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Evaluation of the cytotoxic and antioxidant activities *Buxus wallichiana* Baill leaf extract against U 87 glioblastoma

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Abstract: The scientific community has been drawn towards natural plant resources due to the rising success rates. This work aimed to explore the phytochemical, antioxidant and anti-cancer properties of an ethanolic extract derived from the leaves of *Buxus wallichiana Baill*. which is utilized in traditional medicine in the treatment of various disease such as respiratory disorders, GI disorders and inflammation. Chemical composition of the ethanolic extract of *BW* leaves was evaluated by using Gas chromatography-mass spectrometry and phytochemical analysis. Moreover, anti-oxidant and anticancer activity was carried out through 2,2-diphenyl-1-picryl hydrazyl scavenging activity method and 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide assay against human glioblastoma cell line. ANOVA test was used to analyze the results followed by Bonferoni's post hoc. Results were significant at $P<0.05$ and demonstrated that, blocking the production of free radicals also caused tumor cell proliferation on the U 87 MG. Thus, the results show the same pattern of toxicity as indicated by the American National Cancer Institute. The minimum dose for cytotoxic activity of the crude extract was less than 30µg/mL. *BW* leave extract is considered to have promising anticancer potential as well as antioxidant due to the presence of potent compounds and could become a source of treatment with minimum side effects.

Keywords: Anticancer, antioxidant *Buxus wallichiana Baill*, human glioblastoma cell line, phytochemicals.

INTRODUCTION

Being the second greatest cause of death worldwide, after cardiovascular diseases, cancer poses a serious threat to public health (Henley *et al.,* 2020). The American Cancer Society estimates that in 2016, there were 1,685,210 new cases of cancer discovered nationwide and approximately 600,000 deaths attributable to cancer occurred in the USA. As well as, every year, more than 3 million new instances of cancer are reported in Europe (Rock *et al.*, 2022). Numerous physiological processes, such as inflammation, apoptosis, oxidant/antioxidant balance, differentiation and angiogenesis, are impacted by alterations in cancer. It has been established that oxidative stress, which is characterized as a chronic disruption between the generation of free radicals and antioxidant defenses, plays a significant role in the development of cancer. (Bardelcíkova *et al.,* 2023) Research on a variety of cancer types, including carcinoma, brain, prostate, breast, melanoma, colon and melanoma, has shown that aberrant expression of oxidative stress players occurs in cancers, impacting both the phenotypic (biological behaviour) and responsiveness of cancer cells to treatment interventions (Hayes *et al.,* 2020).

In the central nervous system, glioblastoma multiforme (GBM) represents 47.7% of all cases and is the most frequent malignant brain tumor (Liu *et al.,* 2020). The usual age of diagnosis is 65 years old, but it can happen at any age. With roughly 40% survival in the first year and 17% in the second year following diagnosis, the survival rate is poor. However, Standard treatment options involve chemotherapy, radiotherapy (RT), immunotherapy, and surgical resection, with low survival rates and high recurrence rates with adverse effects. Thus, it is imperative to discover more effective glioblastoma treatments to enhance this type of tumor's prognosis (Grochans *et al.,* 2022; Kumari *et al.,* 2023).

BW is a highly potent medicinal plant belonging to the *Buxaceae* family, commonly known as Himalayan boxwood or boxwood. In Asia, Europe, North Africa, and North America, the *BW* family is extensively dispersed. (Wani *et al.,* 2023) In Pakistan, it is usually found in mountains and hilly areas in Azad Kashmir, Rawalpindi, Murree, Islamabad, Hazara, Chitral and Smack regions (Ali *et al.,* 2023) Leaves of the *Buxus* plant are eagerly obviated and oblanceolate and constrict at the base or apiculate at the apex (Wani *et al.,* 2023) *Buxus* leaves have a bitter taste due to the presence of alkaloids like *buxine*. Moreover, the *Buxaceae* family is used due to its multiple pharmacological effects such as purgative,

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diaphoretic, antioxidant and anti-microbial. Traditionally, *BW* had been widely used as a bitter tonic for anti-rheumatic, analgesic, diuretic, anti-leprotic, diaphoretic, and antiepileptic effects. It is also used to cure GI motility disorders, airway diseases, vascular and respiratory tract infections (Ali *et al.,* 2023).

