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ANTICANCER POTENTIAL OF *CRYPTOLEPIS BUCHANANII* PLANT BARK EXTRACT: CYTOTOXICITY AND EXPRESSION OF *VEGFR2* AND *SOAT1* GENES

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Abstract

Plants are an important natural source of medicines worldwide. The current major issues, such as antibiotic resistance and increased side effects of synthetic medicines, demand for herbal-based medicines. *Cryptolepis buchananii* plant, commonly known as the wax-leaved climber, is an important medicinal plant. Cancer continues to represent a significant global health challenge, characterized by high mortality rates. Current therapeutic strategies are often costly and associated with considerable safety concerns, including moderate to severe adverse effects. In the current study, bark extract of *C. buchananii* plant was evaluated for its therapeutic potential through selected phytochemicals, antioxidant activity, acute oral toxicity, and cytotoxicity of cancer cell lines. Results of *C. buchananii* bark extract revealed the total flavonoid content as 1.09 mg RE/g and total phenolic content as 7.02 mg GAE/g dry weight in the methanolic extract, which were higher than in ethanolic and aqueous extracts. The FTIR spectrum identified alcohols, phenols, alkanes, alkenes, aromatic, and aliphatic compounds. The GC-MS results revealed important metabolites with their potential broad-spectrum biological activities, like anticancerous, antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and neuroprotectors. The acute oral toxicity results showed that oral administration of *C. buchananii* bark extract, in Swiss Albino mice, exhibits no mortality up to a dose of 5000 mg/kg. MTT tests (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium) showed a significant cytotoxic potential against tumor cell lines U-87 and HepG2. q RT-PCR analysis resulted in the up-regulation of pro-apoptotic gene *BAX* (1.5-fold) and down-regulation (0.6-fold less) of anti-apoptotic gene *BCL2* in U-87 (Brain cancer cell lines) compared to control. Angiogenesis-related gene *VEGFR2* and cholesterol metabolism-related gene *SOAT1* were down-regulated (0.8 and 0.9-fold less) in HepG2 (liver cancer cell lines) compared to the control. These findings suggest that the bark of *C. buchananii* contains promising bioactive compounds, such as phenols, which may have potential therapeutic applications in future anticancer drugs.

Key words: Acute oral toxicity assay; Antioxidant activity; Cancer; Cytotoxicity assay; DPPH assay; FTIR analysis; GCMS analysis

Introduction

Cancer is a deadly disease in which the cells start dividing in an abnormal and uncontrolled manner (Matthews *et al*., 2022). It is responsible for a large number of deaths worldwide (Hanahan, 2022; Sung *et al.,* 2020). A total of 19.3 million new cancer cases and 10 million cancer-related deaths have been reported in the year 2020. It is estimated that the global cancer burden will rise to approximately 28.4 million cases by 2040, representing an increase of about 47% compared to 2020 (Yuan *et al.,* 2022). While significant strides have undoubtedly occurred in cancer research over the recent decades through novel diagnostic methods, a deeper understanding of malignant cellular behavior, and some treatment protocols, limitations persist that constrain therapeutic benefits. Despite cutting-edge pharmaceutical innovations, the non-targeted damage of healthy tissues by chemotherapy still diminishes patient well-being significantly (Ren *et al.,* 2023). Moreover, the current healthcare system is facing a great challenge due to the troublesome "late effect" of cancer survivors (Schmidt *et al.,* 2022). In addition, cancer patients residing in resource-limited or underserved regions exhibit comparatively lower survival rates (Kumar & Bhasker, 2015). There is a dire need for research into anticancer medications that are more targeted and less harmful, such as herbal-based ones.

A variety of therapeutic approaches, such as radiotherapy, chemotherapy, gene therapy, molecularly targeted therapy, immunotherapy, embolotherapy, and phototherapy, have been widely used to treat cancer. However, still, cancer treatment is complicated, as all these treatment modalities have severe consequences for cancer patients due to their side effects and the risk of recurrence. It is essential to develop therapeutic agents that have minimal or no side effects, are predictable, cost-effective, and readily available.

To improve health and lifestyle and to protect against several diseases, plant extracts have extensively been used by humans. Various research groups worldwide are investigating medicinal plants with therapeutic potential as alternative cancer treatments, many of which have long been utilized in traditional medicines in the form of plant extracts. Many of these medicinal plants, like *Polygonum*

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minus, *Punica granatum, Catharanthus roseus*, *Taxus brevifolia*, *Curcuma longa* and *Evodia rutaecarpa,* are already widely used in traditional folk remedies in the extract form (Chandra *et al.,* 2023; Quintero-Rincon *et al*., 2025). Medicinal plants are also known as the richest source of drugs in traditional medical systems. The practice of adding phytonutrient supplements to diets is becoming more and more popular as a means of prevention against cancer and other diseases. These phytonutrients interact with the membranes of malignant cells or their receptors to halt the expansion and movement of tumors through cytotoxicity and programmed cell death (Chen & Liu, 2018). These phytonutrients contain several benefits over standard chemotherapeutic medicines, such as increased selectivity, reduced toxicity towards healthy cells, and high ability to permeate tissues and bind with targets. While cancer cells can evolve resistance to chemotherapy, research hints that phytonutrients may circumvent some resistance mechanisms, thus opening new doors for adjuvant treatments (Soldati *et al.,* 2018).

Medicinal plants for example, *Nigella sativa* (black seed), *Azadirachta indica* (neem), and *Phyllanthus emblica* (amla) are currently an essential component of our overall health. Finding and evaluating their chemical constituents can help to identify their various important biological functions (Zeeshan*,* 2025; Prananda *et al.,* 2023). Plant-derived products are particularly beneficial since their extracts contain a range of secondary metabolites, such as flavonoids and phenols, which are more beneficial to humans than synthetic compounds (Alhadidy, 2023; Shrinet *et al.,* 2021). Clinical studies have revealed that an increased risk of chronic diseases is associated with a diet low in fruits and vegetables but enrich in refined sugar, meat, and processed food. However, plant-based diets rich in fibers, antioxidants, and bioactive compounds such as phenolic acids and flavonoids, have been found to reduce the risk of life-threatening diseases including neurological disorders, cardiovascular diseases, diabetes, and cancer, by reducing the inflammation, oxidative stress, and cellular DNA damage (Catalano *et al.,* 2016). This is because phytochemicals containing these protective effects have been reported to show anti-cancer effects (Roy *et al.,* 2022). Several substances, including vincristine, paclitaxel, and combretastatin, are important cytotoxic pharmacological antimitotic agents that are derived from plants (Raheel *et al.,* 2017).

In medicinal plants, the different phytoconstituents include flavonoids, alkaloids, phenols, tannins, and saponins (Parekh & Chanda, 2007). Phenolic compounds are a major group of secondary metabolites that include many subgroups, such as tannins, phenolic acids (e.g., gallic acid), flavonoids (e.g., quercetin), coumarins, and stilbenes (e.g., resveratrol). These subgroups are recognized for their potent pharmacological properties (Xu *et al*., 2025). They are invaluable sources of antioxidants and are of great interest because of their well-acknowledged protective effects in a number of diseases, including diabetes and cancer (Tatipamula & Kukavica, 2021). Many new drugs, containing mostly phenolic compounds, are used for treating and/or preventing cancer (Yaglioglu & Eser, 2017). Plant phenolic compounds have various biological effects, ranging from protection of neuronal cells against apoptosis to anticancerous properties by inhibiting cell proliferation (Panja *et al.,* 2020).

Cryptolepis buchananii, commonly known as wax-leaved climber, is an important medicinal plant that belongs to the family Apocynaceae and subfamily Periplocoideae. Hot and deciduous forests are the common habitats of *C. buchananii* plant (Tayung & Saikia, 2020). It is a large shrub that either climbs or straggles with leaves whose length is 2-5 cm long, and are elliptic-oblong. It has flowers in axillary cymes, which are yellowish-green in color. The traditional uses of *Cryptolepis buchananii* plant include curing bone fractures, loss of appetite, fever, skin diseases, cough, and diarrhea. This plant is considered as an antibacterial, blood purifier, and anti-inflammatory as well (Arthan & Yenjai*,* 2023). In Thailand, since ancient times, it has been used as a treatment for inflammatory disorders like joint and muscular pains (Venkateswara *et al.,* 1987). The *C. buchananii* plant's roots and leaves reportedly contain cardiac glycosides. These compounds exhibit potential anticancer activity (Faisal *et al.,* 2018). We hypothesize that methanolic bark extract of *C. buchananii* exhibits cytotoxic potential against U-87 and HepG2 cancer cell lines through modulation of apoptotic and angiogenesis-related genes.

