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Research Article

Phytochemical Screening and Anticancer Activity of *Blumea lacera*: A Promising Ayurvedic Medicinal Plant

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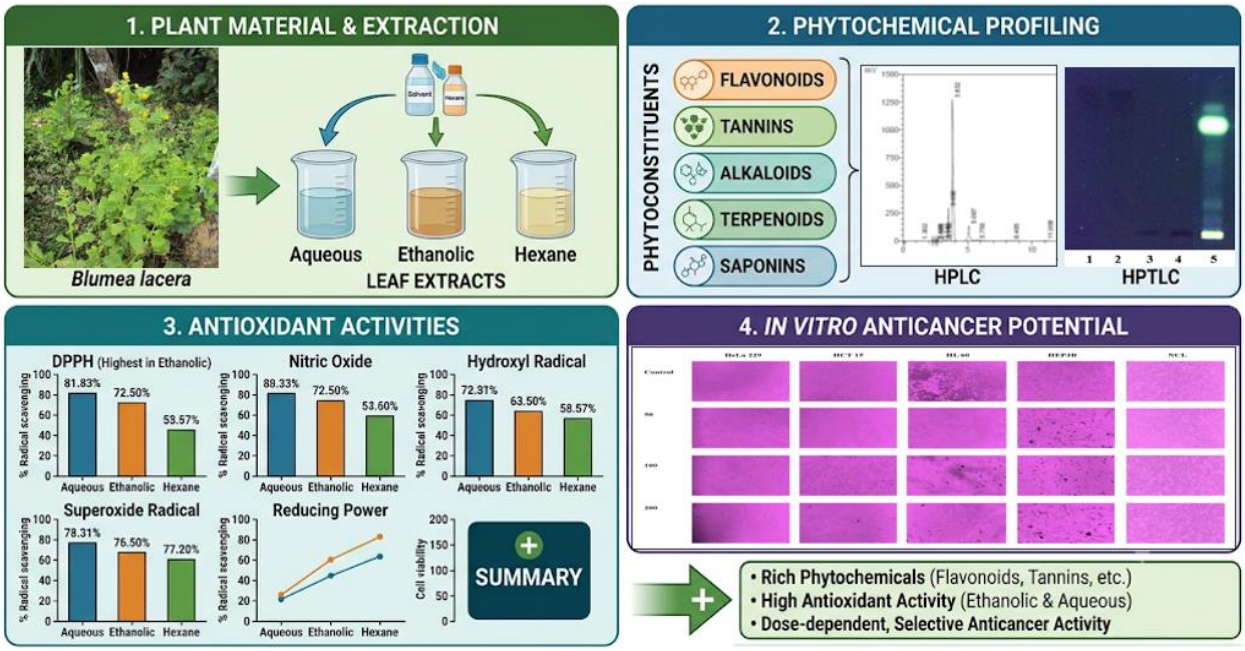
Abstract:
Blumea lacera is a medicinal plant traditionally used for various therapeutic purposes and considered a potential source of bioactive compounds. This study evaluated the phytochemical composition, antioxidant activity, and anticancer potential of aqueous, ethanolic, and hexane leaf extracts of B. lacera. Phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, terpenoids, and saponins, predominantly in the aqueous and ethanolic extracts. HPLC analysis showed a complex phytochemical profile, including major peaks corresponding to gallic acid and rutin, while HPTLC profiling further confirmed the presence of phenolic and flavonoid constituents. Antioxidant activity was assessed using DPPH, hydroxyl radical, superoxide radical, nitric oxide scavenging, and reducing power assays. The ethanolic extract exhibited the highest DPPH scavenging activity (81.83 ± 1.36%), nitric oxide scavenging activity (72.50 ± 0.72%), and reducing power (287.10 ± 1.62), whereas the aqueous extract showed superior hydroxyl and superoxide radical scavenging activities. In vitro anticancer activity, evaluated by MTT assay, demonstrated dose-dependent cytotoxicity against HeLa 229, HCT-15, HL-60, and HEP 3B cancer cell lines. Among these, HeLa 229 cells were the most sensitive to the extract, while normal Chang liver cells showed comparatively lower toxicity, indicating selective anticancer activity. The results suggest that B. lacera possesses significant antioxidant and anticancer properties, owing to its rich phytochemical composition, and may serve as a promising candidate for the development of plant- based therapeutic agents against cancer.

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Graphical Abstract



1. Introduction

Cancer is the most challenging non-communicable disorder and continues to represent a major global health burden. The disease arises from the uncontrolled growth and division of abnormal cells, which can infiltrate surrounding tissues and spread to distant organs through metastasis. Global cancer statistics indicate a persistent increase in cancer incidence, largely attributed to population aging, environmental pollution, unhealthy lifestyles, tobacco consumption, alcohol abuse, radiation exposure, and genetic predisposition [1]. Although substantial progress has been achieved in cancer diagnosis and treatment through chemotherapy, radiotherapy, targeted therapy, and immunotherapy, these therapeutic approaches are frequently associated with adverse effects, systemic toxicity, multidrug resistance, and high treatment costs [2]. Consequently, the search for safer, affordable, and more effective anticancer agents remains an important focus of current biomedical research.

Natural products derived from medicinal plants have played a significant role in disease management for centuries and continue to serve as valuable resources for drug discovery. Traditional healthcare systems such as Ayurveda, Siddha, Unani, and Traditional Chinese Medicine have extensively utilized medicinal plants for treating cancer and other chronic disorders. It is estimated that nearly 60% of currently available anticancer drugs originate from natural sources, particularly plants [3]. Notable examples include vincristine and vinblastine obtained from *Catharanthus roseus*, paclitaxel from *Taxus brevifolia*, camptothecin from *Camptotheca acuminata*, and podophyllotoxin from *Podophyllum* species [4]. The successful development of these plant-derived therapeutics highlights the immense potential of medicinal plants as sources of novel anticancer compounds [4].