However, evaluation and identification of different pharmacological or medicinal uses of *BW* leaves to enlighten the benefits of this part of the plant will be the principal goal of the present study. Specifically, evaluate the anti-cancer effect on GBM and anti-antioxidant activities of ethanol leaf extract also, identified the chemical composition of the ethanolic extract of *BW* leaves was by using Gas chromatography-mass spectrometry (GC-MS) and phytochemical analysis.

MATERIALS AND METHODS

Plant material collection and authentication.
Leaves of *BW* were collected from Swat, region of Pakistan in October 2019 and identified by the Botany Department, Karachi University, Pakistan with voucher number G.H. No. 95564, dated 02/10/2019. The leaves were washed with distilled water, dried, powdered and stored.

Preparation of extract
Ethanolic extract was prepared by using simple extraction method *BW* leaves dry powder material (50g) was macerated in 700 mL of ethanol for 15 days at room temperature. The mixtures of *BW* leave extract were strained by using muslin cloth and for further filtration by using filter paper (Whatman NO.1). The filtrate was left to dryness under condense pressure on rotary evaporator (Heidolph, Germany) at 0°C after proper extraction of extract the subsequent sample was placed in a drying oven for removal of water content. The final powder extract was kept in refrigerator and in an airtight jar for future purposes. (Sabir *et al.,* 2020)

Calculating the yield (%) of plant extract
The formula for calculating the yield percentage (w/w) from the dried extracts is yield (Naz *et al.,* 2020).
(%) = (W1 × 100) ÷ W2
Where
W1 is the extract's dry weight following solvent evaporation
W2 is the weight of the soaking leaf powder.

Chemicals and Drugs
Analytical grade chemicals and drugs were used for the study, including methanol, ethanol Ascorbic Acid (BDH Laboratory Supplies, England), 2-diphenyl-1-picryl hydrazyl solution (Sigma –Aldrich), American Type Culture Collection (ATCC), human main glioblastoma cell line U 87 MG, 3-(4, 5-dimethylthiazol-2-yl)5, 5-diphenyltetrazolium bromide (Merck), Temozolomide (Merck) and Dimethyl Sulfoxide (Sigma-Aldrich).

Preliminary examination of phytochemicals
To identify different types of secondary compounds, including terpenes, alkaloids, phenolics, flavonoids, tannins, saponins, phlobatannins and coumarins, the *BW* leaf extract was subjected to qualitative phytochemical analysis (Bokhari *et al.,* 2020).

GC-MS phytoconstituent profiling of the B.W Leave Extract
Organic crude extracts were analyzed by using GC-MS technology with the help of previously described protocol by Naz.*et al.,* (2020). The composition percentage of the constituents in the crude extract was expressed using the percentage by peak area. Chemical components in various crude extracts were identified and characterized based on the length of the GC retention period. The mass spectra and standards from mass spectrum libraries were computer matched.

To analyze mass spectrum GC-MC data, the National Institute of Standards and Technology (NIST) database was utilized. Along with comparing retention times, the spectrums of the unknown and known components that were kept in the NIST library were compared. It was determined what each component of the test material was elucidated, its molecular weight, and its structure (Naz *et al.,* 2020).

