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# *BALANITES AEGYPTIACA* SEED EXTRACT AND ITS ACTIVE COMPOUND, BALANITIN 6, PROMOTE CASPASE-DEPENDENT APOPTOSIS, LEADING TO ANTICANCER ACTIVITY

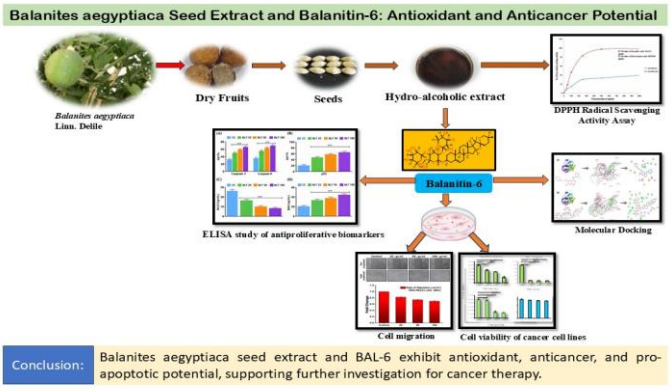
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## ABSTRACT

*Balanites aegyptiaca* Linn. Delile (BA) is a well-known medicinal plant that has been traditionally used for treating several human ailments, notably as an antibacterial, in skin disorders, for wound healing, and as an antioxidant. In this study, both the phytochemical profile and cytotoxic potential of BA were investigated using standard methodologies. Briefly, a hydro-alcoholic (50:50) extract of seeds was prepared and subjected to phytochemical investigation. The cytotoxicity was evaluated by a cell proliferation assay (MTT) against MCF-7, MDA-MB-231, FaDu, and human PBMCs. To further elucidate the mechanism of action, an ELISA was performed for caspases and Bcl family proteins. The results revealed that the hydro-alcoholic seed extract of BA possesses significant antioxidant and anticancer activities. Notably, Balanitin-6 (BAL-6) exhibited potent anticancer effects against MCF-7 cells through a caspase/p53/Bcl family-mediated apoptotic pathway. These findings validate the ethnomedicinal claims of BA and, for the first time, highlight its novelty as a promising source for the isolation of lead molecules with potent antioxidant and anticancer potential.

**Graphical Abstract:**




***KEYWORDS*:** *Balanites aegyptiaca*; Ingudi; Hingot; DPPH free radical; Anticancer, Antioxidant.

## INTRODUCTION

Cancers are caused by mutations that may be inherited, induced by environmental factors, or result from errors in DNA replication [1]. Cancer is the leading cause of death, approximately. 18.1 million cancer cases were found in 2018, with 9.6 million deaths occurring worldwide, and it is projected to increase to 29 million by 2040 [2]. The economic cost of cancer treatment in the United States was estimated to be USD 161.2 billion in 2017, compared to USD 27 billion in 1990 [3], highlighting the importance of developing anticancer molecules. The Atharva Veda contains references to signs of advanced cancer as well as prayers and rituals that seek supernatural cures [4], whereas research on complementary and alternative medicine, especially dealing with cancer management, has recently received more attention. Numerous studies on plants have been done for a variety of ethnobotanical reasons. Since its inception, the Indian traditional medicine known as Ayurveda has been successful in avoiding or suppressing a variety of tumors by employing several therapeutic modalities. Two well-known Ayurvedic classics, the Charaka [5] and Sushruta [6] Samhitas, refer to cancer as either an inflammatory or non-inflammatory swelling and refer to it as either Granthi (benign) or Arbuda (malignant). These texts mention the use of surgery and herbal medications for such diseases. Approximately 80% of the population of the world depends on herbal remedies for healthcare in different ethnic groups. In the ancient history of India, the usage of plants and their formulations was explained in the Rig Veda (2000 BC) [7]. In the modern age, the secondary metabolites of