This research study was designed to screen for the selected phytochemical compounds and to investigate their antioxidant activity through DPPH assay and functional groups through GC-MS. Further, to investigate the anticancer properties of *C. buchananii* bark methanolic extract on brain cancer (U87) and liver cancer (HepG2) cell lines which is rarely reported through different tests, such as acute oral toxicity in Swiss albino mice and cytotoxicity assays against cancer cell lines. Additionally, the expression of selected genes involved in brain and liver cancer was also studied for the first time.

Materials and Methods

The research work related to phytochemical analysis and antioxidant activity was performed in the Department of Biotechnology, COMSATS University Islamabad Abbottabad Campus, while the research on cancer cell lines was carried out at the Institute of Pharmaceutical Sciences, Khyber Medical University, Peshawar, Pakistan.

Ethical statement: All the protocols used in this research work were duly approved by the Institutional Animal Ethical Committee of Khyber Medical University, Pakistan (No: KMU/IPS/PG/IREB/2nd Meeting/2024/9).

Sampling and identification of the selected plant: *C. buchananii* plant was collected from the Margalla Hills (3.74444°N 73.04167°E), at an altitude of approximately 2000 feet. About 8 kg of *C. buchananii* stem was collected and identified by plant taxonomist Dr. Syed Afzal Shah, Department of Biological Sciences, NUMS University Islamabad. For future reference, the accession number is NUMS00025. The plant tissues were cleaned with running tap water and then air-dried in the shade. The stem bark was separated, finely ground using a blender and stored in an airtight container.

Preparation of methanolic bark extract of *C. buchananii*: Methanolic bark extract was prepared by soaking 500 g of powdered bark in one liter of methanol and concentrating the solution using a rotary evaporator (Heidolph, Germany). The extraction process was repeated three times. The crude methanolic extract was then concentrated using a rotary evaporator, yielding a dark brownish solid, in accordance with the protocol described by (Faisal *et al.,* 2018).

Initially, various types of extracts were prepared and compared based on their total flavonoid content (TFC), total phenolic content (TPC), and antioxidant activity. Among them, the methanolic extract consistently showed the highest TFC, TPC, and antioxidant potential and was selected further for biological evaluations and analysis.

Phytochemical screening of *C. buchananii* bark extract

Quantification of total flavonoid and total phenolic contents

Determination of total phenolic content: The total phenolic content was quantified using the Folin-Ciocalteu reagent, following the method of Singleton *et al*., (1999) using a spectrophotometer. The blank reagent was used as a standard. An extract of 80 mg bark sample was prepared in 2 mL chilled methanol (85%). Then, 200 µL FC reagent (10%), 200 µL of plant extract, and 1.6 mL of $NaCO_3$ (700 mM) were added to the sample and incubated for 90 min in the dark. The absorbance was noted at 765 nm. Gallic acid was used as the internal standard, and the results were presented as Gallic acid equivalent (GAE) per gram of the extract.

The total flavonoid content: Flavonoids are a sub group of phenolic compounds, TPC was carried out to determine total phenolic compounds including flavonoids. Additionally, to support our hypothesis, total flavonoids were also measured separately in the plant's extract as these flavonoids are mainly reported as anticancer agents.

The total flavonoid content was determined with the use of the aluminum chloride colorimetric method as reported by Olajire & Azeez (2011). The blank reagent was used as a standard. The spectrophotometer was used to measure reagent blank and standard readings at 765 nm wavelength. The plant bark extract was added to the sample tube, while distilled water was added to the reagent blank tube. Rutin was used as an internal standard with a volume of 250 µL and concentration of 5 mg/5 mL dissolved in 85% concentrated methanol. Subsequently, distilled water, 1.25 mL, was added to both the standard and sample tubes. $NaNO_2$ (5%) and $AlCl_3$ (10%) solutions were also added to the tubes. Finally, 500 µL NaOH and 275 µL distilled water were added. Using a spectrophotometer, the absorbance was noted at 510 nm wavelength. The final results were calculated as milligrams of rutin equivalent per gram of the extract.

GC-MS analysis of the bark extract of the *C. buchananii*: To find the natural compounds present in the bark extract of the *C. buchananii* plant, Gas Chromatography-Mass Spectrometry (Shimadzu QP2010PLUS, Japan) was used. The GC-MS analysis was performed at PCSIR, Peshawar, following the AOAC (19th Edition, 2012) method. The Helium gas with a flow rate of 40.7 cm/s was used as the carrier gas in GC-MS analysis. Briefly, a 1 µL sample of bark extract was injected with the injector while keeping the injector temperature maintained at 250°C in the splitless mode. The initial temperature of the oven was set at 50°C, while later it was gradually increased to 230°C at a rate of 6°C/min, without a hold time at the final temperature. The ion source and interface temperatures were both set at 280°C with a solvent cut time 4 minutes while Mass spectra were acquired over a 40–80 m/z range. The spectrum of each compound was obtained, and subsequent analysis was performed using the area normalization method and mass spectral library matching approach.

Determination of antioxidants in bark extract by DPPH radical scavenging activity: Total antioxidant activity in the bark extract of C. buchananii plant was determined by DPPH radical scavenging activity assay using Brand-Williams' protocol (Brand-Williams *et al*., 1995) with some modifications. Freshly prepared DPPH (2.7 ml, 0.004%) was added to the powder plant sample. The resultant mixture was vortexed and left for about 1 hour in a dark room at a temperature of 25°C. To determine the antioxidants, the absorbance was recorded at 517 nm wavelength using a UV-visible spectrophotometer (PerkinElmer Lambda 35 UV/Vis Spectrophotometer).

Fourier transform infrared spectrometry analysis (FTIR) of *C. buchananii* bark extract: To find the FTIR spectra and functional groups in the methanolic extract of *C. buchananii* bark, FTIR analysis was performed. This was carried out using an Agilent Cary 630 FTIR spectrophotometer (Shimadzu, Japan) in the range of 400 to 4000 cm^{-1}. The identification of functional groups in the extract was carried out by comparing the FTIR spectral data with standard reference spectra according to the method reported by Shaheen *et al*., (2022).

Acute toxicity studies of *C. buchananii* methanolic bark extract: Before use of plant extract as an anticancer or as a treatment against diseases, it is important to evaluate its potential toxic effects prior to considering it for therapeutic applications. For this purpose, animal models, such as the Swiss albino, are frequently used. To evaluate the toxicological effects of *C. buchananii* methanolic extract, an acute oral toxicity assay was carried out using Swiss albino mice following the guidelines of the Organization for Economic Cooperation and Development. The animals were maintained at a constant temperature of 25 ± 2°C, relative humidity of 55–65% and a 12-hour light/dark cycle. These animals were fed a standard diet and water. Before the experiment, all the test animals were subjected to an acclimatization process for five days. The experimental animals were kept on a fast for 12 hours by following fixed-dose procedures. However, the control group received normal saline only. The test extracts (bark extract of *C. buchananii* plant) were administered through the oral gavage method, and the maximum dose given was 5000 mg/kg according to body weight. For investigation, the test animals were divided into eight groups with three mice per group. Though this number is small but still the

objective of the research could be addressed. Initially, three groups were administered with the test dose of 10 mg/kg, 100 mg/kg, and 1000 mg/kg and in subsequent round four groups were treated with test doses of 2000 mg/kg, 3000 mg/kg, 4000 mg/kg, and 5000 mg/kg by weight. After the treatment, test animals were observed continuously for 24 hours for the appearance of symptoms, signs of toxicity, and mortality as per the protocol of Ali *et al*., (2014).

Cytotoxic activity of crude methanolic bark extract of *C. buchananii*: For any plant-based extract to be used as medicine against cancer, it is required to evaluate its potential against the given cancer cell lines, known as cytotoxicity activity. In this study, the cytotoxicity activity of the bark extract of *C. buchananii* plant was assessed against the brain cancer cell line U87 (Glioblastoma cell line) and HepG2 (Hepatocellular carcinoma cell line).

