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PHYTOCHEMICALS, *IN-VITRO* CYTOTOXICITY, ANTIOXIDANT, AND ANTIMICROBIAL EVALUATION OF *BARLERIA CRISTATA* L: PHILIPPINE VIOLET

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Keywords:

Barleria cristata, Phytochemical analysis, Antioxidant, Antimicrobial, Anticancer activity

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ABSTRACT: The current study aims to find out how effective different extractions of *Barleria cristata* leaves are at fighting cancer, free radicals, and bacteria. Physical and chemical qualities and preliminary phytochemical analysis will also be assessed. It also quantifies several active ingredients by total phenolic and flavonoid. Five leaf extracts of *Barleria cristata* were made with ethyl acetate, chloroform, acetone, ethanol, and water. They were then tested for their antioxidant potential using reducing power and free radical scavenging methods, such as DPPH and NO radicals. They were also tested for antibacterial activity against Gram-positive and Gram-negative bacteria. The MTT assay measured how successfully the medication killed MCF-7 breast cancer cell line and Hep-G2 hepatoma cancer cell line. We calculated IC_{50}. Initial phytochemical analysis was performed on all extracts. The total extract was abundant in protein, carbs, tannins, flavonoids, and phenolics. Acetone extract had higher phenolic and flavonoid components than ethyl acetate, chloroform, ethanol, and water. *Barleria cristata* acetone extract was antioxidant rich. Plant extracts also had antibacterial properties. Extracts inhibited MCF-7 breast cancer cell line and Hep-G2 hepatoma cancer cell line *in-vitro*. This study showed that *Barleria cristata* leaves can prevent cancer cell proliferation. It also revealed that leaf phytochemicals combat free radicals and bacteria well. These compounds could be used to produce novel antimicrobials or cancer medicines.

INTRODUCTION: *Barleria cristata*, often called Philippine violet, is a compact, erect, hairy-stemmed, evergreen subshrub in the acanthus family. It usually grows to a height of 3-4 feet and spreads 2-3 feet wide. It is a shrub distributed in subtropical Himalaya, Sikkim, Central and South India, and Khasi hills at a height of 1.350 m. This shrub may be grown in Florida, Southern Texas, Louisiana, Arizona, and California in the US.

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It is regarded as a potential invasive plant species in waste areas and along the side of the road [1, 2, 3]. Several studies of plants have indicated that they contain active metabolites such as alkaloids, flavonoids, tannins, and phenolic compounds, which show good antimicrobial activity [4, 5, 6].

It has been used as a traditional herbal medicine for treating various disorders, including anaemia, toothache, cough, fever, asthma, bronchitis, and diabetes [7]. Leaves were used to reduce swelling and inflammation. Leaves and root juice are used to treat colds and coughs [8]. Whole plant is boiled in water for treatment of cold and flu [9]. Pharmacologically, the plant is reported to possess hepatoprotective [10], antibacterial [11, 12], antifungal [13],

antidiabetic, hypolipidemic [14], anti-inflammatory [15], and antioxidant activities [16], Cardio-protective [17]. Medicinal plants represent a significant source of medicinal properties and natural phytochemicals. Several ailments, such as cancer, diabetes, chronic inflammatory disorders, and tumorigenesis disorders, can be prevented or treated with the help of these beneficial properties [18]. Indigenous pharmaceuticals and the application of therapeutic plant-based medicine for the treatment of diverse ailments have been utilized since antiquity and will persist in providing humanity with novel cures [19].

Cancer constitutes the most extensive category of diseases, resulting in the mortality of 9.6 million individual's globally [20]. The predominant kinds of cancer in females include breast, cervical, colorectal, lung, and thyroid cancer. Cervical cancer ranks as the fourth highest cause of mortality among women, resulting in 270,000 deaths each year [21]. 74 percent of anticancer medications are derived from diverse plant species [22]. Hepatocellular carcinoma, or liver cancer, is the most prevalent kind of cancer, constituting nearly 90% of cases [23]. It results in elevated annual mortality rates, particularly in Southeast Asia, a region with a significant prevalence of hepatitis [24]. Among current therapeutic approaches are chemotherapy, radiotherapy, and Pharma-cologically synthesized agents. Nonetheless, interventions such as chemotherapy may induce numerous side effects and negatively impact the overall well-being of patients [25]. Statistics indicate that more than 60% of the global population, and approximately 80% in developing nations, rely on traditional and medicinal plants for their therapeutic needs [26]. So, the goal of this study is to look into the anticancer, antioxidant, antimicrobial, and phytochemical properties of *Barleria cristata* leaves in different solvents.

