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Chemical characterization and anti-proliferative potential of Hexene fraction of *Sauromatum venosum* (Dryand. ex Alton) Kunth

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Abstract

The present study investigates the antioxidant, anti-inflammatory, quantitative, anti-proliferation activity and spectral characterization of phytochemicals present in the *Sauromatum venosum* (SV) medicinal plant. The objective of the current study was to determine the phytoconstituents and their potential of *Sauromatum venosum* (SV) tuber by performing the antioxidant assay, and anti-inflammatory activity in the hexene fraction (eSV1) of extract. UV-Vis, FTIR analysis spectral characterization of phytochemicals showed the presence of carbohydrates, carboxylic acid, phenols, and flavonoids. The hexene fraction showed a dose-dependent manner of increasing antioxidant activity and *in-vitro* anti-inflammatory activity. The anti-proliferative activity at lower IC_{50}-98.59 ± 1.98 µg/ml was observed against prostate cancer cells (DU145). A considerable decrease (50 %) in cell proliferation. The results indicate that the hexene fraction of S. venosum tuber extract (eSV1) possesses significant potential to effectively neutralize oxidative radicals, with its efficacy varying according to dosage. This antioxidative

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capability is likely attributed to the presence of phytochemical constituents, suggesting their contribution to the anti-proliferation activity leading to pharmacological and antioxidant properties.

Key words : *Sauromatum venosum*, anti-inflammatory, FTIR, Voodoo Lily, phytochemicals.

Abbreviations : SV - *Sauromatum venosum,* ABTS- 2,2'azinobis (3-ethylbenz-thiazoline-6-sulphonic acid) diammonium salt, FTIR-Fourier transform infrared spectroscopy.

The plant kingdom continues to be a reservoir of remarkable biodiversity, encompassing species that intrigue botanists, ecologists, and chemists alike. One such captivating member of this kingdom is *Sauromatum venosum*, an enigmatic plant renowned for, its potential pharmacological significance[1]. Commonly known as the "Voodoo Lily," *S. venosum* has captured the curiosity of researchers due to its intriguing biological features and historical use in traditional medicine[23]. However, despite its intriguing attributes, the comprehensive chemical composition of this plant remains largely unexplored. *S. venosum* belongs to the Araceae family and is native to regions spanning from Southeast Asia to India[2,4]. *SV* holds potential as a source of valuable phytochemicals with diverse pharmacological applications[5]. While various members of the Araceae family have been investigated for their bioactive compounds, the detailed chemical characterization of *S. venosum* remains a gap in current botanical knowledge. Unraveling the chemical constituents present in this plant could offer insights into its potential medicinal properties and provide leads for novel drug development.

Prostate cancer ranks as the fifth leading contributor to cancer-related mortality among diverse cancer types in men. Responsible for approximately 6.6% of male deaths (around 307,000 fatalities)[6,7], it has drawn significant attention. Multiple studies have emphasized the notably higher survival rates in cases of localized prostate cancer when compared to metastasized instances[7–9]. Its metastasized can extend to various organs such as the liver, bone, brain, and lungs[10]. Finding medications that offer a complete cure for prostate cancer remains a compelling area of research, given its high propensity for metastatic spread. Since, several chemotherapeutic drugs derived from taxanes, notably docetaxel and cabazitaxel, have shown promise in treating prostate cancer [11,12]. Nonetheless, only limited studies have delved into probing the impacts of plant extracts and their underlying mechanisms concerning the advancement of metastatic prostate cancer.

To unravel the potential of hexene fraction (eSV1) of *S. venosum* plant extract as a viable approach for addressing human carcinomas, particularly prostate cancer, we conducted an investigated a comprehensive examination of hexene fraction of plant tuber of SV, we endeavour to elucidate the complex biochemical profile. By shedding light on the chemical makeup of *SV*, this study contributes to our understanding of unveils the potential pharmacological significance of its bioactive compounds.

In the following sections, we delve into the extraction, and identification of the diverse

phytochemicals present within *S. venosum* along with their potential to kill the prostate cancer cell. Our findings not only extend the frontiers of botanical knowledge but also lay the foundation for future studies focusing on harnessing the therapeutic potential of this unique plant. Through the meticulous chemical characterization and anticancer activity of *SV*, we hope to unlock a treasure trove of natural compounds that may hold promise for a range of applications, from traditional medicine to modern drug discovery.