The crude methanolic bark extract of *C. buchananii* plant was evaluated for its antiproliferative activity according to the protocol of Costa *et al*., (2020). HepG2 cell line was kindly provided by Dr. Ishaq Khan, originally procured from Texas A & M University, U.S.A., and U87 cell line was provided by Dr. Chen Quan from China. All the cell lines were revived and were cultured in DMEM.F12 medium (Gibco, USA), supplemented with 10% FBS (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA), respectively. When the cells became confluent, they were counted using a hemocytometer (Neubauer, China) and seeded in a 96-well plate, and about 5000 cells per well were seeded. Cells were allowed to grow overnight at 37 °C and 5% CO_2. In the meantime, the crude methanolic extract was dissolved in DMSO and water (3 mg/ml). The cells were treated with different doses in triplicate. Doses were applied in a serial dilution manner, i.e., 400 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL, whereas one well was left untreated as a negative control as per the protocol of Rauf *et al*., (2023). The cells were further incubated at 37°C in a CO_2 incubator for 24 hours. MTT assay (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium) was used to carry out the *In vitro* cytotoxicity assay according to the protocol reported by Nasir *et al*., (2023). After the incubation period, each well was supplemented with 100 µL of new media containing MTT solution with a concentration of 5 mg/mL. The plates were incubated at a temperature of 37°C with 5% CO_2. 100 µL of DMSO (100%) was added to dissolve the insoluble formazan salts. The percentage viability of the samples was computed using the absorbance readings recorded by a microplate reader at a wavelength of 630 nm.

q RT-PCR analysis of selected genes in brain and liver cancer cell lines: The impact of *C. buchananii* bark methanolic extract on glioblastoma (U-87) and hepatocellular carcinoma (HepG2) cell lines was assessed using quantitative real-time PCR. Expression of *BCL-2, BAX* genes in U-87 cell lines and *VEGFR2*, *SOAT1* genes in HepG2 cell lines was studied using q RT PCR using the AriaMx qRT-PCR system. These genes were selected due to their well-established roles in key pathways involved in cancer progression, including apoptosis, angiogenesis, and cholesterol metabolism, particularly in glioblastoma and hepatocellular carcinoma. U-87 and HepG2 cells were initially seeded in six-well plates and incubated until the desired confluency was reached. Half of the wells were treated with the methanolic extract, while the rest served as untreated controls. After treatment, the six-well plates were incubated at 37°C in a 5% CO_2 atmosphere for approximately 24 hours.

For gene expression study, high-quality RNA was extracted from both treated and untreated cells using the TRIzol-chloroform method. RNA purity and concentration were confirmed through NanoDrop and gel electrophoresis. cDNA was synthesized using Reverse transcription of extracted RNA by using the Fine Biotech Script cDNA synthesis kit.

The reaction mixture for q RT PCR was 20 µL containing 1 µL of each of the forward and reverse primers, 10 µL SYBR green reagent (Thermo Fisher Scientific Maxima, USA), 2 µL cDNA template, and 6 µL of nuclease-free water. The primer sequences of selected genes used in q RT PCR are given in Table 1. q RT PCR temperature profile started with initial denaturation for 10 min, followed by 40 cycles at 95ºC for 10 s, 60ºC for 30 s, and 72ºC for 30 s. Agilent Aria software v2.0 was used for the analysis of data, while fold change of gene expression was calculated by the 2^-ΔΔCT method. *β-actin* and *GAPDH* were used as reference genes.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 9.5.0. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare group means, with statistical significance set at $p<0.05$. Additionally, t-test was used for pairwise comparison between the groups.

Table 1. Primer sequences used for quantitative real-time PCR analysis.

Genes	Accession number	Primer	Sequence
GAPDH	NM_001289745.3	F	5' GTCTCCTCTGACTTCAACAGCG 3'
		R	5' ACCACCCTGTTGCTGTAGCCAA 3'
BAX	NM_138764.5	F	5' TCAGGATGCGTCCACCAAGAAG 3'
		R	5' TGTGTCCACGGCGGCAATCATC 3'
BCL-2	NM_000633.3	F	5' ATCGCCCTGTGGATGACTGAGT 3'
		R	5' GCCAGGAGAATCAAACAGAGG 3'
β- actin	NM_001101.5	F	5' CACCATTGGCAATGAGCGGTTC 3'
		R	5' AGGTCTTTGCGGATGTCCACGT 3'
VEGFR2	NM_002253.4	F	5' GGAACCTCACTATCCGCAGAGT 3'
		R	5' CCAAGTTCGTCTTTTCCTGGGC 3'
SOAT1	NM_001252512.2	F	5' GCTCGTGTTCTGGTCCTATGTG 3'
		R	5' TAGAACATCCTGTCACCAAAGCG 3'

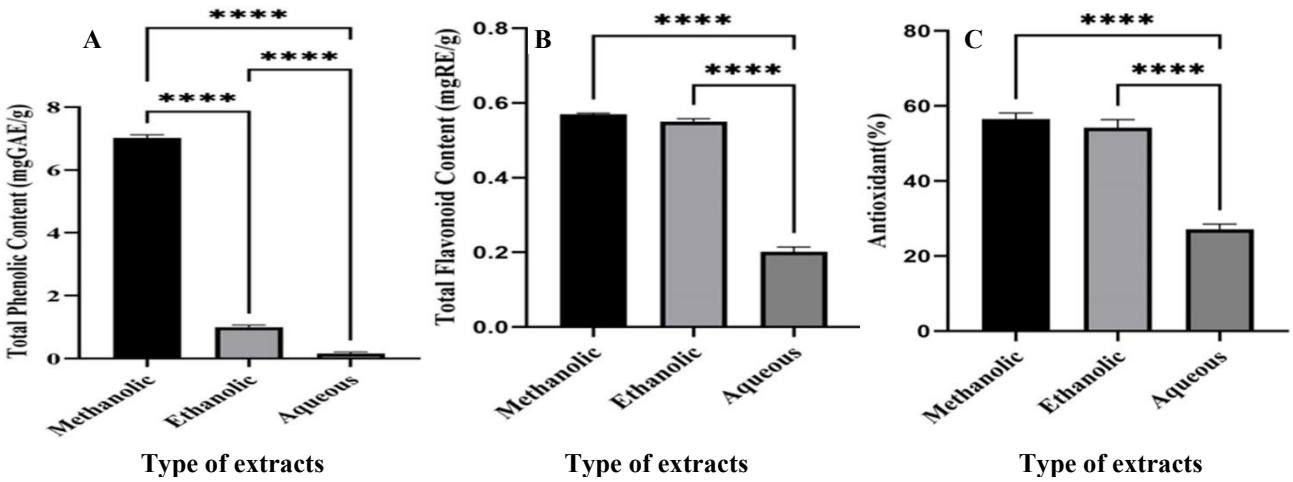

Fig. 1. A Total phenolic content (TPC) and B. flavonoid content (TFC) (mg GAE/g) and (mg RE/g) dry weight in the methanolic, ethanolic, and aqueous extracts of *C. buchananii* bark. C. *In vitro* antioxidant activity of methanolic, ethanolic, and aqueous extracts of *C. buchananii* bark. The data are represented in terms of mean ± SD of triplicate. **** indicates $p<0.0001$.

Results

Determination of total phenolic content (TPC), total flavonoid content (TFC), and *In vitro* antioxidant activity assay: The total phenolic and flavonoid contents of the *C. buchananii* bark methanolic, ethanolic, and aqueous extracts were quantified as milligrams of gallic acid equivalent/gram and milligrams of rutin equivalent/gram of dry weight (Fig. 1A, B). The highest TFC (0.57 ± 0.002 mg/g) was in methanolic extract, while the lowest TFC (0.20 ± 0.01mg/g) was in aqueous extract, followed by (0.55 ± 0.007 mg/g) in ethanolic extract. The highest TPC (7.02 ± 0.03 mg/g) was in methanolic extract, while the lowest TPC (0.16 ± 0.04 mg/g) was in aqueous extract, followed by (0.99 ± 0.06 mg/g) in ethanolic extract. The free radical scavenging activity or antioxidant activity of *C. buchananii* methanolic crude extract, ethanolic, and water extracts was evaluated by using the DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl-Hydrate) assay (Fig. 1C). The methanolic extract showed the highest antioxidant activity (56.49%), followed by ethanolic (54.17%) and aqueous extract (27.16%).