MATERIAL AND METHODS:

Chemicals and Solvents: The study utilized high-quality solvents and chemicals sourced from recognized organizations, including Sigma-Aldrich (USA), Merck (Germany), E-Merck, and other established producers.

Collection and Identification of Plants: The plant sample was acquired from an herbal trader in Nagpur and authenticated by the taxonomist head of the Department of Botany, RTMNU, Nagpur. A voucher specimen was given the number 10423. The plant leaves were cleaned with water, dried in the shade, and then finely pulverized using a mixer grinder for subsequent analysis.

Preparation of Extract: We defatted the dried leaf powder using petroleum ether as the solvent and a Soxhlet apparatus to remove fat. Then it was extracted using ethyl acetate, chloroform, acetone, ethanol, and water. The extract was subjected to solvent evaporation using a rotary evaporator and then dried. The extract was stored in a desiccator for subsequent analysis.

Phytochemical Standardization:

Qualitative Phytochemical Analysis: Using official methods, the chemical makeup of extracts of EABC, CBC, ABC, EBC, and WBC was first looked at to see what chemicals they contained [27, 28].

Quantitative Phytochemical Analysis:

Total Phenolic Content (TPC): We measured the total amount of phenolic compounds in all the extracts using the Folin-Ciocalteu method, with Gallic acid as the standard, following the steps Hagerman described in 1998 [29]. The result is expressed as g Gallic acid equivalent (GAE)/milligram plant extract.

Total Flavonoid Content: The total flavonoid content was found using rutin as a standard, following Tambe's method from 2014 [30]. The values were given as micrograms of rutin equivalent (RE) per milligram of plant extract.

Antioxidant Assay: Because plant extracts are complex and have several defensive mechanisms, it is not feasible to evaluate antioxidant activity using just one approach [31]. Consequently, antioxidant activity was evaluated employing several methods. All readings were conducted in triplicate, and the inhibition percentage was determined as the IC_{50} value.

DPPH Radical Scavenging Activity: The antioxidant efficacy of the plant extract was assessed based on its capacity to neutralize the stable DPPH free radical [32]. Ascorbic acid functioned as the reference compound in the DPPH assay, with absorbance recorded at 517 nm.

Nitric Oxide Scavenging Activity: The Griess-Illosvoy reaction is typically employed to identify nitrile ions generated by sodium nitroprusside in an aqueous solution at physiological pH through its interaction with nitric oxide and oxygen. An absorbance measurement at 550 nm was performed with a UV spectrophotometer to compare the test solution with the blank solution. The 50% inhibition concentration was found using a linear regression curve and graphical analysis [33].

Assay of Reducing Power: The potassium ferrocyanide method was employed to assess the reducing power of the plant extract. Absorbance was recorded at 700 nm, with the highest absorbance signifying a strong reducing capacity [34].

Antimicrobial Evaluation: We got four types of bacteria from the Rajiv Gandhi Biotechnology Centre at LIT, Nagpur, to test their antibacterial activity. These were pure cultures of *Klebsiella pneumonia, Bacillus subtilis, Escherichia coli*, and *Staphylococcus aureus*. The Indian Pharmacopoeia's agar well diffusion test [35] will be used to see how well the medicine kills certain strains of *Klebsiella pneumonia, Bacillus subtilis, Escherichia coli*, and *Staphylococcus aureus*. Mullier-Hinton plates from Hi-Media, Mumbai, were used for the test. Each plate had 5 mm wells filled with 0.1 ml of plant extract at a concentration of 100 mg/ml in dimethylformamide (DMF). The extract permeated the medium for a duration of one hour at the ambient temperature. The bacteria were cultured at 37 degrees Celsius for 24 hours. Growth inhibition zones surrounding the disc were measured in mm to determine the results. The standard utilized was gentamycin sulphate. The presence of distinct zones of inhibition around the discs indicates antibacterial activity. The inhibitory zone (mm) was measured and represented as the mean ± standard error of three measurements after 24 hours of incubation.