Chemicals and Reagents :

Methods :

Isolation and fractionation :

The extraction of plant tuber was performed using the Soxhlet hot extraction method and concentrated until a syrup consistency was obtained and dried using a rotary evaporator**.** Plant extract was labelled appropriately and Subsequent fractionation of plant extract was done by physical fractionation (Fig. 1) using solvents hexene and water[13,14].

UV visualization :

UV-spectroscopy is the most common and convenient technique for the determination of plant extract and its phytoconstituents. In order to check the purity and preliminary characterization of plant crude extract, the absorption was recorded by using UV-visible spectroscopy (UV-1800 Shimadzu, Japan) from the wavelength of 200 to 800 nm. For using this procedure, the hexene fraction (eSV1) was dissolved in respective solvents before taking the absorbance making it as a final concentration of 1mg/ml.

Identification of molecule by Fourier transform infrared spectroscopy (FTIR)

The active functional groups present in eSV1 were identified using FT-IR spectroscopy. eSV1 sample was applied onto the gold surface of an FT-IR spectrophotometer (Bruker's Alpha), and spectra were recorded in the range of 4000 cm^{-1} to 500 cm^{-1}.

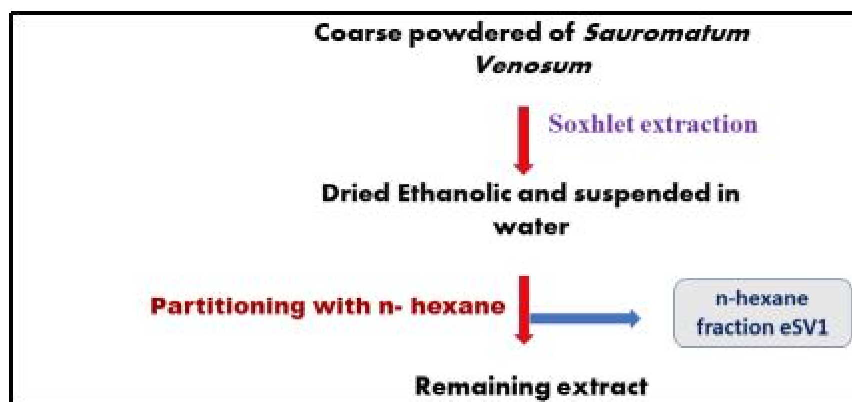

Fig. 1. Fractionation of ethanolic extract of *S. venosum*

Determination of Secondary metabolites (Total phenolic/flavonoid contents) :

Total phenolic contents were determined according to Folin and Ciocalteu's method (1927)[15]. A concentration of 20-1000µg/ml of Gallic acid was prepared in methanol. The same procedure was followed for the preparation of plant extract. For the making, 200 µl of total mixture working solution 13µl of sample and 94µl of 10% diluted Folin-Ciocalteu reagent was mixed. After 5 min of incubation 94 µl of 5% sodium carbonate was added. The mixture was allowed to incubate at room temperature (RT) for 2hr in dark conditions. Absorbance was taken at wavelength λ 760. Experiments were performed in triplicates with gallic acid utilized as the positive control. The total phenolic content was expressed as gallic acid equivalent (GAE). Total flavonoid was determined using the method of Miliauskas, Venstetonis and Beck (2004)[15]. 50 µl of 2% $AlCl_3$ in ethanol was added to 50 µl of extract (concentration of extract 20-1000µg/ml). After 1h incubation at RT absorbance was measured at wavelength λ 420. The total flavonoid contents were calculated as mg QUE/gm of extract.

Measurement of antioxidant activity :

ABTS radical discoloration assay :

Briefly ABTS radical solution was prepared (in distilled water 7mM of ABTS) was mixed with potassium persulfate ($K_2S_2O_8$, 2.45 mM in distilled water) and mixed both solution in the ratio of 2:1. The mixture was then incubated for 12-24 h in dark place at RT. Then the ABTS radical solution was diluted with the ethanol in the ratio of 1:10 to obtained the absorbance of 0.7 ± 0.005 at 595 nm. This radical solution (150 µl) was added to eSV1 (50µl) in a 96 well plate. This reaction mixture was incubated at RT in dark for 30 min then the absorbance was measured at 595 nm. The percentage of ABTS scavenging activity were determined by using this formula[16].

$$\% \text{ of Free Radical Scavenging activity} = (Ac - As/Ac) \times 100$$

Where, Ac is absorbance of control and As is the absorbance of sample or standard.