Medicinal plants are rich reservoirs of biologically active secondary metabolites, including alkaloids, flavonoids, phenolic compounds, terpenoids, tannins, saponins, glycosides, and steroids, many of which exhibit diverse pharmacological activities [5]. These phytochemicals demonstrate anticancer effects via multiple mechanisms, including the activation of apoptosis, reduction of cellular proliferation, cell-cycle arrest, prevention of angiogenesis and metastasis, regulation of signaling pathways, and scavenging of ROS [6]. Oxidative stress resulting from excessive ROS generation is recognized as a major contributor to carcinogenesis because it promotes DNA damage, mutations, and cellular transformation [7]. Therefore, medicinal plants possessing strong antioxidant activity may offer considerable benefits in cancer prevention and treatment [7].

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Increasing scientific interest in plant-based therapeutics has encouraged extensive investigations using in vitro, in vivo, and molecular approaches. Numerous studies have reported significant cytotoxic and antiproliferative activities of plant extracts and isolated phytochemicals against various cancer cell lines, such as breast cancer (MCF-7), cervical cancer (HeLa), liver cancer (HepG2), colon cancer (HCT-15), and leukemia (HL-60) cells [8]. The ability of many phytochemicals to selectively target malignant cells while exerting comparatively lower toxicity toward normal cells has further strengthened interest in the development of herbal-based anticancer therapies [8].

Blumea lacera (*Burm.f.*) DC. is an aromatic medicinal herb belonging to the family Asteraceae and is widely distributed throughout India, Bangladesh, Sri Lanka, and several Southeast Asian countries [9]. Traditionally, the plant has been used for the management of fever, bronchitis, skin disorders, inflammation, gastrointestinal ailments, wounds, helminthic infections, and diarrhea [9]. Ethnobotanical studies have documented the therapeutic use of different plant parts in various folk medicinal systems. Phytochemical investigations have revealed the presence of flavonoids, tannins, alkaloids, terpenoids, glycosides, phenolic acids, and essential oils, which are considered responsible for its diverse biological activities [10].

Despite significant advances in synthetic anticancer drug development, natural products remain indispensable sources of lead molecules for novel therapeutic agents. Plant-derived compounds may provide enhanced therapeutic efficacy through synergistic interactions while potentially reducing the adverse effects associated with conventional chemotherapy. Nevertheless, numerous medicinal plants remain insufficiently explored, and further research is needed to identify their active constituents, molecular targets, mechanisms of action, and clinical significance. Therefore, systematic scientific evaluation of medicinal plants, including Blumea lacera, is essential for discovering safe and effective plant-based anticancer therapies and expanding the repertoire of anticancer drug candidates.

2. Materials and methods

2.1 Materials

Rutin, Gallic Acid, DPPH (1, 1-diphenyl-2-picryl hydrazyl),1-10 phenanthroline, Phenazine methosulphate (PMS), Nitroblue tetrazolium (NBT), Nicotinamide Adenine Dinucleotide (NADH), sodium nitroprusside (SNP), sulphanilamide, napthyl ethylenediamine dihydrochloride (NED), 3,5- dinitrosalicylic acid (DNSA), DMSO. All chemicals and reagents used were AR grade.

2.2 Collection, identification, and authentication of the selected medicinal plants

Fresh leaves of *Blumea lacera* were collected from the Western Ghats region in the vicinity of Pune, Maharashtra, India, during July 2022. The botanical identity of the plant was established and authenticated by a qualified taxonomist at the Botanical Survey of India (BSI). A voucher specimen of the authenticated plant material was deposited in the herbarium of the host institution for future reference. The collected leaves were thoroughly cleaned, shade-dried under ambient conditions, and pulverized into a coarse powder. Both the authenticated plant specimens and the dried powdered material were preserved under suitable storage conditions and utilized for subsequent phytochemical, antioxidant, and anticancer investigations.

2.3 Extraction of the plant sample

The collected leaves of *Blumea lacera* were shade-dried, finely powdered, and subjected to successive solvent extraction using a Soxhlet apparatus. Briefly, 10 g of the powdered plant material was extracted sequentially with water, ethanol, and hexane based on the increasing extraction efficiency and boiling characteristics of the respective solvents. Each extraction cycle was continued for approximately 4 h to ensure exhaustive recovery of phytoconstituents [11,12].

Following extraction, the solvent fractions were concentrated under reduced pressure at room temperature to obtain the crude extracts. The percentage yield of each extract was determined gravimetrically

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after complete solvent removal. The dried extracts were then transferred to airtight containers and stored at 4°C until further phytochemical, antioxidant, and anticancer studies were performed.

2.4 Phytochemical Analysis

Preliminary phytochemical analysis of the plant extracts was conducted utilizing established qualitative techniques to ascertain the presence of principal categories of secondary metabolites, including terpenoids, flavonoids, saponins, tannins, and alkaloids [13].

The Salkowski test was employed to assess the presence of terpenoids. In summary, 0.5 g of the extract was combined with 2 mL of chloroform, accompanied by the meticulous incorporation of 3 mL of pure sulfuric acid along the interior wall of the test tube. The emergence of a reddish-brown interface signified the existence of terpenoid compounds.

For flavonoid detection, 0.5 g of the extract was heated with 10 mL of ethyl acetate on a steam bath for 3 min and subsequently filtered. To 4 mL of the filtrate, 1 mL of dilute ammonia solution was added. Development of a yellow coloration was considered a positive indication of flavonoids.

Saponins were detected by combining 5 mL of distilled water with 0.5 g of the extract and agitating the mixture thoroughly. The development of a stable and enduring froth indicated the existence of saponins. Additional confirmation was achieved by introducing several drops of olive oil and noting the development of a stable emulsion following agitation.

To test the presence of tannins, 0.5 g of the extract was subjected to boiling in 10 mL of distilled water and subsequently filtered. The filtrate was subjected to a few drops of 0.1% ferric chloride solution. The emergence of a brownish-green or blue-black hue validated the existence of tannins.

Alkaloids were identified by dissolving 0.5 g of the extract in acidified alcohol, followed by boiling and subsequent filtration. The filtrate was subjected to treatment with dilute ammonia and subsequently extracted using chloroform. The chloroform layer was then partitioned with acetic acid and separated into two sections. One sample was subjected to Mayer's reagent, whilst the other was subjected to Dragendorff's reagent. The emergence of a cream-colored precipitate with Mayer's reagent or a reddish-brown precipitate with Dragendorff's reagent shows the presence of alkaloids.