Cytotoxicity Studies:

MTT Assay: The cytotoxicity of the supplied sample on the MCF-7 and Hep-G2 cell lines (obtained from NCCS Pune) was assessed using the MTT assay. Ten thousand cells per well were cultured in a 96-well plate and incubated for 24 hours in DMEM medium (Dulbecco's Modified Eagle Medium - AT149 - 1L - HIMEDIA) supplemented with 10% FBS (Fatal Bovine Serum-HHIMEDIA-RM 10432) and 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781) at 37°C with 5% CO2 in an air-jacketed CO2 incubator (Heal Force-HF90).

On the subsequent day, cells were treated with varying amounts of the formulations, as specified in the Excel sheet. A stock solution of materials was produced in DMSO and subsequently diluted to obtain various quantities in incomplete cell culture medium (without FBS). Untreated cells were designated as controls, while cells without MTT were classified as blank.

Upon completion of the experiments, the culture supernatant was discarded, and the cell layer matrix was solubilized in 100 µL of Dimethyl Sulfoxide (DMSO) and analyzed using an ELISA plate reader (IMark, Biorad, USA) at 540 nm. The IC_{50} was determined. Results were expressed as mean ± SEM.

$$\% \text{ Viable cell} = (A_{test} / A_{control}) \times 100$$

(A_{test} = Absorbance of test sample)

($A_{control}$ = Absorbance of control)

RESULT AND DISCUSSION:

Physicochemical Analysis and Extractive Value: **Table 1** shows *Barleria cristata* leaf extractive values. The outcomes of the following tests: water-soluble, alcohol-soluble, acid-soluble, and insoluble ash; extractive value; moisture content; and sulphated ash of leaves of *Barleria cristata* are given in **Table 2.**

TABLE 1: EXTRACTIVE VALUES OF *BARLERIA CRISTATA* LEAF EXTRACTS

Sr. no.	Extract	Sample Name	Solvent	% yield
1	*Barleria cristata* leaf Ethyl acetate extract	BCEA	Ethyl acetate	3.9
2	*Barleria cristata* leaf chloroform extract	BCC	Chloroform	3.2
3	*Barleria cristata* leaf acetone extract	BCA	Acetone	2.4
4	*Barleria cristata* leaf ethanol extract	BCE	Ethanol	4.2
5	*Barleria cristata* leaf water extract	BCW	Water	5.8

TABLE 2: PHYSICOCHEMICAL ANALYSIS OF *BARLERIA CRISTATA* LEAVES

Sr. no.	Test	Wt. of sample taken (g)	% value
1	Total Ash content	2.0	7.8±0.578
2	Acid insoluble ash content	2.0	0.85±0.624
3	Water soluble ash content	2.0	5.6±0.321
4	Water soluble extractive value	5.0	32.8±0.678
5	Alcohol soluble extractive value	5.0	1.5±0.456
6	Moisture content	5.0	8.3±0.308
7	Sulphated ash content	1.0	10.05±0.236

Values are expressed in Mean ±SEM of 3 parallel measurement

Qualitative and Quantitative Physicochemical Analysis: The phytochemical composition of the five plant extracts is presented in **Table 3** and **4**. The qualitative analysis indicated the presence of alkaloids, carbohydrates, tannins, saponins, steroids, flavonoids, and phenolic compounds. Observations indicated that the levels of phenolic compounds and flavonoids were elevated in acetone extract as compared to ethyl acetate, chloroform, ethanol, and water extract. The TPC content in the acetone extract was found to be 99.76±0.318 mg/g, equivalent to Gallic acid. The ethyl acetate extract of *Barleria cristata* had a phenolic content of 58.81±0.247 mg/g GAE. The chloroform extract had 39.17±0.368 mg/g GAE, the ethanol extract had 68.63±0.486 mg/g GAE, and the water extract of *Barleria cristata* had 45.64±0.387 mg/g GAE. An ethyl acetate extract of *Barleria cristata* was found to contain 53.74±0.399 mg of rutin per gram of dry weight. The chloroform extract was determined to have a concentration of 44.42±0.724 mg/g RE, the ethanol extract had a concentration of 67.04±0.515 mg/g of RE, and the acetone extract had a concentration of 92.85±0.483 mg/g RE, and the water extract was 25.77±0.548 mg/g RE.