In vitro pharmacological activities
Antioxidant assay
Assay for scavenging free radicals using DPPH
This test is a simple, fast and delicate approach to analyze antioxidant activity of a particular compound or plant extract. The DPPH radical scavenging activity was used to assess the antioxidant property of *BW* extract as previously described by Gulcin *et al.,* (2023). The antioxidant activity was compared there upon with natural antioxidant, vitamin C (Ascorbic acid). DPPH showed a dose dependent response against the concentration of *B.W* extract. The free radical scavenging activity of every sample was expressed in the form of IC50 and was computed from the graph when plotting inhibition (%) against extract concentration. DPPH assay was performed after some modifications to the previously developed protocol (Gulcin *et al.,* 2023*;* Islam *et al.,* 2023) mL of 0.1 mM 2,2-diphenyl-1-picryl hydrazyl solution was mixed with 1.5 mlL of different serial diluted concentrations (0.01, 0.1,1, 100, 500,1000 µg/mL) of *BW* leave extract. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. A UV Spectrophotometer (Shimadzu) was used to detect the absorbance at 517 nm, which indicated the decrease of the free radical. Solution only have DPPH with methanol was consider as control and the solution with ascorbic acid was considered as positive controls.

DPPH free radical percentage scavenging activity was measured by the formula (Islam *et al.,* 2023).

Table 1: Therapeutic efficacy of different part of BW plant in various extract

B.W Extract	Model	Pharmacological activities	Therapeutic effect	References
Leaf water extract	nickel oxide nanoparticles	*Anti-bacterial and antioxidant activity*	Provides anti-bacterial activity against gram positive and negative bacteria as well as antioxidant against activity against ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)	(Din *et al.*, 2022)
Leaf water extract	Tin Dioxide Nanoparticle	Photocatalytic Activity	Provides Photocatalytic Activity	(Haq *et al*., 2022)
Leaf water extract	Tin Dioxide Nanoparticle	Photocatalytic Activity	Provides highest photocatalytic activity.	(Ehsan *et al.*, 2022)
Bark methanolic extract.	Wistar albino rat	*Wound healing and Antioxidant activities*	Provides great free radical scavenging activity and Healing progresses	(Khan *et al.*, 2010)
Wood various extract	Cup plate method	Anti-microbial activity	Provides Antimicrobial activity *against Klebsiella, bacillus* and *proteus* species	(Nandeesh *et al.*, 2017)
Wood various extracts	Invitro study	Antioxidant activities	Provides great free radical scavenging activity	(Nandeesh *et al.*, 2017)
Wood crude extract	*in vitro*	Gastrointestinal, Respiratory and Vascular disorders	Provides efficacy in constipation, bronchitis, asthma, and hypertension	*(*Hussain. *et al.*, 2015).
Wood various extract	Rat model	Hair growth activity	Rapidly increased hair growth	(Nandeesh *et al*,.2009)
Wood various extract	Rat model	Inflammation-reducing activity	Provides anti-inflammatory activity	(Nandeesh *et al.*, 2008)