2.5 HPLC analysis

The instrument was equipped with an autosampler, an isocratic mobile phase of acetonitrile: orthophosphoric acid 0.1% (75:25 v/v), a Nucleodur C18 column (250 × 4.6 mm, 5 µm) at 30 °C, and LC solution software. Plant sample and standard solutions (Rutin, Gallic acid) were prepared in methanol as the diluent, and detection was performed at 210 nm using a PDA detector. The calibration curve was created by plotting peak areas versus concentration in MS Excel to quantify phenolic and flavanol compounds.

2.6 HPTLC analysis

HPTLC profiling of the plant extracts was carried out using a CAMAG HPTLC system (Germany). Precoated silica gel 60 F254 TLC plates (20 cm × 10 cm; Merck) were initially cleaned with methanol and activated by heating at 100°C for 10 min before use. Extract samples were prepared at a concentration of 1 mg/mL, and 10 µL of each sample was applied as bands onto the activated plates using a CAMAG Linomat 5 sample applicator.

Rutin and gallic acid were employed as reference standards at concentrations of 2 µg/mL and 4 µg/mL, respectively. Chromatographic separation of phenolic and flavonoid constituents was achieved using a mobile phase consisting of ethyl acetate, dichloromethane, and glacial acetic acid (10:2.5:200 µL). Following chromatographic development, the plates were examined under ultraviolet light at 254 nm using a CAMAG UV visualization chamber. The chromatograms were subsequently recorded and documented with a CAMAG photo documentation system for qualitative fingerprint analysis.

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2.7 Antioxidant Study

2.7.1 DPPH radical scavenging assay

The free radical scavenging activity of the plant extracts was assessed using the DPPH assay with minor modifications to a reported method [14]. Briefly, 1 mL of extract solution was mixed with 1 mL of 0.1 mM DPPH solution in ethanol and incubated in the dark at room temperature for 20 min. The decrease in absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Ascorbic acid (1 mM) served as the reference standard. The antioxidant activity was expressed based on the percentage inhibition of DPPH radicals relative to the control.

2.7.2 Hydroxyl (OH) radical scavenging assay

Hydroxyl radical scavenging activity was determined using the Fenton reaction method with minor modifications [15]. The reaction mixture contained $FeCl_2$ (1 mM), 1,10-phenanthroline (1 mM), phosphate buffer (0.2 M, pH 7.8), H_2O_2 (0.17 M), and the test extract (1 mg/mL). The reaction was initiated by adding H_2O_2 and incubated at room temperature for 5 min. Absorbance was measured at 560 nm using a UV–Visible spectrophotometer. Ascorbic acid (1 mM) was used as the standard, and hydroxyl radical scavenging activity was calculated by comparing the absorbance of the test samples with that of the control

2.7.3 Superoxide radical (SOR) scavenging assay

SRS activity of the plant extracts was evaluated using the phenazine methosulfate–nicotinamide adenine dinucleotide (PMS–NADH) non-enzymatic system following a previously reported method with slight modifications [16]. In this assay, superoxide anion radicals were generated through the interaction of PMS, NADH, and molecular oxygen, while the reduction of nitro blue tetrazolium (NBT) served as an indicator of radical formation.

The reaction mixture consisted of 3 mL of Tris-HCl buffer (100 mM, pH 7.4), 0.75 mL of NBT solution (300 μM), 0.75 mL of NADH solution (936 μM), and 0.3 mL of the test extract (1 mg/mL). The reaction was initiated by the addition of 0.75 mL of PMS solution (120 μM). Following incubation at room temperature for 5 min, the absorbance was recorded at 560 nm using a UV–Visible spectrophotometer. Ascorbic acid (1 mM) was employed as the reference antioxidant standard. The SRS activity of the extracts was determined by measuring their ability to inhibit NBT reduction in comparison with the control and standard solutions.

2.7.4 Nitric oxide (NO) radical scavenging assay

Nitric oxide scavenging activity was assessed using the sodium nitroprusside (SNP)-Griess reaction method with minor modifications [17]. The reaction mixture containing SNP (10 mM), phosphate-buffered saline (pH 7.4), and the test extract (25 μg/mL) was incubated at 25°C for 150 min. After incubation, sulphanilamide solution (0.33% in 20% glacial acetic acid) and N-(1-naphthyl)ethylenediamine dihydrochloride (NED, 0.1% w/v) were added sequentially to develop the characteristic pink azo chromophore. The absorbance was measured at 540 nm against a blank using a UV–Visible spectrophotometer. Ascorbic acid (1 mM) was used as the reference standard, and nitric oxide scavenging activity was calculated based on the inhibition of nitrite formation relative to the control.

2.7.5 Reducing activity (RA) assay

The reducing power of the plant extracts was evaluated by measuring their ferric ion (Fe^{3+}) reducing capacity using a previously reported method with slight modifications [18]. This assay is based on the ability of antioxidant compounds to reduce ferric ions to their ferrous form, which reflects their electron-donating potential.

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Briefly, 0.5 mL of the test extract solution (25 µg/mL) was mixed with 0.5 mL of phosphate buffer (0.2 M, pH 6.6) and 0.5 mL of potassium hexacyanoferrate solution (0.1%). The reaction mixture was incubated in a water bath at 50°C for 20 min to facilitate reduction of ferric ions. Following incubation, 0.5 mL of 10% trichloroacetic acid (TCA) was added to terminate the reaction.

Subsequently, 1 mL of the upper layer of the reaction mixture was transferred and combined with 1 mL of distilled water and 0.1 mL of 0.01% ferric chloride ($FeCl_3$) solution. The resulting mixture was allowed to stand at room temperature for 10 min to permit color development. Absorbance was then measured at 700 nm against an appropriate blank using a UV–Visible spectrophotometer. Ascorbic acid (1 mM) was employed as the positive control. An increase in absorbance indicated greater reducing power and, consequently, stronger antioxidant activity of the tested extracts.