TABLE 3: PRELIMINARY SCREENING OF PHYTOCHEMICALS OF THE INVESTIGATED EXTRACTS *BARLERIA CRISTATA* LEAVES

Sr. no.	Phytochemicals	Method	BCEA	BCC	BCA	BCE	BCW
1	Carbohydrate	Molisch's test	+	-	-	+	+
		Fehling test	+	-	-	+	+
		Benedict's test	+	-	-	+	+
2	Protein	Biuret test,	+	-	+	+	+
		Xanthoproteic test, Millon's	+	-	+	+	+
		test	+	-	+	+	+
3	Amino acid	Ninhydrin test	-	-	+	+	+
4	Saponin	Foam test	-	-	-	+	+
5	Tannin and phenolic compound	Ferric chloride test	+	+	+	+	+
6	Steroid	Salkowski test	-	-	-	+	+
7	Alkaloid	Dragendorff's test	-	-	+	+	+
		Mayer's test.	-	-	+	+	+
		Wagner's test	-	-	+	+	+
		Mayer's test	-	-	+	+	+
8	Glycoside	Keller Killiani test.	+	+	+	+	+
9	Flavonoid	Alkaline test	+	+	+	+	+

+ = Present, - = Absent

TABLE 4: TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT OF *BARLERIA CRISTATA* LEAF EXTRACT IN VARIOUS SOLVENTS

Sr. no.	Phytoconstituents	Ethyl acetate extract	Chloroform extract	Acetone extract	Ethanol Extract	Water extract
1	Total Phenolic content (mg/g Gallic acid equivalent)	58.81±0.247	39.17±0.368	99.76±0.318	68.63±0.486	45.64±0.387
2	Total Flavanoid content (mg/g Rutin equivalent)	48.25±0.399	44.42±0.729	92.85±0.483	57.04±0.515	25.77±0.548

Values are mean ± SEM of 3 parallel measurements.

Antioxidant Potency: To find out how good DPPH is as an antioxidant, its free radical scavenging activity was tested. Its IC_{50} values were found to be significant when compared to ascorbic

acid 9 µg/mL. The IC_{50} values for the different extracts were as follows: ethyl acetate 110µg/mL, chloroform 113µg/mL, **ethanol 98 µg/mL**, acetone 51µg/mL, and water 180µg/mL. Nitric oxide is an unstable species that reacts with oxygen molecules to form stable nitrate, which may be measured using Griess reagent. The acetone extract of *Barleria cristata* demonstrated significant nitric oxide scavenging activity, with an IC_{50} value of 441 g/mL, when compared to standard ascorbic acid, which has an IC_{50} value of 322 g/mL. The IC_{50} values for the extracts of ethyl acetate, chloroform, ethanol, and water were 522, 862, 462, and 541, respectively. The reducing power assay involved the reduction of Fe3+ to Fe2+ and evaluated the extract's ability to reduce the compound, potentially indicating its antioxidant activity. The IC_{50} values for iron chelating activity were as follows: ethyl acetate extract 173µg/mL, chloroform extract 180µg/mL, acetone extract 160µg/mL, ethanol extract 167µg/mL, and water extract 197µg/mL. Ascorbic acid exhibited an IC_{50} value of 142 µg/mL. The results were disclosed in **Fig. 1, 2, 3**, and **Table 5.**