In-vitro anti-inflammatory assay :

In-vitro anti-inflammatory activity of plant crude extract was evaluated by protein denaturation method[17]. Salicylic acid used as standard drug, the reaction mixture consisting of 150 µl of different concentrations of plant crude extract (50 to 800 µg/ml) and standard was taken in the same concentration. 60µl of phosphate buffered saline (pH 6.4) was mixed with 40µl of egg albumin and incubated at 27°C for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. The mixture was cooled and absorbance was measured at 660 nm. The experimental samples were analysed in triplicates and PBS used as blank. The inhibition percentage of protein denaturation was calculated by using this formula :

$$\text{Inhibition } (\%) = (A_C - A_t/Ac) \times 100$$

Where, A_t= Absorbance of the test sample, A_c= Absorbance of control.

Cell proliferation assay :

A prostate cancer cell line (DU145)

was acquired from the National Centre for Cell Science (NCCS) in Pune. The cells were cultivated in a controlled laboratory environment with 5% CO_2 and a temperature of 37°C. The prostate cancer cells (DU145) were seeded at a density of 7500 cells/well in 96-well plates. Following an overnight incubation, the next day, the culture media was replaced with 100 µl of fresh media containing plant samples. eSV1 were provided at varying concentrations ranging from 20 to 100 µg/ml for 24 h. Subsequently, 10 µl of MTT solution (5 mg/ml) was added to each well. The cells were then incubated for a period of 3-4 hours under dark conditions. The MTT assay was employed to assess cell proliferation. The absorbance using EPOCH Elisa plate reader of the resulting solution was measured at a wavelength of 595 nm. Using the obtained absorbance data, the half-maximal inhibitory concentration (IC_{50}) was calculated. The IC_{50} value indicates the concentration of the eSV1 at which the growth of the prostate cancer cells is inhibited by 50%.

Statistical analysis :

Each assay was performed in triplicated independent experiments. The results are expressed as mean values ± SEM (standard error of mean). GraphPad prism version 8 software was used for statistical analysis.

UV visualization :

The UV-Vis spectroscopic analysis of the hexene fraction (eSV1) of *S. venosum* (Fig. 2) revealed distinct absorption patterns at various wavelengths. At 230 nm, absorption suggested the presence of aromatic compounds, potentially phenolic compounds or flavonoids[18,19]. Absorption peaks at 250 nm indicated the existence of compounds with conjugated double bonds, possibly aromatic molecules. Peaks at 300 nm and 320 nm implied the presence of compounds with extended conjugated systems, such as flavonoids[20] and phenolic acids[21–23] (Table-1). These findings provide initial insights into the diverse chemical composition of the plant extract.

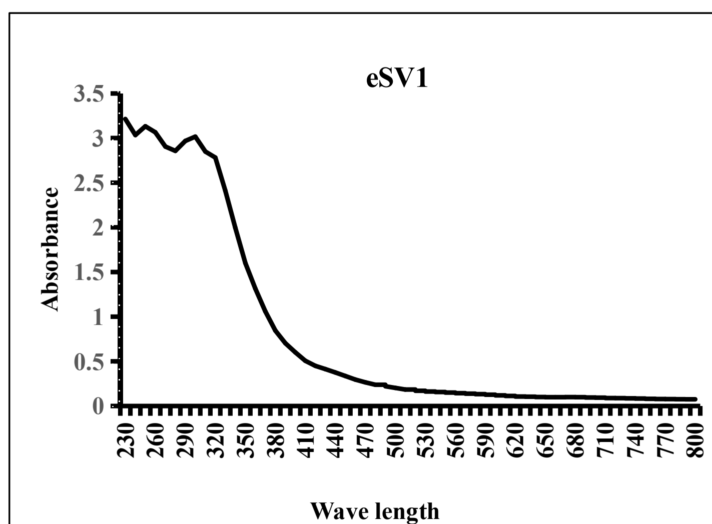

Fig. 2. UV-Vis spectroscopic Spectral peaks of hexene fraction of *S. Venosum* (eSV1)

Table-1. Major picks observed under the UV spectroscopic analysis of hexene fraction of *S. Venosum* (eSV1)