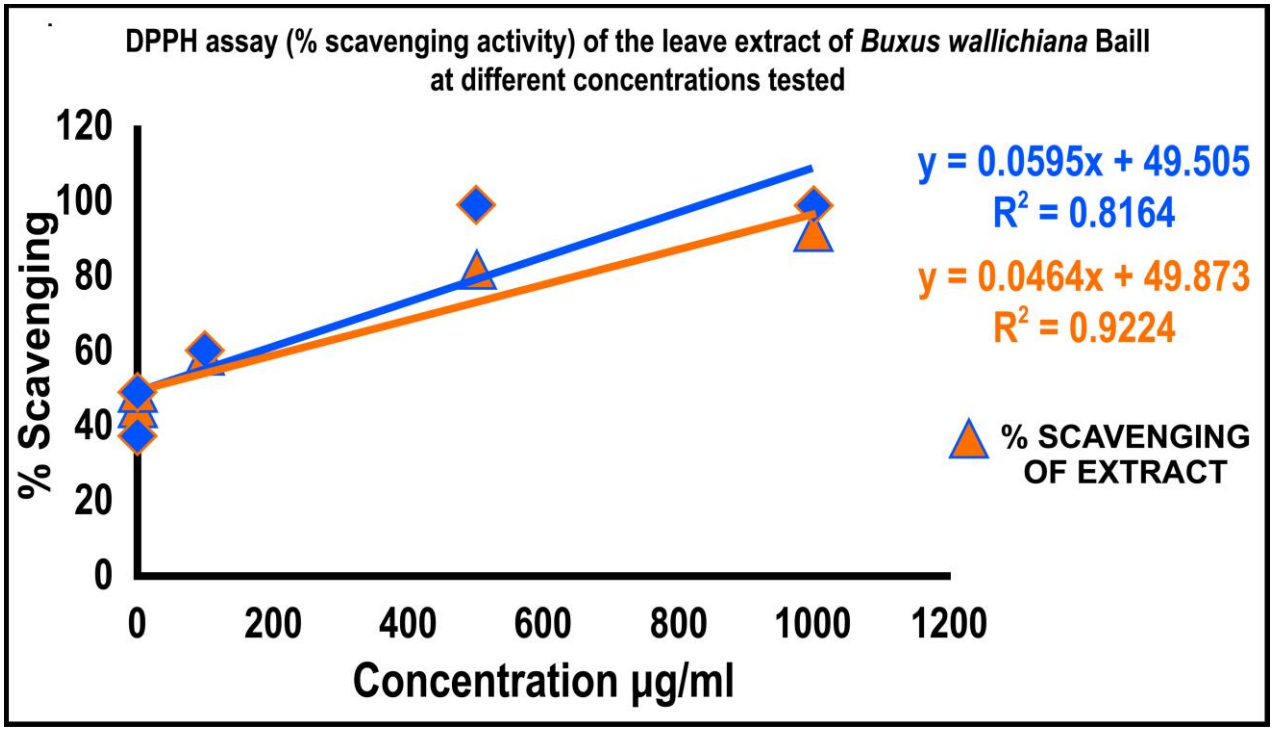

Fig. 1: Linear graph representation of % scavenging activity of the leave extract of *BW* at Different concentrations

The yellow and blue lines are indicating the *BW* and Ascorbic acid comparison according to linear regression equation applied mentioned that as shown in table 4

% Scavenging = [(Absc – Abst) / Absc] × 100
Where,
$Abs_{C=}$ Absorbance of ascorbic acid
Abs_T= Absorbance of Extract (Reaction Mixture)
All tests were repeated two time, and the average value was calculated.

Estimation of IC50 value
Inhibitory concentration was calculated via linear regression equation for the analysis of results from DPPH assay. The discoloration of sample was compared against ascorbic acid concentration to calculate the value of IC_{50}. The concentrations of samples account for 50% of reduction of the preliminary activity of DPPH (Martinez-Morales *et al.,* 2020).

In vitro cytotoxic activity on glioblastoma cell line
Glioblastoma cell line (U 87 MG) was procured from American Type Culture Collection (ATCC). Glioblastomas are one of the malignant tumors. U 87 was first originated from malignant glioma from a female patient (Bilal *et al.,* 2021). For analyzing the toxic effects of extract, normal cell line 3T3 cells (Primary mouse embryonic fibroblast cells) were used in this experiment.

Cell culture
L-glutamine, amphotericin B, penicillin, streptomycin, 10% fetal bovine serum, 1% sodium pyruvate, and Dulbecco's Modified Eagle's medium (DMEM, GIBCO) were the growth media used for the cell line. Growing at 37°C and 5% CO_2, the cell line was maintained in a humidified environment (Bilal *et al.,* 2021).

Cell viability and morphology
Cells were checked for their normal growth, viability and morphology under phase contrast microscope on regular basis. The media was changed every alternate day. The cells were trypsinized when 90% confluency was achieved. Cell viability was analyzed by the Trypan Blue extraction method.