2.8 MTT cytotoxicity assay for in vitro anticancer study

The cytotoxic activity of the test samples was evaluated using the MTT assay on HeLa 229, HL60, HCT 15, HEP 3B, and a Normal Chang Liver cell line [19]. Test samples were sterilized with 0.22 µm membrane filters and subsequently diluted in a double-dilution series using Minimum Essential Medium enriched with fetal bovine serum. The cell lines were cultivated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum and incubated at 37°C in a humidified environment with 5% CO_2, with medium alterations occurring every 2–3 days. Cells were inoculated in 96-well microtiter plates at a density of 1×10^5 cells per well and incubated for 24 hours to facilitate adhesion and the establishment of a semi-confluent monolayer. The culture medium was thereafter discarded, and cells were exposed to varying amounts of the test substance, while control wells were administered solely culture medium. Following 24 hours of treatment, cells were subjected to an additional 24-hour incubation and subsequently analyzed microscopically for morphological alterations. Thereafter, the medium was substituted with fresh medium containing MTT reagent and incubated for 4 hours to facilitate the production of purple formazan crystals. The crystals were solubilized using a solution (DMSO or isopropanol), and absorbance was quantified at 570 nm using a microplate reader. Cell vitality was determined by comparing the absorbance of treated cells to that of control cells. The percentage of cell survival was determined using the formula,

$$\% \text{ cell survival} = 1 - \frac{T}{C} \times 100$$

Where, T = Test sample, C = Control

3. Results and Discussion

3.1 Qualitative Phytochemical Analysis of Selected Plant Species

Phytochemical screening identified the presence or absence of major including terpenoids, flavonoids, saponins, tannins, and alkaloids, in water, ethanol, and hexane extracts (Table 1). Both water and ethanol extracts contained all tested phytochemicals, terpenoids, flavonoids, saponins, tannins, and alkaloids. These findings indicate that water and ethanol are effective solvents for extracting a broad spectrum of bioactive compounds from plants. The presence of these phytochemicals suggests that the extracts may exhibit a range of biological activities, including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects. For instance, flavonoids and tannins are associated with antioxidant properties, while alkaloids and terpenoids are linked to pharmacological activities such as cytotoxicity and antimicrobial effects.

Table 1. Qualitative phytochemical analysis of *B. lacera*

Plant Extract	Phytochemical test				
	Terpenoid	Flavonoids	Saponin	Tannins	Alkaloids
Water	+	+	+	+	+
Ethanol	+	+	+	+	+

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Hexane	-	+	-	+	+

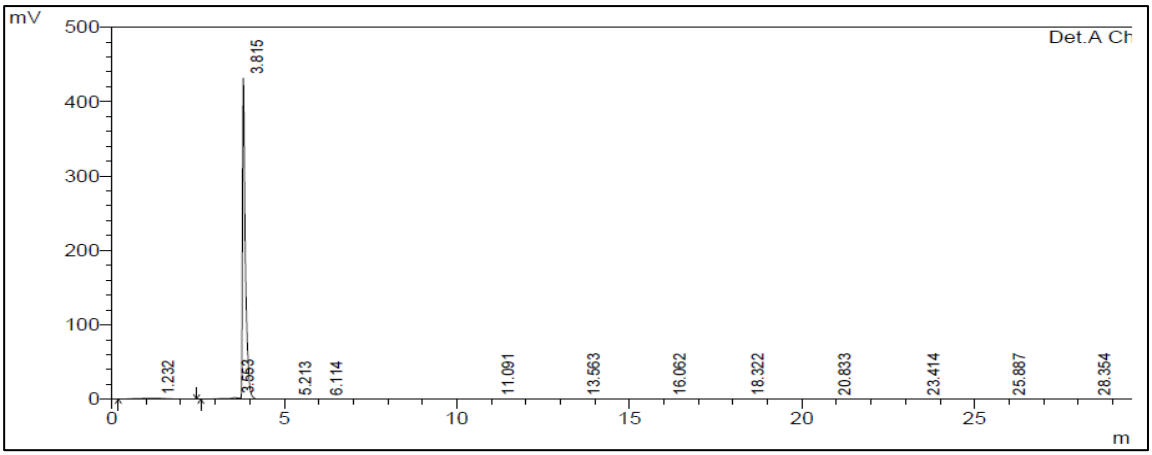
(+) Presence of phytochemical, (-) Absence of phytochemical

In contrast, the hexane extract lacked terpenoids and saponins but contained flavonoids, tannins, and alkaloids. This outcome is likely attributable to hexane's nonpolar nature, which primarily extracts nonpolar or less polar compounds. As saponins are generally polar, their absence in the hexane extract is expected. Similarly, certain terpenoids may not be efficiently extracted with hexane due to differences in polarity and chemical structure.

Among the three solvents, ethanol and water extracts demonstrated the most diverse phytochemical content. These results suggest that polar solvents are more effective for extracting a wide range of secondary metabolites from plants. The presence of multiple bioactive compounds in these extracts may contribute to synergistic biological or pharmacological effects. Furthermore, the choice of solvent significantly influences both the extraction efficiency and the phytochemical profile of the extracts.