S. N.	Wavelen-gth (nm)	Absor-bance	Remark
1.	230	3.2141	Phenolics and flavonoids
2.	250	3.1326	aromatic compounds, including phenolic compounds, flavonoids, and other polyphenols,
3.	300	3.0166	Polyenes
4.	320	2.782	Phenolics and flavonoids

Fourier transform infrared spectroscopy (FTIR) analysis :

In Fourier Transform Infrared (FTIR) spectroscopy, the emergence of peaks within a spectrum furnishes crucial insights concerning the molecular constitution of the subjected sample. Every peak corresponds to a distinct vibrational mode exhibited by the constituent atoms within the sample's molecules. Which aid in the discernment of prevailing chemical bonds and facilitate the elucidation of functional groups within the analysed sample[24]. In FTIR spectroscopy, absorption peaks at specific wavenumbers provide valuable insights into a sample's molecular composition (Fig. 3). Around 2921.7 cm^-1, aliphatic C-H stretching vibrations from compounds like alkanes, alkenes, and alkynes are observed[25]. At 2854.3 cm^-1, symmetric stretching of aliphatic C-H bonds is common, indicating alkanes and aliphatic hydrocarbons. A peak near 1724 cm^-1 indicates C=O stretching, seen in ketones, aldehydes, acids, esters, and amides[1]. Peaks around 1589.2 cm^-1 signify aromatic rings and conjugated double bonds. Other wavenumbers indicate methyl (1454.7 cm^-1) and methylene (1369.5 cm^-1) bending vibrations, C-C-C, C-C-N, and C-O-C bending (1272.98 cm^-1), and C-C, C-N, and C-O stretching (1122.8 cm^-1 and 1025.8 cm^-1)[26]. Peaks at 1072 cm^-1 point to C-O bonds in alcohols, ethers, esters, and carboxylic acids. Frequencies around 858 cm^-1, 741 cm^-1, 704 cm^-1, and 657 cm^-1 involve C-H and N-H or S-H bonds, indicating specific molecular structures[1,27] (Table-2).

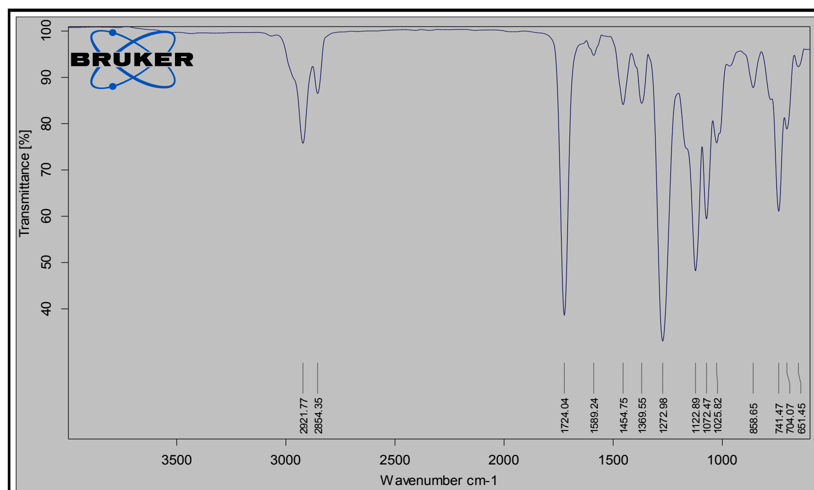

Fig. 3. Fourier transform infrared spectroscopy (FTIR) spectra of hexene fraction of *S. Venosum* (eSV1)

Table-2. Fourier transform infrared spectroscopy (FTIR) stretches frequency range and their functional groups of hexene fraction of *S. Venosum* (eSV1)

S.N.	Strech frequency range (cm^{-1})	Functional group
1.	2921.77	Aliphatic C-H/ alkanes, alkenes, and alkynes
2.	2854.35	Aliphatic C-H/ single-bonded hydrocarbons
3.	1724.04	Carbonyl (C=O)/ ketones, aldehydes, carboxylic acids, esters, and amides
4.	1589.24	Aromatic rings
5.	1454.75	Methyl (CH_3) group
6.	1369.55	Methylene (CH_2) group
7.	1272.98	C-C-C, C-C-N, C-O-C/ alkanes and other hydrocarbons
8.	1122.89	C-C, C-N, C-O
9.	1072.45	C-O/alcohol, ether, ester, carboxylic acid etc.
10.	858.85	C-H bending
11.	741.47	C-H, N-H
12.	704.07	C-H, S-H, C-O-H
13.	657.45	C-H, C-N-H