Percentage cell viability was determined by the following formula (Larsson *et al.,* 2020)
Percentage viable cells = [1.00 – (Number of blue cells ÷ Number of total cells)]*100

Anti-proliferative (MTT) assay
Cellular metabolic activity is measured by the MTT 3-(4, 5-dimethyl thiazol-2-yl)-2, 5- diphenyl tetrazolium bromide assay, which reveals cell viability, proliferation, and cytotoxicity. The colorimetric assay's basic idea is that living cells can change a yellow tetrazolium salt into purple formazan crystals. Once the cells reached 90% confluency, they were trypsinized. In 96-well plates, cells were seeded at a density of 1 × 105 cells/mL. Different concentrations of the supplied extract (25, 50, 75, 100, and 125µg/mL) were used for treatment over the course of 24 and 48 h. After adding 10µL of MTT dye (final concentration 0.5 mg/mL) to each well, the cells were incubated for 3h at 37ºC. After dissolving the MTT-formazan product in DMSO, the absorbance at 570 nm was determined using spectrophotometer (Shimadzu). The assay was repeated in triplicates for confirmation of results.

The percentage inhibition and IC_{50} was calculated by the following formulas (Forouhandeh *et al.,* 2023)
Percentage cell inhibition: 100 – {(Atest – Ablank) /Acontrol – Ablank)} *100
Where,
$A._{test}$. comprising of cell media plus drug
$A._{blank}$. containing only media plus drug.
$A._{control.}$ Containing cell media plus vehicle

IC50 = (X2 – X1) x(50 –Y1) / (Y2- Y1) + X1
Where,
X_1: {Higher concentration is used}
X_2: {Lower concentration is used}
Y_1: {Mean percentage of viable cells at the higher concentration (X_1) used}
Y_2: Mean percentage of viable cells at the lower concentration (X_2)

STATISTICAL ANALYSIS

Mean ± SD was used to express the data. SPSS version 22 was used to analyze the data. One-way Anova is used for multiple comparison post hoc statistical computations, the Bonferroni test was used. Any p-value below 0.05 was regarded as significant. Moreover, IC_{50} values were determined using linear regression analysis.

RESULTS

Phytochemical compounds identified in the ethanolic extracts of Buxus wallichiana Baill leave
Determination of phytochemicals
Plant extracts are intricate concoctions of chemicals that are known to be crucial to several biological processes. All the leaf extract in this study contained coumarin, alkaloids, glycosides, terpenoids, flavonoids, phenols and tannins, according to a qualitative examination of the phytochemicals of *BW* (table 2).

Table 2: Presence of phytochemicals groups in ethanolic extract of *BW* leave

S. No.	Class of metabolites	Results
1	Flavonoids	+ve
2	Alkaloids	+ve
3	Phenols	+ve
4	Glycoside	+ve
5	Coumarin	+ve
6	Tannins	+ve
7	Terpenoids	+ve

GC-MS phytoconstituent analysis
Based on their phytochemical composition, medicinal plants can be used to cure a wide range of human ailments.

Table 3: GC-MS data of ethanolic extract of *BW* leave extract, provided with retention time (min) and %age of the detected compound

S. No	Retention Time (Min)	% Age of Total	Compound Name	Molecular Formula
1	9.012	0.84	N-carbobenzyloxy-I-tyrosyl-I-.Ivaline	$C_{22}H_{26}N_2O_6$.
2	16.431	1.1	2-Furanmethanol, 5 -ethenyltetrahydro- α, α,5-trimethyl-, cis-	$C_{10}H_{18}O_2$
3	20.914	13.73	2-Cyclohexen.-1-one, 4-(3-hydroxy-1butenyl)-3,5,5-trimethyl-	$C_{13}H_{20}O_2$.
4	28.516	9.14	9,12-Octadecadienoyl. chloride ,(Z, Z)-	$C_{18}H_{31}ClO$
5	61.412	17.29	2-[4-methyl.-6-2(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en.-1-Carboxaldehyde	$C_{23}H_{32}O$.
6	65.899	100	Lupeol	$C_{30}H_{50}O$
7	71.737	71.48	Betulin	$C_{30}H_{50}O_2$