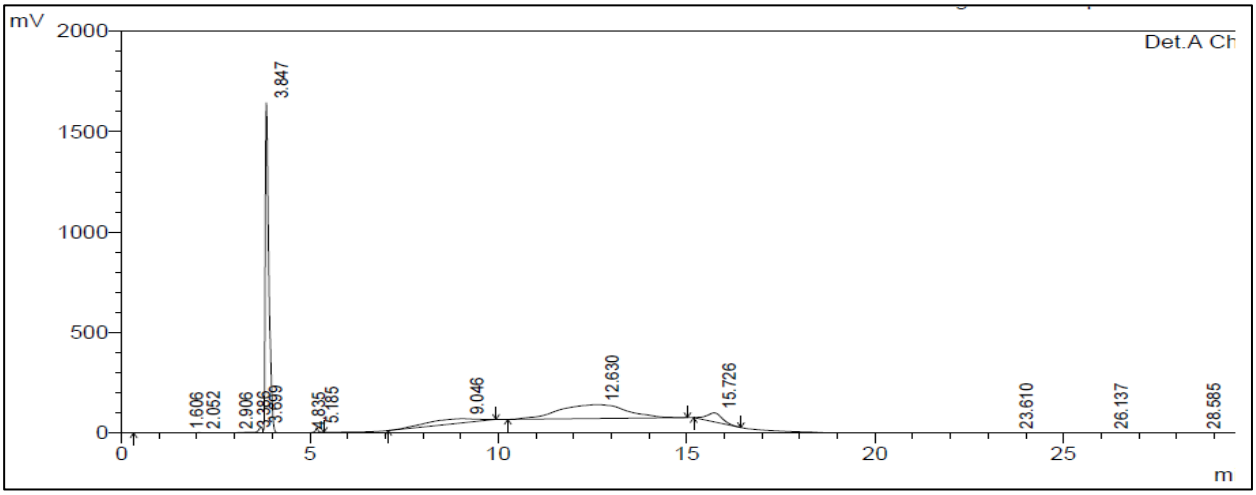
3.2 HPLC analysis of *B. lacera*

HPLC analysis of *B. lacera* extract at 272 nm revealed a complex phytochemical profile, with 18 distinct peaks indicating the presence of multiple constituents (Figure 1). The chromatogram illustrated a predominant peak at a retention time (RT) of 3.832 minutes, accounting for 62.034% of the total area. Additional significant peaks appeared at RTs of 5.097 minutes (9.808%) and 3.498 minutes (7.988%). Comparative analysis with reference standards demonstrated that gallic acid produced a peak at RT 3.847 minutes (44.94%), while rutin produced a peak at RT 3.815 minutes (72.15%). The close similarity in retention times between the major peak of the extract and those of gallic acid and rutin suggests the presence of phenolic and flavonoid compounds in *B. lacera*. The multiple peaks observed in the extract, relative to the standards, confirm its complex composition and indicate a mixture of bioactive constituents that may contribute to its antioxidant and anticancer activities.

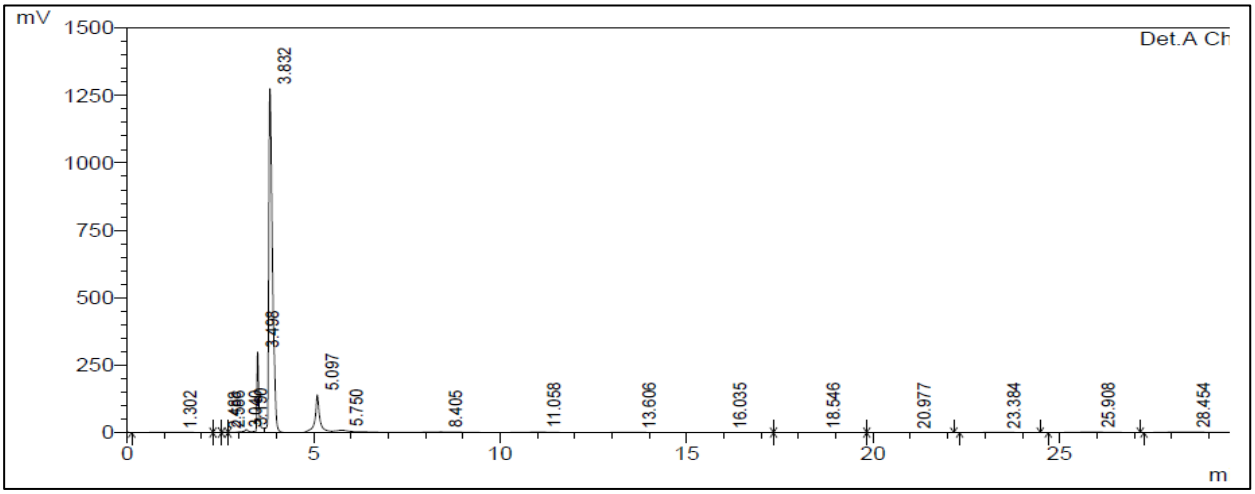


(A)

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(B)



(C)

Figure 1. HPLC chromatogram of samples. A= Rutin, B= Gallic acid, C= *B. lacera*

3.3 HPTLC profile of *B. lacera*

Figure 2 presents the High-Performance Thin Layer Chromatography (HPTLC) fingerprint profile of the ethanol extract of *B. lacera*, with gallic acid and rutin as standard marker compounds. In the chromatogram, tracks 1 and 2 correspond to gallic acid standards at concentrations of 2 µg and 4 µg, respectively, while Tracks 3 and 4 correspond to rutin standards at the same concentrations. Track 5 represents the ethanol extract of *B. lacera*. The presence of distinct bands in Track 5 suggests the existence of phytoconstituents analogous to gallic acid and rutin within the plant extract. The identification of these bioactive compounds in *B. lacera* highlights the plant's phytochemical diversity and may account for its reported antioxidant and anticancer activities.

Furthermore, the HPTLC fingerprint profile provides a distinctive chemical marker for authentication, quality control, and standardization of the plant extract. The multiple bands observed in Track 5 further indicate the presence of additional phytochemical constituents, reflecting the complex composition of the ethanol extract.