such described medicinal plants have served as natural resources for many incipient drug candidates due to their vast chemical diversity. The secondary metabolites of medicinal plants build up and invigorate physical systems, especially immunity, which can then effectively fight pathogenic invaders and avail to destroy offending antigens without engendering any rigorous side effects. In recent years, many secondary metabolites have been reported as antioxidants that inhibit oxidative damage and prevent inflammatory conditions [8, 9]. On the other hand, naturally occurring secondary metabolites from plants are frequently used for drug discovery to save money and time. Therefore, one clever strategy to strengthen the anticancer efficacy and selectivity of these more promising chemical moieties, improve their absorption, distribution, metabolism, and excretion capabilities, and lessen their toxicity and side effects is to change their chemical structure [10]. Due to the adverse side effects of synthetic drugs, today, pharmaceutical industries are using traditionally used herbal drugs for the development of drug candidates efficiently.

The locally known Ingudi/Hingot (*Balanites aegyptiaca* Linn. Delile) is a very small tree with glabrous or puberulous branches having very sharp ascending spines and bifoliate leaves of two elliptical or obovate, coriaceous leaflets. Africa, the Middle East, Rajasthan, Gujarat, Madhya Pradesh, and the Deccan are the regions where it is most prevalent [11]. In ancient times, Ingudi was used in traditional medicine, and its medicinal importance was mentioned in the Vedas, Puranas, Upanishads, and Ayurvedic literature. In the Sanskrit textbook, Abhigyan Shakuntalam mentioned that the people who lived in forest areas used 'Hingot' as hair oil and for wound healing [12]. The hydro-alcoholic extract of Ingudi is a potential source of natural compounds containing cytotoxic and antioxidant activities. This may be the originality of this ethnopharmacological investigative work. In an earlier study, the hydro-alcoholic extract of Ingudi was screened for secondary metabolites having antimicrobial properties [13]. The present study showed the antioxidant and anticancer activity of the hydro-alcoholic extract of Ingudi, which has not been investigated previously for its anticancer property in both the hydro-alcoholic extract and the isolated compound BAL-6.

## MATERIALS AND METHODS

### 2.1 Collection and extraction of plant materials

Ingudi (*Balanites aegyptiaca* Linn. Delile) fruit seeds were obtained from Munaganj village, Etawah district, Uttar Pradesh, India, after scientific authentication at Dravyaguna Department, Faculty of Ayurveda, Banaras Hindu University, Varanasi, with accession number DG / 1819 / 194 (seeds) (Figure 1A). The seed was once pulverized in the mechanical grinder to a plausible excellent powder to undergo microscopic research and was once preserved in a nicely closed hermetic glass vessel for integration analysis. All reagents and chemical compounds utilized for the extraction of information were of analytical grade [14, 15]. Now take 5 gm of seeds and air-dry them at 37 °C. All seeds were coarsely powdered (Figure 1B), macerated with 100 mL of hydro-alcohol (40:60) in a closed conical flask for twenty-four hours, shaking regularly at intervals of six hours, and allowed to stand for 18 hours. It was rapidly filtered through Whatman filter paper, taking safety measures to prevent loss of ethanol. 25 mL of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish (Temp. 105 °C), and its weight was determined (Figure 1. C). After that, the share of ethanol-soluble extract used to be as soon as calculated concerning the air-dried drug.