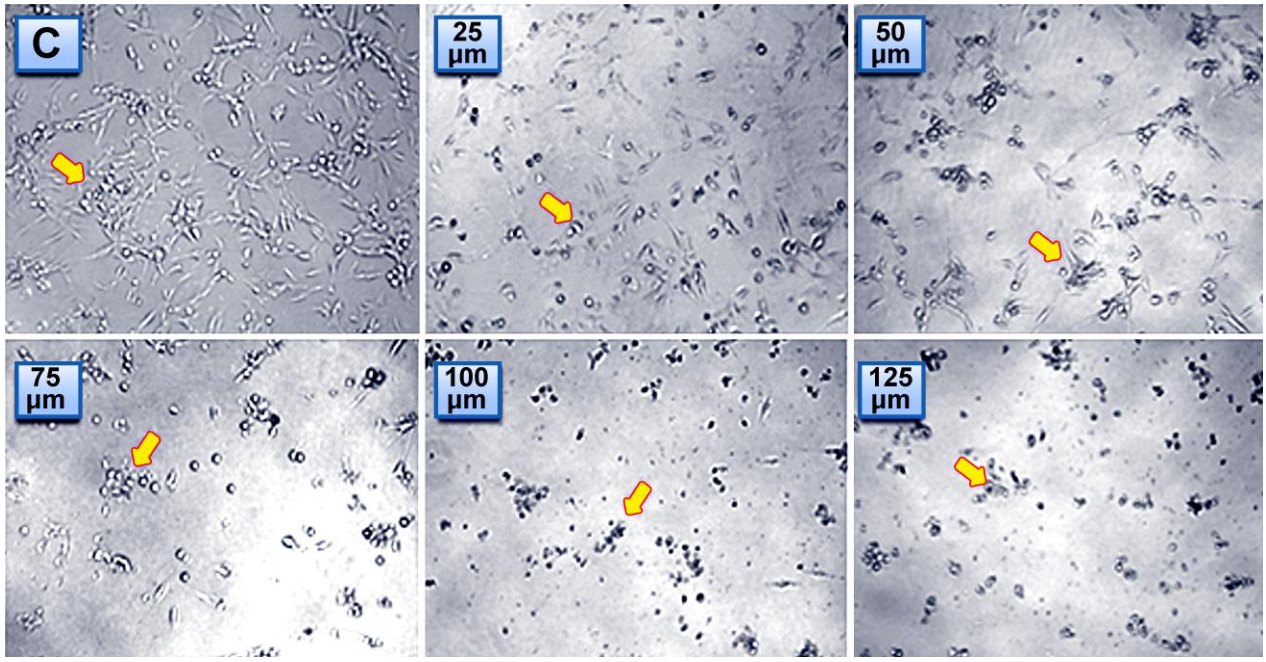

Fig. 2: Inhibitory effect of *Buxus wallichiana Baill* leave extract on glioblastoma (U 87 cell line)

In this fig. arrows indicating disrupt morphology of U87 glioblastoma cells and highlight the dead cells at different concentrations

Significant bioactive components with varying retention durations were found in the ethanolic extract of BW by gas chromatography-mass spectrometry (GC-MS) (table 3). Within the ethanolic extracts, the following bioactive substances were discovered: Lupeol and Betulin2-furanmethanol 5-ethenyltetrahydro-α, α,5-trimethyl-cis-, 4-(3-hydroxy- 1butenyl) and 2-Cyclohexen-1-one3.4,5-trimethyl-, 9.12-Octadecadienoyl chloride, (Z, Z)- and 2-[4-methyl-6-2(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]Phenolhex-1-en-1- carboxaldehyde.

Assessment of Antioxidant activity

DPPH free radical scavenging assay

This assay determines the presence of antioxidants in a plant extract by decolorization of DPPH methanol solution. The dose reaction curve of DPPH radical scavenging activity of crude extract of *BW* leaves was analyzed, comparing with standard ascorbic acid (table 4 and fig. 1). The IC_{50} value was 2.7 3µg/mL and 8.3 µg/mL of leave extract and ascorbic acid respectively.