Total phenolic/flavonoid contents :

Total phenolics and flavonoid contents in hexene fraction are summarized in Table-3. Present study showed that the higher concentration of total phenolics in s. venosum in comparison of flavonoid contents in hexene fraction. 0.231± 0.00 mg gallic acid equivalent/gm of fraction and 0.033 ± 0.00 mg quercetin equivalent/gm of fraction. Polyphenols and their derivatives play a vital role in bolstering the immune system and combatting harmful reactive oxygen species (ROS). This contributes to lowered susceptibility to various conditions like cancer, and other inflammatory disorders[20,28].

Table-3. Total Phenolic and flavonoid contents of eSV1 fraction sample of *S. venosum*

Samples	TPC mg/gm of GAE	TFC mg/gm of QUE
eSV1	0.231± 0.00	0.033 ± 0.00

Values are expressed as mean SEM (n=3). GAE= gallic acid equivalent, QE= Quercetin equivalent.

Measurement of antioxidant activity ABTS radical discoloration assay :

The total antioxidant capacity of eSV1 fraction sample of *S. venosum* was evaluated using the ABTS assay method. The results pertaining to antioxidant activity demonstrated a significant inhibition of free radicals by the hexene fraction. This effect is depicted in Table-4. ABTS scavenging activity was quantified in the terms of the percentage of inhibition. An increasing variation in the percentage of inhibition of various concentration was observed (13.68 -57.313%). The highest inhibition was observed (57.313%) at the concertation of the 400 µg/ml. The observed results indicate that eSV1 displayed notable ABTS scavenging activity, likely owing to the presence of different radical scavenging compound in fraction, recognized for their role in antioxidant activity. These findings suggest that the hexene fraction of SV sample possesses significant antioxidant potential. This attribute can be harnessed to combat conditions like inflammation conditions and other pharmacological conditions, underscoring its valuable role in health research.

Table-4 ABTS scavenging activity of hexene fraction of *S. Venosum* (eSV1)

Concentration µg/ml.	% of inhibition
20	13.68159 ± 0.190676
40	18.40796 ± 0.188953
80	20.60697 ± 0.372021
100	50.1791 ± 0.232753
200	56.2189 ± 0.092131
400	57.31343 ± 0.108922

Values are expressed as mean SEM (n=3).

In-vitro anti-inflammatory assay :

In-vitro anti-inflammatory activity of the hexene fraction (eSV1) of *S. venosum* was evaluated at various concentrations, ranging from 50 µg/ml to 800 µg/ml. The percentage of inhibition exhibited a concentration-dependent trend. At 50 µg/ml, the inhibition percentage (27.68%) was measured whereas the higher percentage of inhibition was observed at the concentration of 400 µg/ml which increased more than 100 %. These results demonstrating a concentration-response relationship (Fig. 4). These results highlight the promising protein denaturation ability of hexene fraction of SV plant extract**.** The study demonstrated that the hexene fraction of SV extract effectively prevented protein denaturation and membrane lysis in inflammatory disorders[29]. This strong inhibition of protein denaturation suggests its potential as a natural anti-arthritic agent, likely due to the presence of beneficial secondary metabolites like polyphenols in the plant tuber[30].

Cell proliferation :

In-vitro cell proliferation were determined by MTT assay of hexene fraction of *S. venosum* extract against the human cancer cell line DU145. A graphical representation of cell proliferation is depicted in figure 5. Briefly, cells were treated with eSV1 sample 20-100 µg/ml for 24 h. According to observed results, the cells treated with eSV1showed 50 % of cell proliferation at the concentration 98.59 ± 1.98 µg/ml (IC_{50}) in comparison to the untreated cells. It was found that the percentage of cell proliferation were decreasing with the increasing concentration. This result demonstrates that eSV1 reduces the survival of prostate cancer cells.