TABLE 5: ANTIOXIDANT ACTIVITY OF *BARLERIA CRISTATA* BY USING DIFFERENT METHODS

Sr. no.	Content	DPPH Method IC_{50} (µg/ml)	Nitric oxide scavenging method IC_{50} (µg/ml)	Reducing power assay method IC_{50} (µg/ml)
1	Ascorbic acid	9	322	142
2	Ethyl acetate	110	522	173
3	Chloroform	113	862	180
4	Acetone	51	441	160
5	Ethanol	98	462	167
5	Water	180	541	197

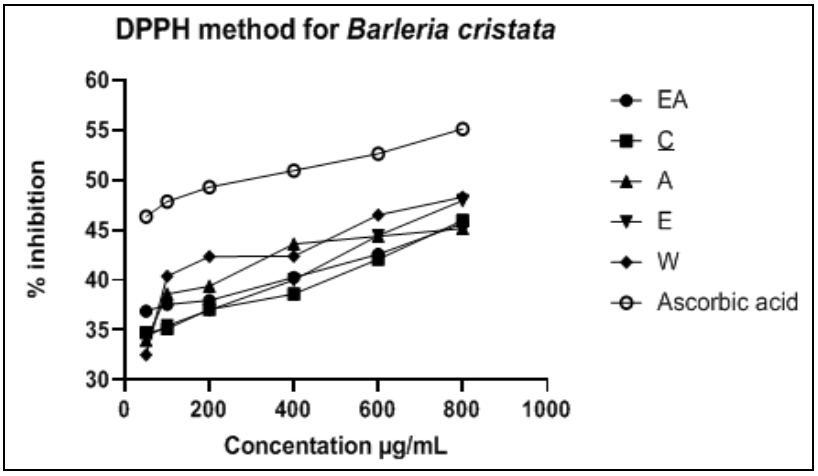


FIG. 1: DPPH ASSAY METHOD FOR *BARLERIA CRISTATA* IN ETHYL ACETATE, CHLOROFORM, ACETONE, ETHANOL, WATER EXTRACT AND ASCORBIC ACID

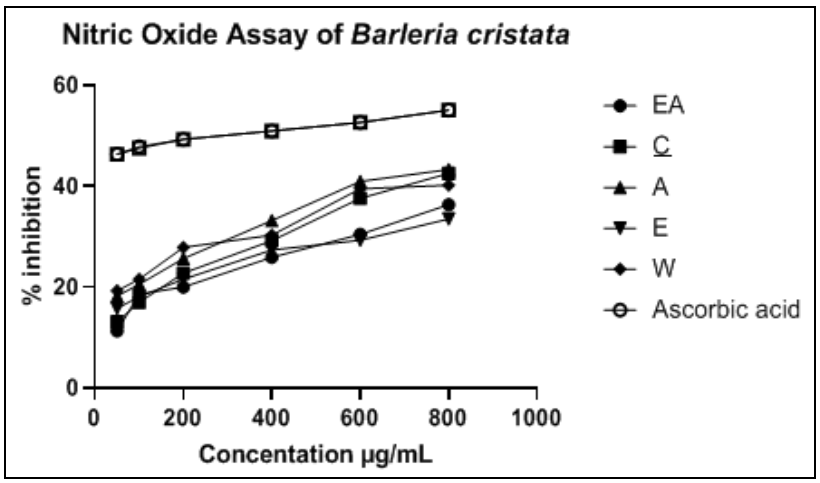


FIG. 2: NITRIC OXIDE SCAVENGING ASSAY METHOD FOR *BARLERIA CRISTATA* IN ETHYL ACETATE, CHLOROFORM, ACETONE, ETHANOL, WATER EXTRACT AND ASCORBIC ACID