Anti-proliferative activity of Buxus wallichiana Baill leaf extract on glioblastoma (U 87 cell)

ANOVA (One Way) and Bonferoni's Post hoc test were used to assess the anti-proliferative activity data. ($F=535.19$; $P<0.001$) for various comparisons. The anti-proliferative activity of leave extract was screened on human glioblastoma cell line U 87. *BW* leave extract did not show 50 percent inhibition within 24 h. The results were significant when cells were treated for 48 h. The percentage growth inhibition of extract and standard Temozolomide are shown in (table 5 and fig. 2). All concentrations of ethanol extract used in the assay have shown significant anti-proliferative activity when

compared with control (***P<0.05). However, 75µg/mlL 100µg/mL and 125µg/mL concentrations have also demonstrated significant results compared with standard (P<0.05). The IC50 value of extract was 19.3ug/mL. The toxic effects of BW leave extract were also analyzed on normal cell line 3T3. There was no significant cytotoxic effect produced by extract as compared to Temozolomide which has shown toxic effects to normal cells.

Table 4: DPPH assay (% scavenging activity) of the leaf extract of *Buxus wallichiana Baill* at different concentrations tested

Concentration (µg/mlL)	% scavenging (*B.W* leave)	% scavenging (Ascorbic acid)
1000	91.54	98.79
500	81.54	98.75
100	59.29	60
1	49.25	49.25
0.1	47.03	48.49
0.01	44.85	37

BW has a dose-dependent effect on U 87 cell migration inhibition. Equally wide lines were etched into comparable cell dishes, which were subsequently subjected to varying *BW* concentrations for 48 hours before being clarified with hematoxylin/eosin staining.

DISCUSSION

A priceless gift from nature to humanity are plants. They can manufacture a wide range of secondary metabolites and are the source of a diversity of phytochemicals. The idea that a given plant's combination of secondary products is taxonomically distinct is supported by the medicinal properties of some plant species or groups. It has been estimated that between 85 and 90 percent of people worldwide use traditional herbal remedies. Studies on the phytochemical components of medicinal plants and their pharmacological effects have drawn a lot of attention in recent years (Diab *et al.,* 2021). In the ethanol extract of *BW*, our recent investigation revealed the existence of medicinal phytochemical elements such as alkaloids, flavonoids, phenols, tannins, terpenoids, coumarin, and glycoside. We identified various phytochemical classes in the ethanolic extracts of *BW* leaves based on these findings. Additionally, we evaluated phytocompounds identification by GC-MS. Using this method, we were able to recognize several active ingredients with beneficial medicinal properties from the extracts of the investigated plant species listed in (table 3). In addition to their anticancer properties against the U 87 Malignant Glioma cell line, they also exhibited significant antioxidant activity.

An antioxidant property of plants can intervene with the oxidation process by reacting with free radicals, chelating and reactant metals and as cancer-preventing agents, and act as oxygen scavengers (Kumar *et al.,* 2022). Reactive oxygen species (H_2O_2) and (HOCl) and free radicals like hydroxyl-radical (OH) and superoxide anion (O^{-2}) are generated as regular molecules of various metabolic pathways. The current study revealed antioxidant activities of *BW* leave extract, which is a novel finding so far according to the literature search. The IC_{50} values of ethanol extract of *BW* leaves for hydroxyl radical scavenging activity were found to be 2.73 µg/mL, while ascorbic acid showed antioxidant activity at dose of 8.3 µg/mL. From this outcome, the ethanolic extract of *BW* leave was found to possess strong antioxidant activity.

When compared with standard. It has been found that hydroxyl radical is produced in large amounts during lipid peroxidation from lipid hydro peroxides in the cell layer. The disturbance in the rate of production of these free radicals and body's response to trap them has justified the use of natural substances or drugs possessing antioxidant potential (Diab *et al.,* 2021). Free radical percentage scavenging activity of the *BW* leave extract and ascorbic acid was 91% and 98% respectively. Moreover, antioxidant effect of *BW* plant is due to the presence of numerous phytochemicals and chemically active compounds mentioned in (table 2 and 3).