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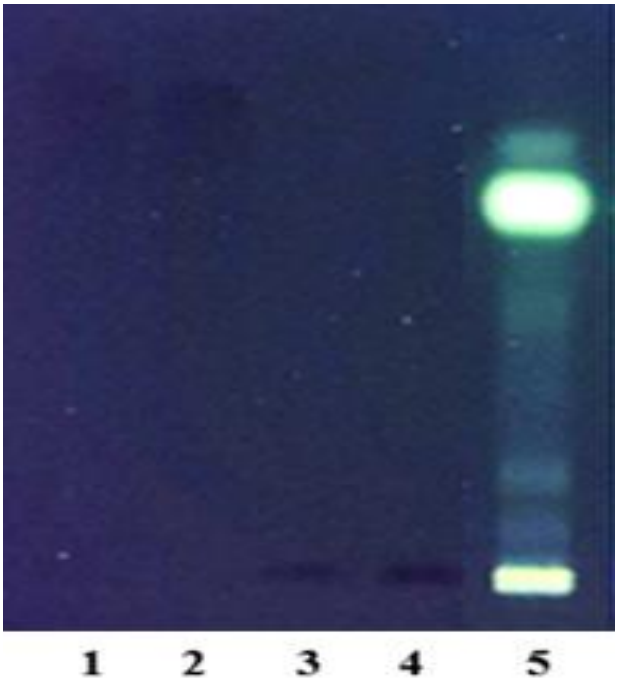


Figure 2. HPTLC profile of flavonoid fingerprints of ethanol extract of *B. lacera* using Rutin and Gallic acid as a marker compound. Track no. 1- Gallic Acid (2 µg), Track no. 2- Gallic Acid (4 µg), Track no. 3- Rutin (2 µg), Track no. 4- Rutin (4 µg), Track no. 5- *B. lacera*

3.4 Antioxidant activity profile of *B. lacera*

The table summarizes the antioxidant potential of plant extracts prepared with water, ethanol, and hexane, as evaluated by multiple assays. The ethanolic extract showed the highest DPPH radical scavenging activity (81.83 ± 1.36%), followed by the aqueous extract (77.30 ± 0.93%), while the hexane extract exhibited the lowest activity (38.67 ± 0.97%). Since DPPH scavenging activity reflects the hydrogen-donating capacity of phytochemicals, the pronounced activity in the ethanolic extract suggests a higher concentration of phenolic and flavonoid compounds with antioxidant properties. The aqueous extract demonstrated the highest hydroxyl radical (OH) scavenging activity (71.37 ± 0.91%), followed by the hexane extract (62.43 ± 1.17%), while the ethanolic extract exhibited lower activity (51.00 ± 1.61%). Hydroxyl radicals are highly reactive species that can cause cellular damage. The substantial scavenging capacity of the aqueous extract suggests a greater potential to protect cells from oxidative stress.

For SRS activity, the aqueous extract showed the highest inhibition (50.43 ± 1.02%), whereas the ethanolic extract showed the lowest (20.73 ± 1.27%). These results indicate that water-soluble antioxidants in the aqueous extract are particularly effective at neutralizing superoxide radicals. The ethanolic extract exhibited the highest nitric oxide (NO) scavenging activity (72.50 ± 0.72%), followed by the aqueous extract (63.46 ± 1.26%) and the hexane extract (59.90 ± 1.63%). Nitric oxide radicals are linked to inflammation and oxidative damage. The pronounced NO-scavenging activity of the ethanolic extract suggests potential anti-inflammatory and antioxidant effects.

Table 2. Radical scavenging potential of *B. lacera*.

Plant sample	% Scavenging activity				
	DPPH	OH	SOR	NO	RA
Water	77.30 ± 0.93	71.37 ± 0.91	50.43 ± 1.02	63.46 ± 1.26	151.00 ± 1.71
Ethanol	81.83 ± 1.36	51.00 ± 1.61	20.73 ± 1.27	72.50 ± 0.72	287.10 ± 1.62
Hexane	38.67 ± 0.97	62.43 ± 1.17	35.23 ± 1.07	59.90 ± 1.63	259.86 ± 1.33

The experiment performed in triplicates (n = 3 ± SD), SD = Standard Deviation, OH=Hydroxyl, SOR= Superoxide Reducing, NO= Nitric Oxide, RA= Reducing activity

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Regarding power reduction, the ethanolic extract also showed the highest value (287.10 ± 1.62), followed by the hexane extract (259.86 ± 1.33), while the aqueous extract showed the lowest (151.00 ± 1.71). Increased reducing power signifies enhanced electron-donating ability and antioxidant potential, suggesting that the ethanolic extract possesses a higher level of active chemicals. Ethanolic extract exhibited the strongest antioxidant effects in DPPH, NO scavenging, and reducing power assays, whereas the aqueous extract was more effective at scavenging hydroxyl and superoxide radicals. The hexane extract exhibited comparatively lower antioxidant activity, likely due to its reduced ability to extract polar antioxidants.

3.5 Anticancer activity evaluation of *B. lacera*

The data in the table demonstrate the cytotoxic effects of the plant extract on several cancer cell lines, including cervical cancer (HeLa 229), colorectal cancer (HCT 15), promyelocytic leukemia (HL 60), and hepatocellular carcinoma (HEP 3B), as well as on normal Chang liver cells. Cell survival decreased consistently with increasing concentrations of the plant extract (50–200 µg/ml), indicating a dose-dependent cytotoxic response. HeLa 229 cells showed the highest sensitivity to the plant extract among the tested cancer cell lines. At 50 µg/ml, cell survival was 53.71 ± 4.23%, decreasing to 41.71 ± 1.25% at 100 µg/ml and 30.56 ± 3.47% at 200 µg/ml. This substantial reduction in cell viability indicates the extract's strong antiproliferative activity against cervical cancer cells. HCT-15 and HL-60 cell lines exhibited moderate sensitivity to the extract, with both showing identical survival percentages declining from 60.08 ± 2.65% at 50 µg/ml to 47.95 ± 3.63% at 200 µg/ml. This gradual decrease in viability suggests that the plant extract inhibits colon cancer and leukemia cells, although these effects are less pronounced than in HeLa 229 cells.

Table 3. Effect of *B. lacera* ethanolic extract on percent cell survival of different cell lines.