Fourier transform infrared spectrometry analysis (FTIR) of *C. buchananii* bark extract: The FTIR analysis separated the different functional groups present in *C. buchananii* bark extract based on their relative intensities in the 400–4000 cm^{-1} infrared radiation spectrum, as shown in Fig. 2A. This comprehensive spectrum suggests a mixture of organic compounds with potential metal oxide interactions. The FTIR spectrum of the crude methanolic extract of *C. buchananii* exhibited distinct maxima at 3283, 2940, 2836, 2120, 1688, 1513, 1599, 1449, 1375, 1114, and 1017 cm^{-1}. A broad peak around 3283.8 cm^{-1} suggests the presence of hydroxyl (O–H) groups, possibly due to alcohols or water, indicating hydrogen bonding within the sample. The peak at 2940.9 cm^{-1} corresponds to C–H stretching vibrations, typical of alkyl groups in aliphatic hydrocarbons. Peaks in the range of 2409.7 to 2346.8 cm^{-1} could be attributed to triple bonds such as nitriles (C≡N) or atmospheric CO_2. A notable peak at 2120.9 cm^{-1} may indicate the presence of alkynes (C≡C) or nitriles (C≡N). In the fingerprint region, a sharp absorption at 1688.5 cm^{-1} could correspond to carbonyl (C=O) stretching or C=C stretching in alkenes. Peaks between 1449.1 cm^{-1} and 1375.4 cm^{-1} are likely due to bending vibrations of C–H bonds, while the absorption between 1267.3 cm^{-1} and 1017.6 cm^{-1} indicates the presence of C–O stretching, which is typical of esters, ethers, or alcohols. The peak at 879.7 cm^{-1} could suggest aromatic C–H out-of-plane bending. The low-frequency peak at 451.0 cm^{-1} points to metal-oxygen (M–O) stretching, hinting at the presence of metal oxides or metal complexes in the sample.

Pharmacological resemblance of FTIR analysis of crude methanolic extract of *C. buchananii*: For the evaluation of the pharmacological resemblance of the crude methanolic extract of *C. buchananii*, the FTIR spectrum was compared with different pharmacological formulations in the pharmaceutical industry (Fig. 2B). FTIR graph of crude methanolic extract showed similarities in the intensities and location of peaks with the FTIR graph of sample named VITACEL L600, when compared in the library containing FTIR of all pharmacological formulations. This suggests that the current crude methanolic extract of *C. buchananii* is similar to it.

GC-MS analysis of crude methanolic extract of *C. buchananii* bark: The GC-MS spectrum of crude methanolic bark extract of *C. buchananii* has revealed the presence of 18 different bioactive compounds (Fig. 3, Table 2). The identified bioactive compounds are classified as alcohols, alkanes, phenols, aromatic aldehydes, ketones, and fatty acids. The concentration of alcohols ranged from 0.51% to 1.41%, while alkanes from 1.3% to 2.06%. Aromatic aldehydes represented the major group, with a concentration of 24.72%. The identified compounds contained ketones in the range of 1.21% to 3.68%, and fatty acids, another major group found, were found in concentrations ranging from 1.08% to 22.82%. Among the identified phenolic compounds, 4-hydroxy-2-methylacetophenone has been reported for its anticancer properties. Many of the detected compounds have previously been reported in other medicinal plants and are known for their potential broad spectrum biological activities, including anticancer, antioxidant, anti-inflammatory, antidiabetic, antimicrobial, antifungal and neuroprotective properties (Table 2).

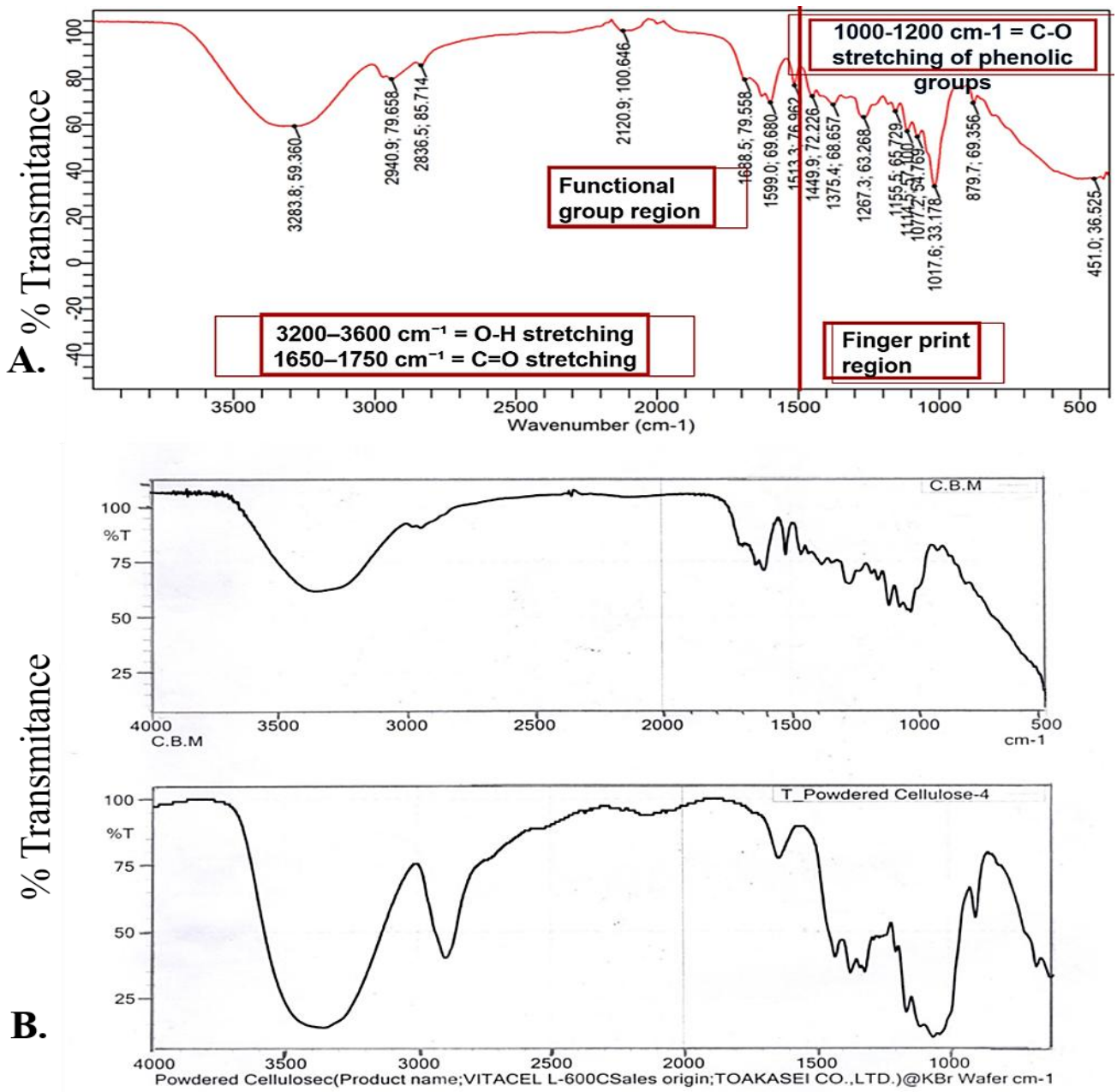

Fig. 2. **A.** The FTIR spectrum of the *C. buchananii* bark methanolic extract. **B.** Comparative FTIR analysis has been reported. It is a resembled pharmacological formulation present in the pharmaceutical industry in parallel to crude extract.

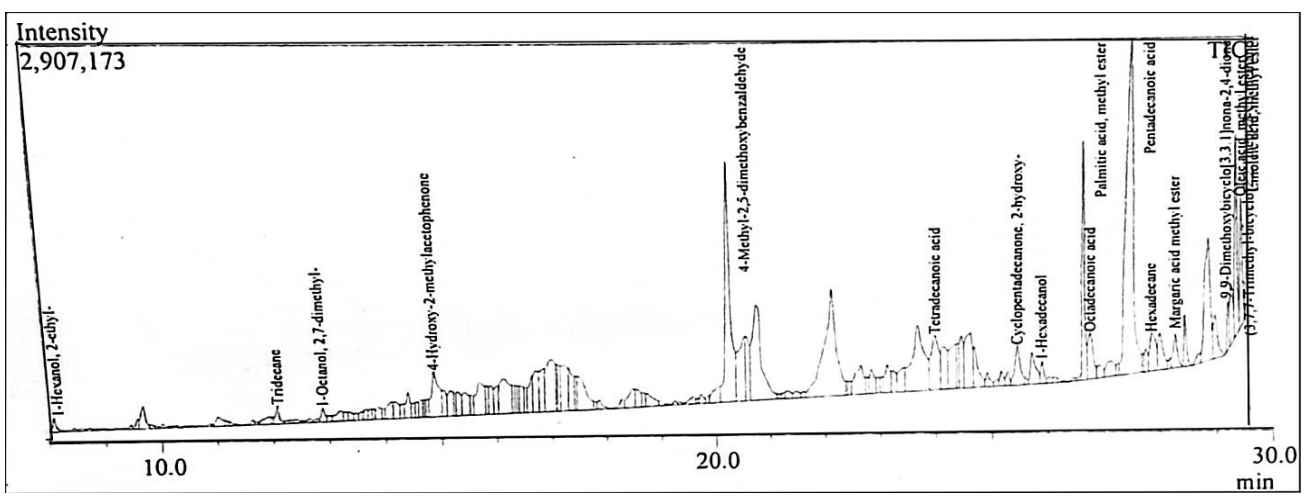

Fig. 3. GC-MS chromatogram of *C. buchananii* plant crude methanolic bark extract.