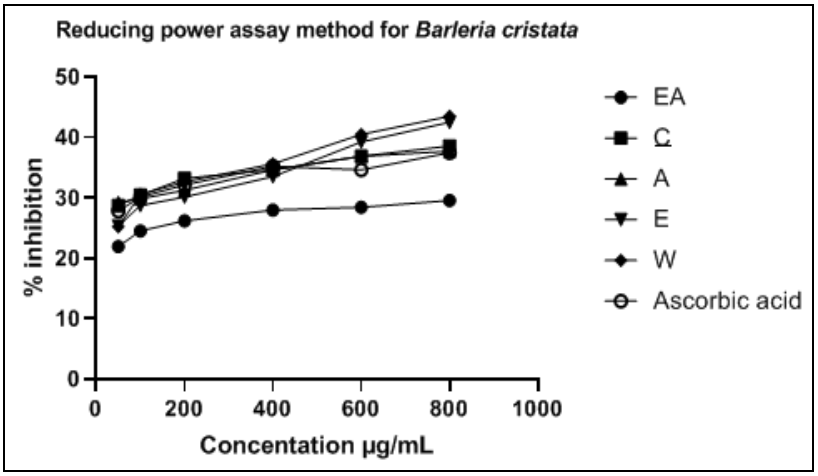


FIG. 3: REDUCING POWER ASSAY METHOD FOR *BARLERIA CRISTATA* IN ETHYL ACETATE, CHLOROFORM, ACETONE, ETHANOL, WATER EXTRACT AND ASCORBIC ACID

Antimicrobial Activity: It was tested against both Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus, Bacillussubtilis, Escherichia coli,* and *Klebsiella pneumonia*, using 100 mg/mL of *Barleria cristata* leaf extract. Ethanol extracts of plants show significant efficacy against all examined bacteria as compared to ethyl acetate, chloroform, acetone, and water. **Table 6** shows antibacterial study results.

TABLE 6: ANTIMICROBIAL ACTIVITY OF *BARLERIA CRISTATA* LEAF EXTRACT IN ETHYL ACETATE, CHLOROFORM, ACETONE, ETHANOL BY USING DISC DIFFUSION METHOD

Sr. no.	Microorganism used	Zone of inhibition in mm					
		Ethyl acetate	Chloroform	Acetone	Ethanol	Water	Gentamycin
1	*Staphylococcus aureus,*	21±0.89	23±0.24	23±0.74	24±0.26	22±0.34	25±0.24
2	*Bacillus subtilis*	19±0.56	18±0.41	18±0.66	19±0.22	18±0.41	24±0.19
3	*Escherichia coli*	18±033	16±0.24	22±0.27	23±0.59	21±0.46	25±0.28
4	*Klebsiella pneumonia*	15±0.45	14±0.67	16±0.45	17±0.23	15±0.24	26±0.46

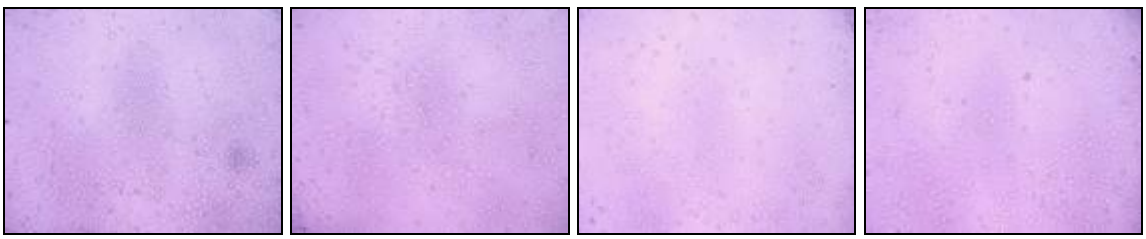
Note: All the value are mean ±SEM of three determinations.

Cytotoxicity Studies:

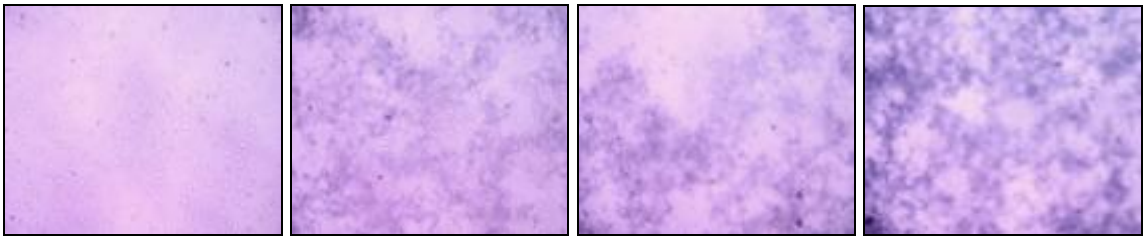
MTT Assay: It was found that the ethyl acetate extract had an IC_{50} of 86.32±0.099 µg/mL for MCF-7 and 110±0.189 µg/mL for Hep-G2. The IC 50 for the chloroform extract was 103±0.098 µg/mL for MCF-7 and 163.0±0.080 for Hep-G2. Acetone extract 66 ± 0.146 µg/mL for MCF-7 and 96.34 ± 0.168 µg/mL for HepG2. Ethanol extract 70.46±0.0985 µg/mL for MCF-7 and 131.3±0.120 µg/mL for Hep-G2. Water extract doesn't show the activity against the cell line. Result are shown in **Table 7** and **Fig. 4, 5, 6, 7.**

