pharmaceuticals. The present investigation indicates that the methanolic extract of the aqueous extract of *Salicornia europaea* exhibits strong, dose-dependent action against HepG2 hepatic cancer cells. *Salicornia europaea* aqueous extract may be a better treatment choice for patients with hepatic cancer due to this 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.

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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

MM, HT. Designed experiments, supervised, AV. Wrote initial draft and edited the manuscript, MM. carried out an investigation, HT. Validation, characterization, and edited the manuscript, AV. wrote the initial draft and edited the manuscript, MM. Funding acquisition, and HT: Characterization and software analysis.

SUMMARY

Altogether, overall results found that *Salicornia europaea's* methanolic extract of its aqueous extract has potent, dose-dependent effects on HepG2 hepatic cancer cells. Because of this special mechanism, *Salicornia europaea* aqueous extract might be a better option for hepatic cancer patients. In therapeutic drug development efforts, it is increasingly crucial to quickly identify new molecules from plant resources that have potent anti-oxidant, anti-inflammatory, anti-cancer, induce apoptotic, and antivenom characteristics. From this study we recommend that *Salicornia europaea* potent to inhibit/prevent the cancer, further we will used as chemotherapeutics drug compound containg plant for further research analysis.

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