Table 2. Compounds identified through GC-MS in *C. buchananii* bark crude methanolic extract.

S. No.	Name	Retention time	Area	% Concentration	Type	Biological activities	References
1.	3,7,7-Trimethyl-bicyclo[2.2.1]hept-2-yl)methanol	29.297	126739	1.41	Bicyclic alcohol	Anti-inflammatory, Neuroprotective	Kalkarni *et al.*, (2019
2.	1-Hexanol,2-ethyl	8.081	110913	1.23	Alcohol	Antifungal	Zhang *et al.*, 2024
3.	1-Octanol,2,7-dimethyl	12.88	67521	0.75	Alcohol	Antimicrobial, Antioxidant	Coêlho *et al.*, 2022
4.	1-Hexadecanol	25.908	45502	0.51	Fatty alcohol	Anticancer, Anti-inflammatory	Gayathiri *et al.*, 2024
5.	Hexadecane	27.86	185254	2.06	Alkane	Antioxidant	Wiraswati *et al.*, 2023
6.	Tridecane	12.071	116957	1.3	Alkane	Antimicrobial	More *et al.*, 2022
7.	4-Methyl-2,5-dimethyoxybenzaldehyde	20.244	2225077	24.72	Aromatic Aldehyde	Antimicrobial, Anticancer	Canh *et al.*, 2022
8.	9,9-Dimethoxybicyclo [2.2.1}hept-2-yl)-methanol	28.893	331150	3.68	Bicyclic di ketone	Anticancer, Anti-inflammatory	Reza *et al.*, 2024
9.	4-Hydroxy-2-methylacetophenone	14.864	245019	2.72	Phenol/ketone derivative	Anticancer, Antifungal	Mamedov *et al.*, 2015
10.	Cyclopentadecanone, 2-hydroxy-	25.473	109087	1.21	Cyclic ketone	Antibiotic, Anticancer	Liu *et al.*, 2022
11.	Pentadecanoic acid	27.538	2054691	22.82	Fatty acid	Anticancer, Anti-inflammatory	To *et al.*, 2020
12.	Palmitic acid, methyl ester	26.655	1259497	13.99	Fatty acid methyl ester	Antioxidant, Antimicrobial, Anticancer	Wang *et al.*, 2023
13.	Methyl lignocerate	28.895	701662	7.79	Fatty acid methyl ester	Anti-diabetic	Shilpa *et al.*, 2009
14.	Octadecanoic acid	20.785	457017	5.08	Fatty acid	Anticancer, Anti-inflammatory	Reza *et al.*, 2021
15.	Linoleic acid, methyl ester	29.383	435176	4.83	Poly unsaturated fatty acid	Anti-inflammatory	Kolar *et al.*, 2019
16.	Oleic acid, methyl ester	29.479	156043	1.73	Fatty acid methyl ester	Antioxidant, Antimicrobial, Anticancer	Djeghim *et al.*, 2024
17.	Margeric acid, metyl ester	28.291	100356	1.11	Fatty acid methyl ester	Antibacterial	Krishnaveni *et al.*, 2014
18.	Tetradecanoic acid	24.001	97011	1.08	Fatty acid	Anticancer, Antioxidant	Ismail *et al.*, 2024

Acute oral toxicity studies of *C. buchananii* bark extract: Before performing the acute oral toxicity studies to check the presence of any potentially harmful compound like cyanide in the methanolic extract of *C. buchananii* bark, FTIR analysis was performed. The FTIR spectrum showed no cyanide-related absorption peaks between 2220 and 2260 cm^{-1}, the characteristic range for cyanide detection, indicating the absence of cyanide in the extract. The concentration of the extract was doses of 10mg/g to a maximum of 5000 mg/kg (Table 3). All treated groups in comparison to the control group, which was given normal saline (0.9%), revealed no mortality but only slight alteration in behavior. The various symptoms were normal up to 3000 mg/kg concentration except for symptoms like pilo erection, analgesia, hyperesthesia, ptosis, respiration, and Strabismus reaction that show slight change after 3000 mg/kg concentration. However, at the maximum (5000 mg/kg) concentration, additional symptoms were observed, including reduced motor activity, analgesia and hyperesthesia. The clinical signs of acute toxicity observed in mice following oral administration of the methanolic bark extract of *C. buchananii* are summarized Table 3.

Cytotoxicity activity of *C. buchananii* bark methanolic extract: The antiproliferative activity of *C. buchananii* methanolic extract was assessed *In vitro* against human Glioblastoma (U-87) and Hepatocellular carcinoma (HepG2) cells (Figs. 4 & 5). The MTT assay images showed the effect of different (DMSO-based) extract concentrations on cancer cells in a dose-dependent manner (Fig. 4A-E, Fig. 5A-E). The dose response curve showed the relationship between the concentration of extract and percent cell viability, where each point in the dataset was the average of three replicates. The dose-dependent inhibition bar graph showed a gradual and significant decrease in cell viability of both U-87 and HepG2 with increasing concentrations of the extract, prepared in both DMSO (Fig. 4A-B, Fig. 5A-B) and sterile water (Fig. 4C-D, Fig. 5C-D). The first image (Fig. 4E(i) and Fig. 5E(i)) represents the negative control group. As evident from images 4E ii-viii and 5E ii-viii, increasing concentration of extract led to a significant reduction in cell viability, whereas, a decrease in concentration resulted in enhanced cancer cell proliferation. The extract resulted in a concentration-dependent reduction in cell viability, as can be evidenced from cytotoxicity graphs for U-87 (Fig. 4A-E), HepG2 (Fig. 5A-E) cells. *C. buchananii* extract exhibited the highest cytotoxicity, as evidenced from IC50 of 47.53 µg/mL in U-87 cells (DMSO-based extract) and IC50 of 81.53 µg/mL (sterile water-based extract) compared to HepG2 cells IC50=59.58 µg/mL (DMSO-based extract) and 100.4 µg/mL (sterile water-based extract) in a dose-dependent manner (Table 4). Among both cell lines, the DMSO-based extract revealed notable potent cytotoxicity as compared to the sterile water-based extract.

Expression of apoptosis and angiogenesis-related genes in U-87 and HepG2 cell lines: q RT-PCR analysis was used for the evaluation of the expression of selected genes involved in apoptosis, angiogenesis, and cholesterol metabolism. Fig. 6A-D illustrate the fold changes in gene expression after 24-hour incubation of cancer cell lines with the methanolic extract. q RT-PCR analysis resulted in the up-regulation of pro-apoptotic gene *BAX* (1.5 fold) (Fig. 6A) and down-regulation (0.6-fold less) (Fig. 6B) of anti-apoptotic gene *BCL2* in U-87 (Brain cancer cell lines) compared to control. Angiogenesis-related gene *VEGFR2* (Fig. 6C) and cholesterol metabolism-related gene *SOAT1* (Fig. 6D) were down-regulated (0.8 and 0.9-fold less) in HepG2 (liver cancer cell lines) compared to the control.

Table 3. Behavioral observation and general appearance of treated and control groups of Swiss Albino mice after acute oral toxicity assay.

Symptoms	Intensity/Remarks							
	Normal saline	Test Substance Doses (mg/kg)						
		10	100	1000	2000	3000	4000	5000
Analgesia	-	-	-	-	-	-	+	++
Anesthesia	-	-	-	-	-	-	-	-
Arching & rolling	-	-	-	-	-	-	-	-
Atexia	-	-	-	-	-	-	-	-
Catatonia	-	-	-	-	-	-	-	-
Clonic convulsion	-	-	-	-	-	-	-	-
Decreased motor activity	-	-	-	-	-	-	-	+
Diarrhea	-	-	-	-	-	-	-	-
Exophthalmos	-	-	-	-	-	-	-	-
Failure	-	-	-	-	-	-	-	-
Hyperesthesia	-	-	-	-	-	-	+	++
Hypnosis	-	-	-	-	-	-	-	-
Increase motor activity	-	-	-	-	-	-	-	-
Lacrimation	-	-	-	-	-	-	-	-
Loss of the righting reflex	-	-	-	-	-	-	-	-
Opisthotonis	-	-	-	-	-	-	-	+
Pilo erection	-	-	-	-	-	-	++	+++
Ptosis	-	-	-	-	-	-	-	++
Respiration	-	-	-	-	-	-	-	+
Salivation watery, Viscid	-	-	-	-	-	-	-	-
Skin blanching flushing cyanosis	-	-	-	-	-	-	-	-
Stimulation	-	-	-	-	-	-	-	-
Straub reaction	-	-	-	-	-	-	++	+++
Tonic extensor	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-

The control group contains normal saline. +, ++, and +++ sign shows the presence of symptoms in one, two, and three mice, respectively

Table 4. IC 50 values and 95% confidence intervals of dose-response curves.