Concentration (µg/ml)	% survival of cell lines				
	Hela 229	**HCT 15**	**HL 60**	**HEP 3B**	**Normal Chang Liver**
50	53.71 ± 4.23	60.08 ± 2.65	60.08 ± 2.65	63.46 ± 7.36	87.65 ± 6.96
100	41.71 ± 1.25	53.74 ± 4.39	53.74 ± 4.39	60.71 ± 6.10	81.21 ± 6.68
200	30.56 ± 3.47	47.95 ± 3.63	47.95 ± 3.63	53.89 ± 5.23	76.37 ± 2.02

The experiment performed in triplicates (n = 3 ± SD), SD = Standard Deviation

In the HEP 3B liver cancer cell line, the extract induced cytotoxicity, with cell survival decreasing from 63.46 ± 7.36% at 50 µg/ml to 53.89 ± 5.23% at 200 µg/ml. This effect was less pronounced than in the other cancer cell lines, indicating lower sensitivity of HEP 3B cells to the treatment. Normal Chang liver cells maintained high survival rates, ranging from 87.65 ± 6.96% at 50 µg/ml to 76.37 ± 2.02% at 200 µg/ml. These results suggest that the plant extract shows selective toxicity toward cancer cells while causing minimal damage to normal cells, a desirable property for potential anticancer agents. Overall, the plant extract demonstrates promising dose-dependent anticancer activity, particularly against HeLa 229 cells, while exhibiting lower cytotoxicity toward normal liver cells. These findings support the potential therapeutic value of the plant extract and warrant further investigation in anticancer studies.

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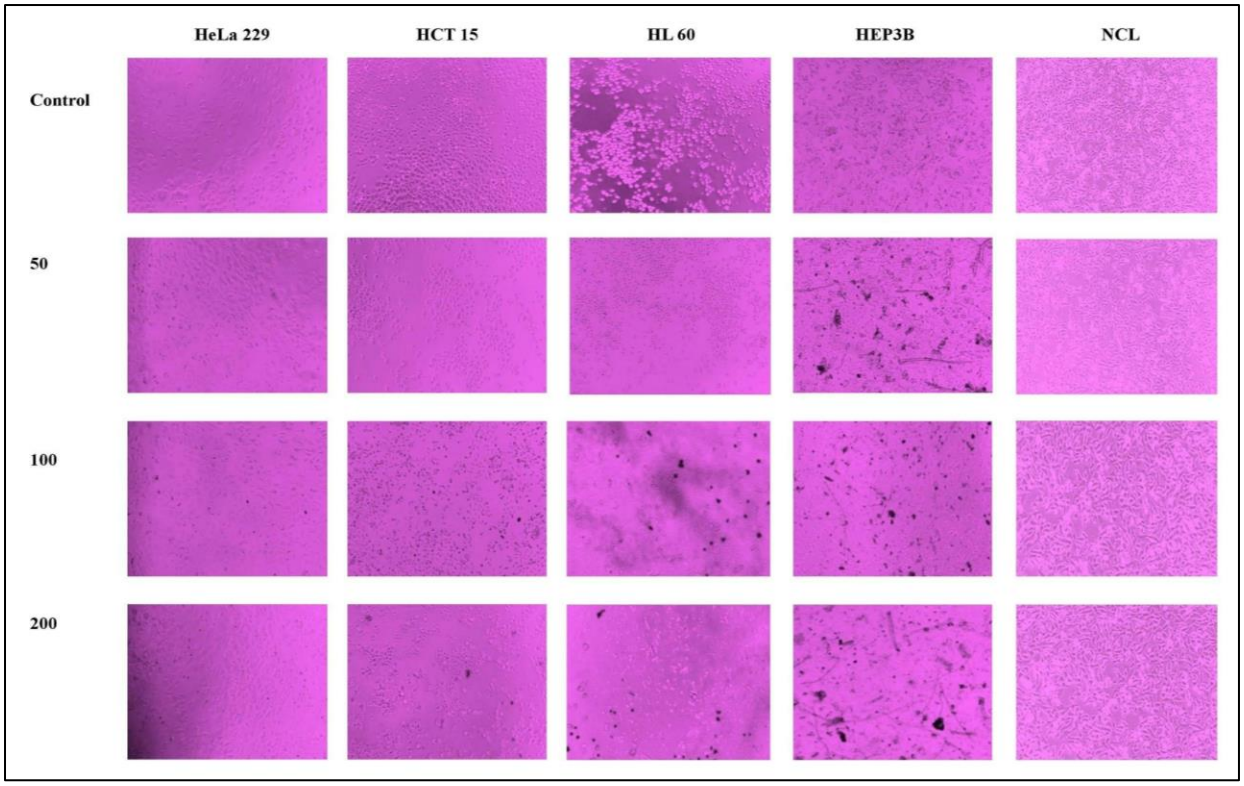


Figure 3. Impact of *B. lacera* ethanolic extract on the viability of several cancer cell lines at different doses (50, 100, and 200 µg/ml). The plant shows dose- dependent cell viability. As the concentration increases, the % cell viability decreases. The NCL (Normal Chang Liver Cell) was used as a comparative cell line.

4. Discussion

The results indicate that *B. lacera* contains a diverse array of phytochemicals with notable antioxidant and anticancer properties, consistent with its traditional medicinal use. Screening demonstrated that both aqueous and ethanolic extracts are rich in secondary metabolites, including flavonoids, tannins, alkaloids, terpenoids, and saponins. In contrast, the hexane extract lacked certain polar compounds, such as saponins and some terpenoids. This observation is consistent with previous studies, emphasizing the influence of solvent selection on the yield and diversity of plant extracts. Water and ethanol, as polar solvents, are particularly effective at extracting phenolic and flavonoid compounds due to their high solubility in these media.

The identification of flavonoids and phenolic compounds in *B. lacera* is significant, as these molecules are well-documented for their antioxidant, anti-inflammatory, and anticancer activities. Flavonoids, for example, can inhibit tumor growth by inducing apoptosis, inhibiting angiogenesis, modulating cell signaling pathways, and reducing oxidative stress. Alkaloids and terpenoids further contribute by disrupting cellular metabolism and DNA replication, resulting in cytotoxic and antimicrobial effects. The presence of multiple phytochemical groups suggests potential synergistic interactions among these bioactive compounds, potentially enhancing the overall biological activity of the plant extract. HPLC analysis of *B. lacera* revealed 18 distinct peaks, each corresponding to a different constituent. The most prominent peak, observed at 3.832 minutes, closely matched the retention times of gallic acid and rutin standards, confirming the presence of these phenolic and flavonoid compounds. Both gallic acid and rutin are recognized for their potent antioxidant and anticancer properties. Gallic acid induces apoptosis and inhibits cancer cell proliferation, while rutin effectively neutralizes free radicals and protects cellular integrity. The discovery of these chemicals establishes a molecular foundation for the observed antioxidant and anticancer actions in our investigation. The identification of several more peaks indicate the existence of other, currently uncharacterized, phytoconstituents that may also influence the biological effects of *B. lacera*.