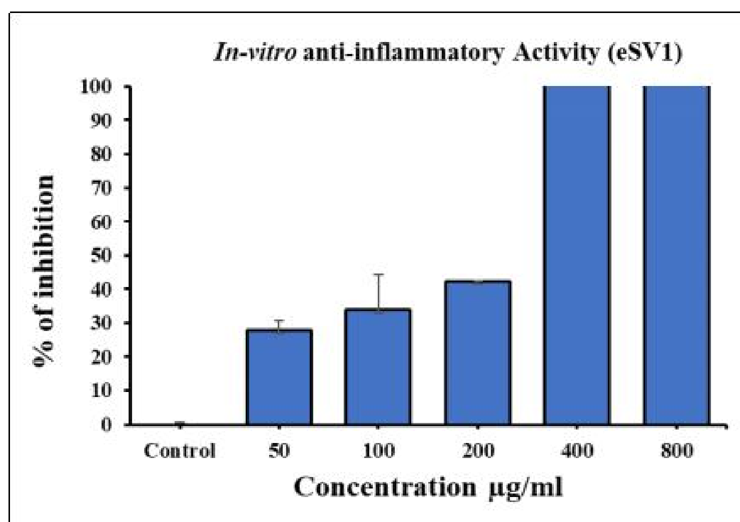

Fig. 4. *In-vitro* anti-inflammatory activity of hexene fraction of *S. venosum* (eSV1)

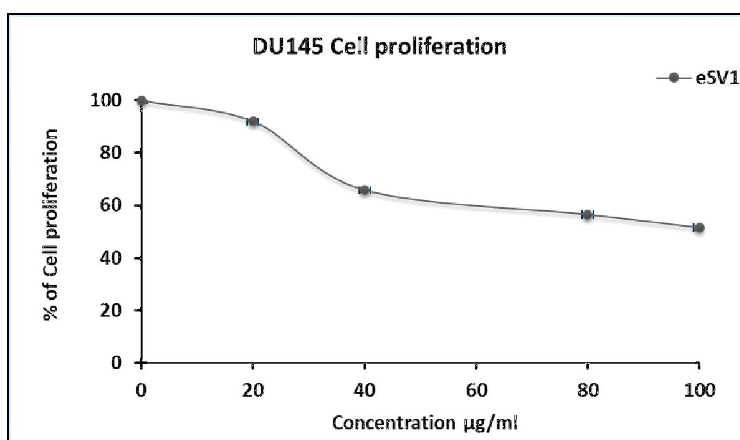

Fig. 5. *In-vitro* antiproliferation activity of hexene fraction of *S. venosum* (eSV1)

In this current study, our findings reveal a significant abundance of phenolic and flavonoid compounds within the examined plant. This, in turn, provides a robust biochemical foundation for the traditional medicinal application of the extracted sample from *S. venosum*. These emerges as a promising reservoir of bioactive compounds, holding immense potential for the development of valuable pharmaceutical agents.

UV-Vis spectroscopy revealed aromatic and conjugated compounds in the plant extract, which aligned with FTIR peaks suggesting aromatic rings and carbonyl groups and polyphenolics compound. These compounds are known for their antioxidant, anti-inflammatory, and anticancer properties. This

antioxidant potential could mitigate oxidative stress, linked to inflammation and cancer. Additionally, the presence of extended conjugation compounds aligns with anti-cancer flavonoids. Combining UV-Vis and FTIR data provides insights into the potential of eSV1 fraction of *S. venosum* as anti-inflammatory, antioxidant and anti-proliferative effects through its diverse molecular composition. The results indicated that hexene fraction possessed the antioxidant potential, these findings highlight the antioxidant compounds present in *S. venosum* and support the utilization of these fraction as potential natural sources of antioxidants in functional pharmaceuticals. Further it anti-proliferative effects study is warranted to identify the specific antioxidant compounds responsible for the observed effects and to explore their potential health benefit.

In summary, our study not only establishes the biochemical rationale behind the ethnomedicinal usage of *S. venosum* extract but also underscores its potential as a prolific source of bioactive substances. These findings collectively advance our understanding of the therapeutic potential and warrant further exploration for the development of novel medicinal interventions.

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Author contributions

NK performed all the experiments, data analysis and manuscript writing; MY manuscript writing; RS and RB final manuscript editing, supervision and planning of experiment.

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Conflict of Interest :

None. For all the authors, there is no potential conflict of interest.

Declarations

Ethical Approval	: Not applicable.
Consent to Participate	: Not applicable.
Consent for Publication	: Not applicable.

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