Cancer cell lines	Solvent	IC_{50} (µg/mL)	95% Confidence interval
U-87	DMSO	47.53	44.73 - 50.00
U-87	Sterile Water	81.53	76.03 - 87.49
$HepG_2$	DMSO	59.58	52.13 - 68.14
$HepG_2$	Sterile Water	100.4	93.94 - 107.4

Discussion

Cancer is a serious human disease responsible for a large number of deaths worldwide. Current treatment strategies such as chemotherapy and radiotherapy, though effective in combating the disease are often associated with side effects, leading to reluctancy among patients towards these therapeutic approaches. To develop more potent anticancer treatments, the medicinal plants are being widely investigated across the world as valuable source for the isolation of anticancer compounds (Chandra *et al*., 2023). *C. buchananii* is a promising medicinal plant, reported to cure various health conditions, such as purifying blood and as an anti-inflammatory and antibacterial agent (Arthan & Yenjai, 2023). There are currently no published reports on *C. buchananii* bark extract as a potential alternative source of herbal medication for cancer. To our knowledge, this is the first study that has explored the pharmacogenetic parameters as well as the anti-cancerous potential of *C. buchananii* bark extract and its effects on the expression of apoptosis, angiogenesis, and cholesterol metabolism-related genes in brain and liver cancer cell lines.

Our study confirmed the presence of clinically significant phytochemicals, including phenols, alkaloids, and tannins, which are well known for their diverse biological activities. The results of our investigation are consistent with a study on the methanolic extract of the aerial parts of *C. buchananii*, where these phytochemicals have been previously identified (Sharma *et al.,* 2012; Tugaonkar *et al.,* 2020). In the present study, the methanolic bark extract exhibited significantly higher antioxidant activity as determined by the DPPH assay. Substantial total phenolic and flavonoid content was also quantified, which showed a strong connection between phenols and antioxidant activities as reported earlier (Foo *et al.,* 2017). Methanolic extracts exhibited higher phenolic and flavonoid compounds and antioxidant activity in comparison to ethanolic or aqueous extracts. This can be linked to methanol's higher polarity compared to ethanol or other solvents, which enables it to extract and dissolve a diverse group of bioactive compounds, including phenols and flavonoids (Salih *et al.,* 2021). Furthermore, the small size of the methanol molecule (methyl radical) compared to ethanol allows it to penetrate deep into plant tissues and more effectively solubilize phenolics and flavonoid compounds by interacting with the hydroxyl groups in phenolic compounds (Mehmood *et al.,* 2022). Elevated levels of antioxidant activity in plant methanolic extracts can be attributed to elevated levels of TPCs and TFCs, well known for their ability to scavenge free radicals and reduce oxidative stress (Mutha *et al.,* 2021).

The FTIR analysis revealed the presence of key functional groups in the methanolic extract. The identified groups have been reported for their anticancerous, antioxidative, and anti-inflammatory qualities. Phenolic and flavonoid compounds are characterized by the presence of a key functional hydroxyl group. This hydroxyl group can scavenge free radicals by donating hydrogen atoms, thus conferring antioxidant potential (Chandrasekar *et al*., 2022; Zhang *et al*., 2022). This antioxidant mechanism is associated with the pharmacological activities like antimicrobial, anti-inflammatory, and anti-cancer by establishing hydrogen bonds, and by modulating cellular pathways and enzymes involved in the progression of these disease pathways (Lobiuc *et al*., 2023; Zhang *et al*, 2022). The FTIR spectrum of the methanolic extract was further compared with the FTIR reference library of known pharmacological formulations used in the pharmaceutical industry. It showed significant similarity with VITACEL L600 formulation, a multivitamin. Though this comparison can be considered exploratory, but it points towards health health-promoting properties of plant extract.

The GC-MS analysis was used to analyze the bioactive compounds present in the *C.buchananii* bark extract. GC-MS results revealed 18 compounds, out of which most were very well known for their pharmacological roles, like antimicrobial, antidiabetic, neuroprotective, anti-inflammatory, antioxidative, and anticancerous. Among identified compounds, 4-methyl-2,5-dimethoxybenzaldehyde (24.72%), pentadecanoic acid (22.82%), palmitic acid, methyl ester (13.99%), methyl lingocerate (7.79%), octadecanoic acid (5.08%), linoleic acid, methyl ester (4.83%), 9, 9-dimethoxy bicyclo [2.2.1] hept-2-yl methanol (3.68%).4-Hydroxy-2-methylacetophenone (2.72%), oleic acid methyl ester (1.73%), cyclopentadecacanone, 2-hydroxy (1.21%), margaric acid methyl ester (1.11%) tetradecanoic acid (1.08%) and 1-hexadecanol (0.51%) have been previously reported in literature for their significant anticancerous potential.