Furthermore, previous studies revealed phytoconstituents effectiveness to produce free radical scavenging activity (Diab *et al.,* 2021; Lahare *et al.,* 2021). Therefore, the results of our study are in accordance with previous studies (Diab *et al.,* 2021; Roy *et al.,* 2023) and it was proposed that *BW* leaves extract could follow the same pathway as that of ascorbic acid (Tyagi *et al.,* 2021).

Buxus leaves possess high anti–oxidant activity due to the presence of aforementioned phytochemicals chemicals which increase its cytotoxic effects as well (Din *et al.,* 2022). The anti- proliferative action of leave extract was analyzed by using MTT (3-(4, 5-dimethylthiazol-2- yl) - 2, 5-diphenyltetrazolium bromide) assay (Promega, USA). U 87 Malignant Glioma cell line was treated with various concentrations of the leave extract. The leave extract demonstrated significant inhibition after 48 h. It has also shown promising results in comparison to standard drug temozolomide (Mutlu *et al.,* 2021). The results show the same pattern of toxicity as shown by our plant extract. It is indicated by the American National Cancer Institute (NCI), the minimum dose for cytotoxic activity of crude extract is less than 30µg/mL (Canga *et al.,* 2022). The IC_{50} value of *BW* leave extract obtained was 19.3µg/mL, which falls in the standard range of NCI anticancer models. *BW* leaves extract is considered to have promising anticancer potential due to presence of potent compounds such as 2-cyclohexen-1-one, 4-(3-hydroxy-1butenyl)-3,5,5-trimethyl-, 2-furanmethanol 5-ethenyltetrahydro-- α, α,5-trimethyl- cis-. These

Table 5: Anti-proliferative activity of *Buxus wallichiana Baill leave* extract on glioblastoma (U 87 cell)

Growth Inhibition (Mean ±S.E.M) % Inhibition (48 h)							
Concentration groups µg/ml	Control.	25 (µg/mL)	50 (µg/mL).	75 (µg/mL).	100 (µg/mL)	125 (µg/m)	Standard (Temozolomide)
% Inhibition	13.958±1.37	62.243±0.79 ***	63.632±0.54 ***	75.061±3.96 ***,###	85.464±0.79 ***,###	89.334±1.34 ***,###	58.598±1.91

This table shows the percentage inhibition in each concentration. Data are represented as Mean±SEM by using Oneway Anova statistical software (df = 12, 100; n = 7). P value was taken as significant when **P*<0.05, ***P*<0.01 and ****P*<0.001 compared to control group while #*P*< 0.05 compared with standard (Temozolamide)

compounds have high anticancer activity as shown in previous studies (Shin *et al.,* 2020). However, conventional chemotherapeutic drugs used for the treatment of various cancers are associated with potential toxic effects. Alkylating agents like temozolomide act by cleaving DNA cross-linkages, in this way repressing DNA and cell replication. Temozolomide is nonspecific in action, which relates to its serious adverse effects. It has demonstrated significant toxic effects on normal cell line 3T3 (Singh *et al.,* 2023) Therefore, *BW* leaves extract could have minimum adverse effects as it has not shown toxic effects on normal cell line.

CONCLUSION

Our study demonstrated the antioxidant, as well as anticancer effects of *BW* in human glioblastoma U 87 MG cells due to the presence of valuable phytoconstituents such as flavonoids, and alkaloids. *BW* treatment acts by blocking the production of free radicals and induces apoptosis by killing tumor cells. However, the *BW* extract was found to have the strongest anticancer and DPPH scavenging properties. The active ingredients in the extracts, such as *buxpiine K* and *buxtauine*, are primarily responsible for their antioxidant and anticancer properties. Additionally, more research is needed to determine the examined extracts' method of action and *in vivo* anticancer effects. It can be hypothesized that *BW* could act as a possible lead molecule for the development of future anticancer medicines considering the findings.

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