TABLE 7: IC_{50} VALUE OF DIFFERENT SOLVENT EXTRACT OF LEAVES OF *BARLERIA CRISTATA*

Sr. no.	Plant Extract	MCF-7	Hep-G2
		IC_{50} µg/mL	
1	Ethyl acetate extract	86.32±0.099	110±0.189
2	Chloroform extract	103±0.098	163.0±0.080
3	Acetone extract	66 ±0.146	96.34±0.168
4	Ethanol extract	70.46±0.0985	131.3±0.120
5	Water extract	Not converged	above maximum dose limit

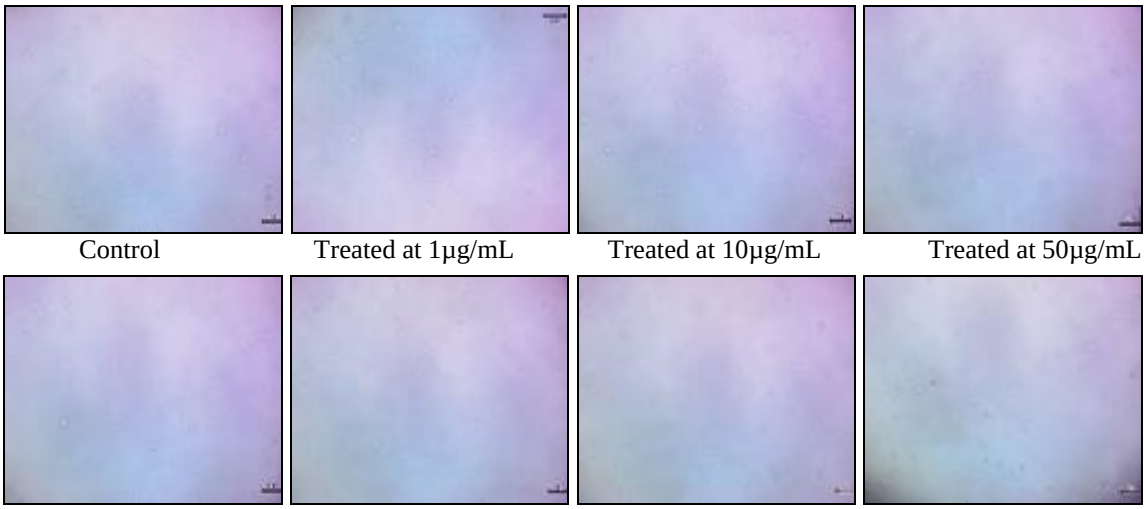


Control Treated as 1µg/mL Treated at 10µg/mL Treated at 50µg/mL



Treated at 100µg/mL Treated at 250µg/mL Treated at 500µg/mL Treated at 10001µg/Ml

FIG. 4: MICROSCOPIC IMAGE OF HEP-G2 CELL AFTER TREATED WITH DIFFERENT CONCENTRATION OF ACETONE EXTRACT OF *BARLERIA CRISTATA* LEAVES



Control Treated at 1µg/mL Treated at 10µg/mL Treated at 50µg/mL

Treated at 100µg/mL Treated at 250µg/mL Treated at 500µg/mL Treated at 1000µg/mL

FIG. 5: MICROSCOPIC IMAGE OF MCF-7 CELL AFTER TREATED WITH DIFFERENT CONCENTRATION OF ACETONE EXTRACT OF *BARLERIA CRISTATA* LEAVES