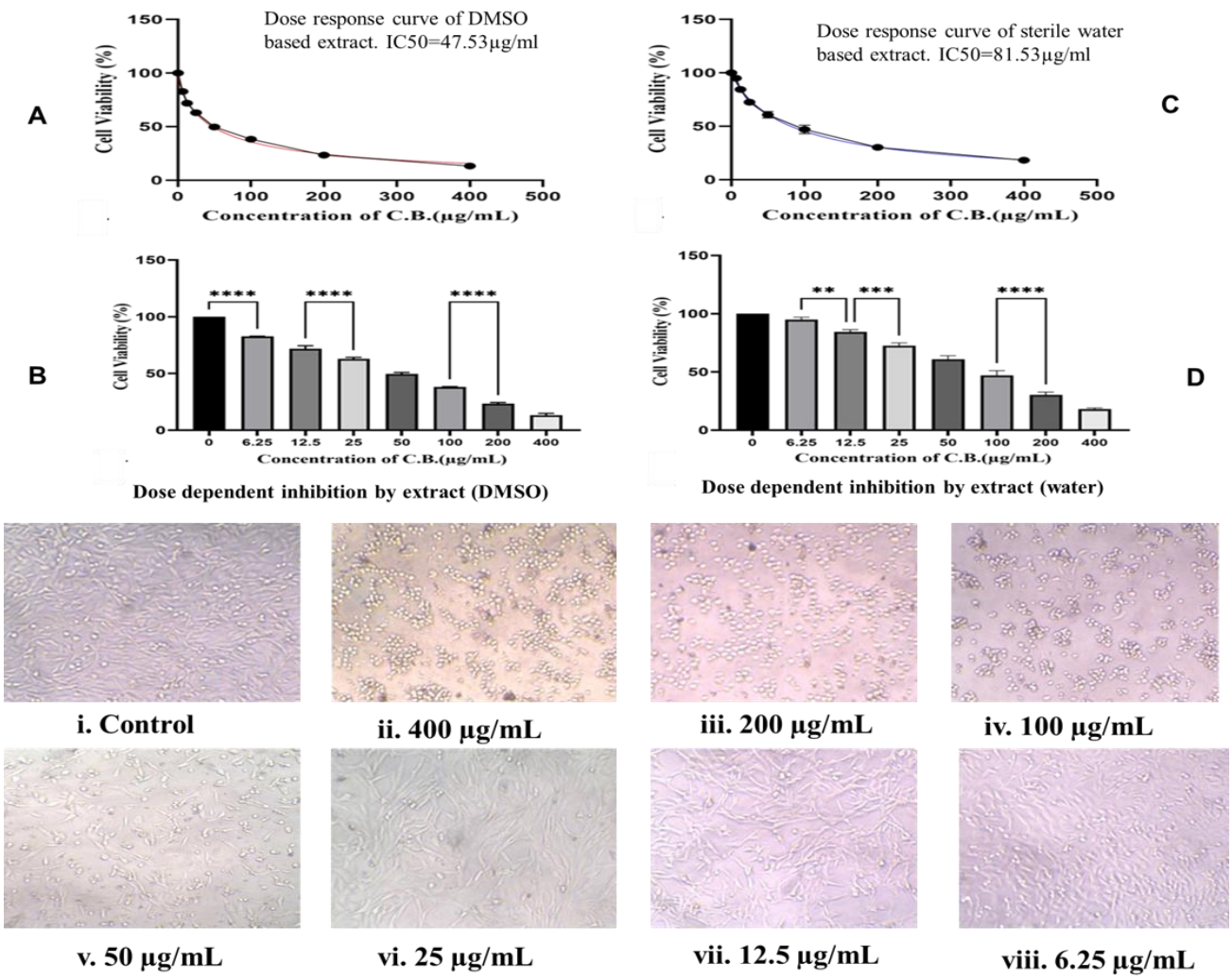

Fig. 4. **(A)** The calculated IC50 value for *Cryptolepis buchananii* bark extract *(*dissolved in DMSO) against U-87 cells is 47.53 µg/mL. IC50 was determined by using non-linear regression in a dose-response curve. **(B)** Bar chart representing dose-dependent inhibition of cell viability. **(C)** The calculated IC50 value for *C. buchananii* bark extract (dissolved in sterile water) against U-87 cells is 81.53 µg/mL **(D)** The bar chart represents the dose-dependent inhibition for water-based extract. **(E)** MTT assay images of U-87 cells (DMSO-based extract). **(i)-**represents the negative control (untreated cells). **(ii-viii)** represents the viability of cells at increasing concentration of *C. buchananii* bark methanolic extract. * Indicates differences when compared to the control group (****:p<0.0001, ***: p<0.001, **: p<0.01, *: p<0.1).

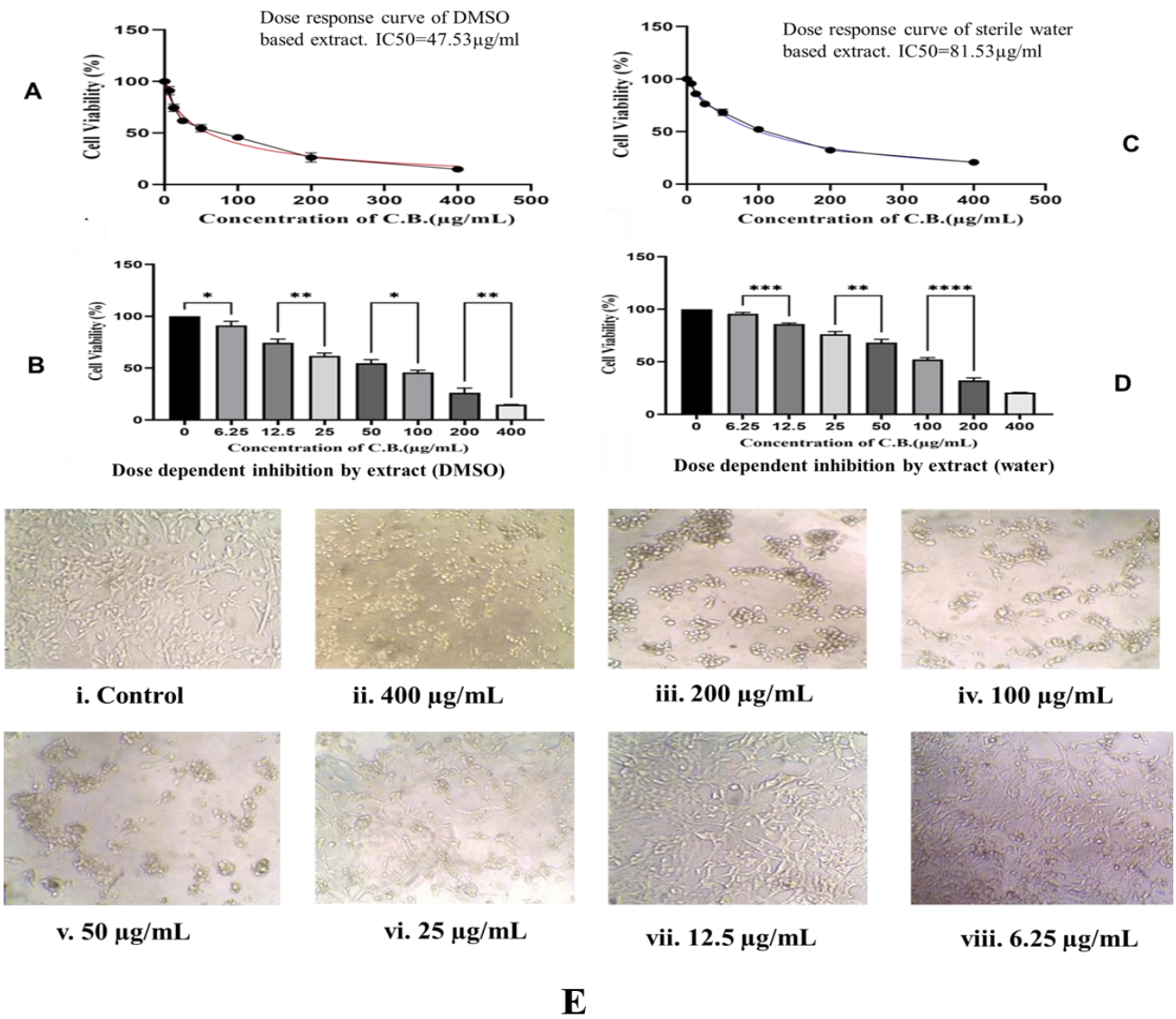

Fig. 5. **(A)** The calculated IC50 value for *Cryptolepis buchananii* bark extract *(*dissolved in DMSO) against HepG2 is 59.58 µg/mL. IC50 was determined by using non-linear regression in a dose-response curve. **(B)** Bar chart representing dose-dependent inhibition of cell viability. **(C)** The calculated IC50 value for *C. buchananii* bark extract (dissolved in sterile water) against HepG2 cells is 100.4 µg/mL **(D)** The bar chart represents the dose-dependent inhibition for sterile water-based extract **(E).** MTT assay images of HepG2 cells (DMSO-based extract). **(i)-** represents the negative control (untreated cells). **(ii-viii)** represents the viability of cells at increasing concentration of *C. buchananii* methanolic bark extract. * Indicates differences when compared to the control group (****:p<0.0001, ***: p<0.001, **: p<0.01, *: p<0.1).

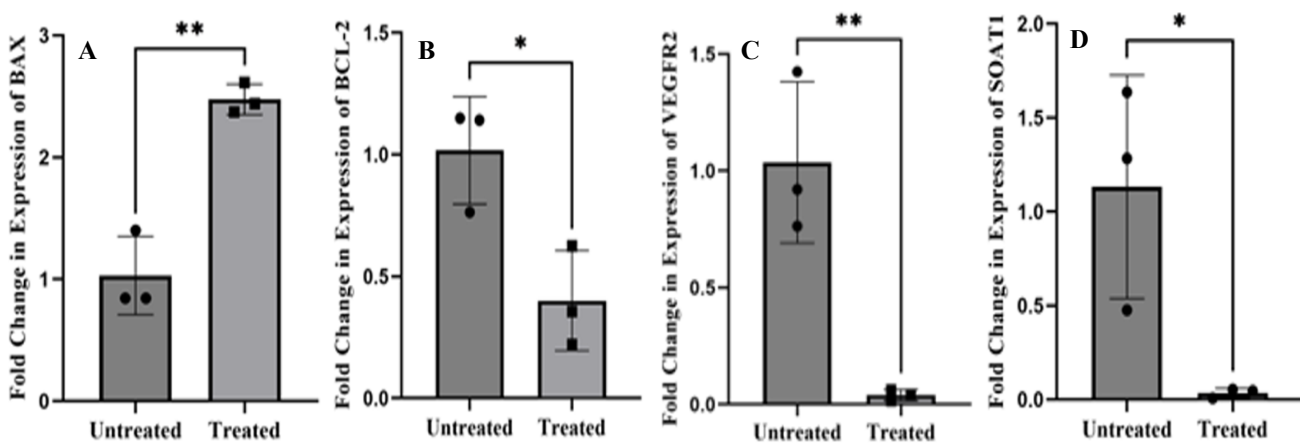

Fig. 6. **A:** Expression (fold change) of *BAX* and *BCL-2* genes in U-87 cell lines. **B:** Expression (fold change) of the *BCL-2* gene in the U-87 cell line. **C:** Expression (fold change) of the *VEGFR2* gene on the HepG2 cancer cell line. **D**: Expression (fold change) of the *SOAT1* gene in the HepG2 cell line. The data is composed of the mean ± standard deviation of three independent experiments performed in replicates. ** p<0.01,*p<0.05 represents the level of significance.