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HPTLC fingerprint profile corroborated the HPLC findings, showing bands corresponding to gallic acid and rutin in the ethanolic extract of *B. lacera*. HPTLC fingerprinting is a validated method for authenticating and assessing the quality of medicinal plants, as it generates characteristic chemical profiles of bioactive constituents [20]. The presence of multiple bands in the chromatogram indicates a diverse phytochemical composition. Comparable HPTLC profiles have been observed in other medicinal plants rich in flavonoids and phenolic compounds, which are associated with antioxidant and anticancer activities [21]. Thus, the

HPTLC results further substantiate the phytochemical diversity and medicinal potential of *B. lacera*. Antioxidant assays demonstrated that the ethanolic extract exhibited superior DPPH radical- scavenging, nitric oxide-scavenging, and reducing power compared with the aqueous and hexane extracts. These findings suggest that ethanol extracts higher concentrations of phenolic and flavonoid compounds, which are effective at donating hydrogen atoms or electrons to neutralize free radicals. Phenolic compounds exert antioxidant effects by terminating free radical chain reactions and preventing oxidative damage to biomolecules [22]. The pronounced reducing power of the ethanolic extract further supports its electron-donating capacity and antioxidant potential.

The aqueous extract exhibited higher hydroxyl and superoxide radical-scavenging activities than the ethanolic extract. This enhanced activity is likely due to the presence of water-soluble antioxidants, including glycosides, tannins, and polysaccharides, which are more efficiently extracted in aqueous media. Hydroxyl radicals are highly reactive oxygen species that can cause significant cellular damage, including lipid peroxidation, DNA fragmentation, and protein oxidation [23]. The substantial scavenging activity observed in the aqueous extract suggests a strong protective potential against oxidative stress-induced cellular injury. Conversely, the lower antioxidant activity in the hexane extract may result from the limited capacity of nonpolar solvents to extract polar antioxidants such as phenolics and flavonoids.

Oxidative stress is strongly linked to carcinogenesis, as reactive oxygen species can induce DNA mutations, genomic instability, and abnormal cell proliferation [24]. Consequently, the pronounced antioxidant activity of *B. lacera* extracts may substantially contribute to their anticancer potential. The presence of gallic acid, rutin, and flavonoid-like compounds in the extract likely plays a pivotal role in mitigating oxidative damage. Evaluation of anticancer activity demonstrated that *B. lacera* extract induced significant, dose-dependent cytotoxicity in HeLa 229, HCT-15, HL-60, and HEP 3B cancer cell lines. HeLa 229 cervical cancer cells exhibited the highest sensitivity, with marked reductions in cell viability as extract concentration increased. Comparable results have been reported for flavonoid-rich medicinal plant extracts, which induce apoptosis and cell cycle arrest in cervical cancer cells by modulating mitochondrial pathways and promoting oxidative stress [25].

The moderate cytotoxicity observed in HCT-15 and HL-60 cells indicates that the extract possesses broad-spectrum antiproliferative activity against both solid tumors and hematological malignancies. The lower sensitivity of HEP 3B liver cancer cells may be attributed to differences in cellular metabolism, membrane permeability, and intrinsic resistance mechanisms among cancer cell types. Notably, the extract exhibited reduced toxicity toward normal Chang liver cells, indicating selective cytotoxicity against cancer cells. Selective toxicity is a crucial characteristic of potential anticancer agents, as it minimizes harm to normal tissues and reduces treatment-related side effects [26]. The selective antiproliferative effect observed in this study may be associated with phytochemicals, such as flavonoids and phenolic acids, that induce apoptosis in cancer cells while sparing normal cells. The broad-spectrum cytotoxicity of *B. lacera* may result from the combined actions of multiple phytoconstituents present in the extract. Previous studies have demonstrated that phenolics, flavonoids, alkaloids, and terpenoids can regulate apoptosis-related proteins, inhibit angiogenesis, suppress metastasis, and interfere with signaling pathways such as NF-κB, PI3K/Akt, and MAPK, which are implicated in tumor progression [27]. Thus, the cytotoxic effects observed in this investigation are likely mediated through multiple molecular mechanisms.

5. Conclusion

In conclusion, the present study demonstrates that Blumea lacera is a rich source of bioactive phytochemicals with significant antioxidant and anticancer potential. Qualitative phytochemical screening confirmed the presence of flavonoids, tannins, alkaloids, terpenoids, and saponins, particularly in the aqueous and ethanolic extracts. HPLC and HPTLC analyses further revealed the presence of important phenolic and flavonoid constituents, including gallic acid and rutin, which are known for their pharmacological activities. Among the tested extracts, the ethanolic extract exhibited superior antioxidant activity in DPPH, nitric oxide scavenging, and reducing power assays, while the aqueous extract showed remarkable hydroxyl and SRS activities. Furthermore, the ethanolic extract demonstrated significant dose-dependent cytotoxicity against HeLa 229, HCT-15, HL-60, and HEP 3B cancer cell lines, with comparatively lower toxicity toward normal Chang liver cells, indicating selective anticancer activity. These findings suggest that the antioxidant and antiproliferative effects of B. lacera are closely associated with its diverse phytochemical composition. Therefore, B. lacera represents a promising candidate for the development of plant-based anticancer therapeutics. Further studies involving bioactive compound isolation, mechanistic investigations, in vivo validation, and clinical evaluation are warranted to establish its therapeutic efficacy and safety.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

Authors contribution includes "Conceptualization, and methodology, software, validation, and formal analysis investigation, resources, data curation, writing—original draft preparation, writing—review and editing, visualization, supervision, project administration and acquisition by Samarth R. Kuldharme, Hiralal B. Sonwane, Rafik U. Shaikh. All authors have read and agreed to the published version of the manuscript.

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