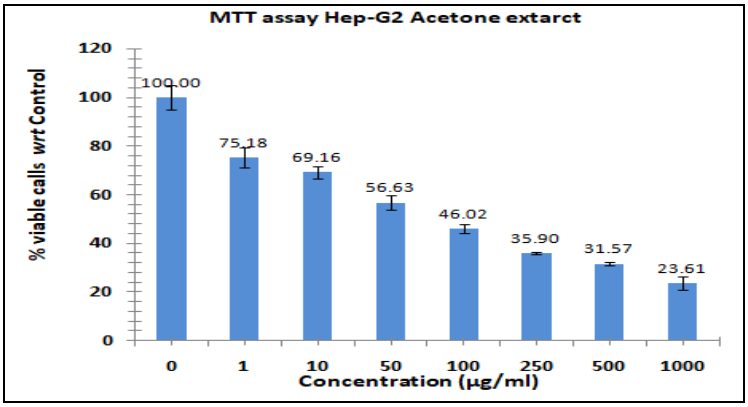


FIG. 6: PERCENT INHIBITION OF VIABLE CELL COUNT HEP-G2 CELL LINE FOR ACETONE EXTRACT OF *BARLERIA CRISTATA* BY MTT ASSAY METHOD

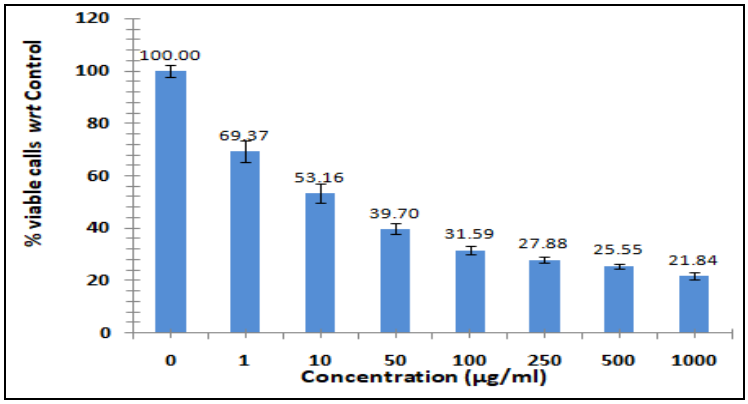


FIG. 7: PERCENT INHIBITION OF VIABLE CELL COUNT MCF-7 FOR ACETONE EXTRACT OF *BARLERIA CRISTATA* BY MTT ASSAY METHOD

DISCUSSION: Herbal plants were initially studied for their many medicinal uses, especially in ancient times. The variation in extract yields might be linked to the polarity of the solvent used, which increases the solubility of phytochemical components [36]. Physicochemical analysis helps evaluate the quality and purity of plants. Plant minerals may be quantified by two techniques: ash content analysis, which involves total, acid-insoluble, and water-soluble components, and moisture determination. The humidity of the medicinal plant is below the minimum level needed, which is 17% [37]. Plant components required moisture, but crude medications should have minimal amounts of it. Ash values reflect the drug's quality, precision, and purity, whereas extractive values reveal details on its elemental makeup [38, 39]. Plants are the important source of potentially beneficial compounds for developing new chemotherapy medications. The first step toward reaching this goal is an *in-vitro* antimicrobial test [40].

CONCLUSION: This study revealed the antioxidant and antibacterial effects of *Barleria cristata*. The study found that leaf extracts had antioxidant capabilities and included a high quantity of flavonoids and phenolic substances and alkaloids [41, 42].

The significant antioxidant activity of the ethanol extract is due to its high polyphenolic content. *Barleria cristata* possesses antimicrobial properties that effectively combat harmful microorganisms. The plant has bioactive compounds with antibacterial properties that can be used to create antimicrobial medicines for treating bacterial infections. Further research should focus on isolating, identifying, and purifying these phytoconstituents, as well as evaluating their therapeutic efficacy and toxicity, to develop novel chemotherapeutic medications.

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CONFLICTS OF INTEREST: The author has declared no conflicts of interest.

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