For 4-methyl-2,5-dimethoxybenzaldehyde, although no extensive previous research is available related to its biological activity, it is reported in the literature as being part of biologically active compounds either as its precursor or as a structural component (Canh *et al.,* 2022). Pentadecanoic acid has previously been reported for its strong anti-inflammatory and anticancer activities. In a study conducted on breast cancer cells (MCF-7), it has shown potential cytotoxicity by reducing invasiveness and stemness. It changes EMT marker expression, induces apoptosis, inhibits JAK2/STAT3 signaling, and alters cancer stem cells, thus suggesting a potential therapeutic role in cancer treatment (To *et al.,* 2020). Pharmacological studies has revealed that palmitic acid not only possesses antioxidant and anti-inflammatory properties but has also shown significant cytotoxic potential against various cancers like cervical cancer, gastric cancer, liver cancer, colorectal cancer and breast cancer by inducing apoptosis (Wang *et al.,* 2023). Octadecanoic acid, oleic acid, and 4-hydroxy-2-methylacetophenone reported in the current study are also known for their potential cytotoxic activity against various cancer cell lines. Compounds like tetradecanoic acid, hexadecane, and 1-Octanol, 2,7-dimethyl have been reported to contribute to the extract's antioxidant and anticancer properties. Thus, the use of *C. buchananii* in folk medicines is supported by its chemical profile. The presence of aromatic aldehydes, hydrocarbons, and fatty acids in its crude extract provides a significant basis for further investigation of its therapeutic efficacy in brain and liver cancer.

Plant-based medicines called phototherapeutics have become popular in basic healthcare, particularly in less developed nations. However, safety testing is necessary the approval of herbal treatments currently available on the market (Habbu *et al.,* 2013). Before any pharmacological validation or the development of a phytomedicine, acute toxicity must be assessed in compliance with established procedures (El-Gazayerly *et al.,* 2014). Consequently, scientists have recently focused on investigating the safety and efficacy of traditional medicines to generate research that meets the rigorous standards necessary to support their global use (Singh *et al*., 2017). In the current study, the safety of a crude methanolic extract of *C. buchananii* was evaluated by acute oral toxicity in Swiss albino mice. No mortality was observed with minimum clinical signs at the highest dose of 5000mg/kg, thus proving its safety in use. Clinical experiments of *C. buchananii* in humans are greatly encouraged due to its negligible toxicity, especially when administered orally, as also proven by previous studies conducted on rats (Arthan & Yenjai*,* 2023). Normal saline was used as the standard since the current study aimed to assess the acute toxicity and mortality pattern of the extract rather than drug-induced effects as reported in a previous study by Ali *et al*., (2014). Histopathological and biochemical analyses are conducted generally in chronic or sub-chronic toxicity studies, where prolonged exposure can reveal tissue-level alterations. Hence, inclusion of a positive control such as doxorubicin was not applicable in this acute toxicity design.

The cytotoxic findings in the current study indicate that *C. buchananii* methanolic bark extract dissolved both in DMSO and sterile water had potential cytotoxic effects and inhibited the proliferation in both cancer cell lines, i.e., U87 and HepG2, with the methanolic extract dissolved in DMSO demonstrating greater efficacy than the in sterile water. These results are consistent with those of the earlier research (Srivastava *et al.,* 2015). The data taken together across all cancer cell lines demonstrated that DMSO-based extract exhibited more potent cytotoxicity compared to sterile water-based extract, thus suggesting that DMSO has a greater ability to dissolve active compounds that are responsible for the cytotoxic effect of plant extract than sterile water. This suggests the importance of solvent selection in cytotoxic assays for maximizing the biological potential of plant extracts. The plant extract exhibited a moderate level of anti-cancer activity when tested on U-87, HepG2 cancer cell lines. Among the major reasons that have resulted in moderate cytotoxicity could be the use of whole plant extract for the assay. In literature, the nanoparticles made from *C. buchananii* have been shown to kill> 90% of HeLa cells (Tayung & Saikia, 2020). If bioactive compounds are separated using bioassay-guided extraction or through spectrometric analysis subsequently evaluated for cytotoxicity, enhanced cytotoxic activity may be revealed, as demonstrated by previous studies on plants from the same family (Ren *et al.,* 2023; Bhandari *et al*., 2017). This study has the limitation of not including a standard drug for direct comparison, which can be addressed in future studies.

q RT-PCR analysis showed that *C. buchananii* methanolic extract has a significant impact on the expression of the genes involved in apoptosis, angiogenesis, and cholesterol metabolism in glioma(U-87) and hepatocellular carcinoma. In the U-87 cancer cell line, a downregulation in expression of anti-apoptotic gene *BCL2*, while upregulation in *BAX* gene, a pro-apoptotic gene, was observed. This *BCL2*/*BAX* ratio indicates the initiation of apoptosis. As *BCL*2, an oncogene is known for its role in the prevention of cell death, while *BAX* is a pro-apoptotic protein; thus, it can be concluded that *C. buchananii* bark extract promotes apoptosis by maintaining the balance towards cell death. These results are consistent with the critical role of *BCL2* and *BAX* in the regulation of the mitochondrial pathway of apoptosis. Previous study confirmed that *Hedyotis diffusa* Wild extract downregulates the *BCL2/BAX* ratio by inducing apoptosis in U-87 cancer cell lines (Zhang *et al.,* 2014). Similar findings were noted for *Urtica dioica* extract-induced apoptosis in HepG2 cell lines (Kardan *et al*., 2020). In HepG2, a significant downregulation was observed in *VEGFR2* (vascular endothelial growth factor receptor 2) and *SOAT1* (sterol O-acyltransferase 1) gene expression. *VEGFR2* plays a key role in angiogenesis by forming new blood vessels and by supplying tumors with nutrients and oxygen. The progression of the tumor can be inhibited by inhibiting the expression of *VEGFR2*. Our findings are consistent with a previous study reporting the inhibitory effect of aqueous extract of *Crocus sativus* L. on the expression of *VEGFR2* gene in MCF-7 cell lines (Mousavi *et al.,* 2014). Similar findings were reported in HUVEC cell lines, where polyphenols such as epigallocatechin gallate from green tea and procyanidin oligomers from apples had significantly inhibited VEGF receptor-2 (*VEGFR*-2) gene (Moyle *et al.,* 2015). The *SOAT1* gene has a critical role in cholesterol metabolism as it catalyzes the formation of cholesterol esters, present in lipids, and can proliferate cancer cell growth by its utilization to meet its metabolic demands. Inhibition of the expression of the *SOAT1* gene can result in significant metabolism in HepG2 Cell lines (Pramfalk *et al.,* 2020). However, till now, no study is available for the evaluation of methanolic extract on the gene expression of *SOAT1*. The downregulation of both *VEGFR2* and *SOAT1* indicates that *C. buchananii* extract can play a role in the regulation of both angiogenic and cholesterol metabolism.

Conclusions

This study provides a comprehensive evaluation of *C. buchananii* methanolic bark extract's pharmacognostic properties and cytotoxic potential. Among the selected solvents, the methanolic bark extract exhibited the highest phenols, flavonoids, and antioxidant capacity, suggesting its significant ability to scavenge free radicals and its contribution in regulating oxidative stress, crucial in both cancer initiation and metastasis. FTIR analysis showed the presence of key functional groups, i.e., hydroxyl and carbonyl. The GC-MS analysis further identified the presence of bioactive compounds, contributing a major role in cytotoxicity by inhibiting cancer cell proliferation. MTT assay revealed that extract from *C. buchananii* bark exhibited promising cytotoxicity against brain (U-87) and liver (HepG2) cancer cell lines. The acute oral toxicity studies confirmed further extract's safety at therapeutic doses. q RT-PCR analysis revealed that *C. buchananii* had a significant effect on the expression of genes related to apoptosis in Glioma and genes related to angiogenesis and cholesterol metabolism in Hepatocellular carcinoma cancer cell lines.

Conclusively, this study provides a solid foundation for advanced molecular research based on plant-based anticancer drug development. This study further provides insight into *C. buchananii's* anticancer mechanism against aggressive tumors supports the development of less toxic, more effective, and economic therapeutic alternatives for cancer, particularly for low-resource countries. *C. buchananii* plant being a potential phytomedicine candidate in the future, can be a valuable addition to the plant-based anticancer agents.

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