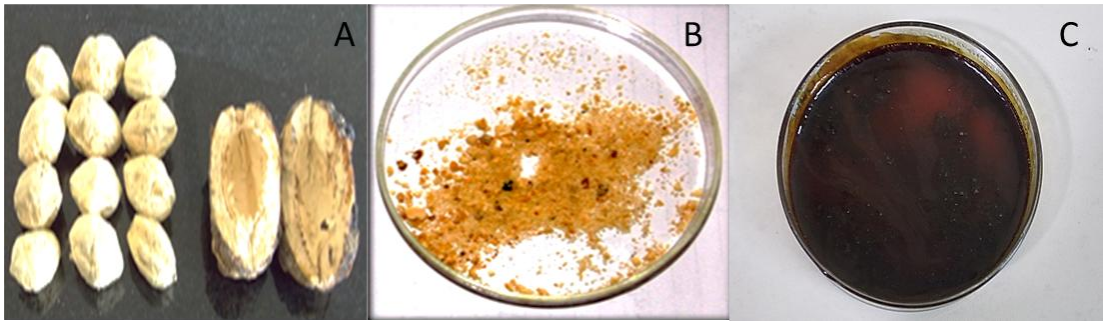



**Figure 1. (A) Seeds of *Balanites aegyptiaca*; (B) Seed powder of *Balanites aegyptiaca*; (C)Hydro-alcoholic extract of Seeds of *Balanites aegyptiaca***

### 2.2 Isolation of Balanitin-6 from seed extract of *Balanites aegyptiaca*:

Further purification of the ethyl acetate fraction of the extract was also carried out to isolate phytoconstituents of BA using silica-gel column chromatography. A total of 18 fractions were obtained from the ethyl acetate extract by elution with the ratio of 80:20 (chloroform: methanol) as the mobile phase, in which fractions 11 and 12 showed positive results for saponin glycoside BAL-6.

### 2.3 Phytochemical analysis of *Balanites aegyptiaca* extract

#### (A) Alkaloid determination

Alkaloid determination was made according to the method reported by Selvakumar et al. [32]. 2 mL of HCl was added to the extract dissolved in dimethyl sulfoxide, and the mixture was filtered. An equal volume of bromocresol green and phosphate buffer was added to the solution. The mixture was shaken by adding chloroform. The absorbance of the solutions containing the extract and atropine used as a standard was read at 470 nm. The alkaloid content was expressed as mg atropine equivalent (AE)/g. All tests were repeated in triplicate. Calibration curves of standards were given in Supplementary Fig. S1.

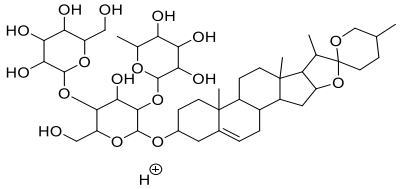


**Fig S1. Structure of Balanitin 6**

**(B)Tannin determination**

Quantitative tannin content in the extracts was tested according to the method suggested by Mital and Jha [30, 31]. 1 mL of extract was mixed with 0.5 mL of Folin reagent, then it was saturated with 1 mL of Na2CO3, and 8 mL of distilled water was added to the final mixture. The solution, which was incubated for 30 min, was centrifuged, and the supernatant was analyzed at 725 nm. Tannic acid was used as a standard, and tannin content was expressed as mg tannic acid equivalent (TAE)/g.

**(C) Folin–Ciocalteu Technique for Calculating Total Phenolic Concentration:**

Solution of gallic acid (0.1 mg/mL): In a glass beaker, 0.1 milligram of gallic acid was prepared by dissolving 1 mL of DL. FC solution: 0.2 N (1 mL of FC solution + 9 mL of DL). A total of 7.5% of $Na_2CO_3$ (Sodium carbonate): 15 g of $Na_2CO_3$ in 200 mL of DL.

Standardized gallic acid (0, 0.2, 2, 4, 10 g/mL) was placed into sterile test tubes as part of the protocol. To obtain a total volume of 500 µL, DL was added. For each test tube, 2.5 mL of Folin–Ciocalteu solution was added, followed by incubation for 5 min at room temperature after thoroughly mixing the solutions. Within every test tube, add 2 mL of 7.5% $Na_2CO_3$ and incubate for 1 h at room temperature. A spectrophotometer was used to determine the absorbance of each specimen at 765 nm. With an empty solution, adjust the absorbance to 0. Establish the standard calibration graph by plotting a graph using protein content on the x-axis and absorbance on the y-axis. Applying the value of the slope of the graph, determine the quantity of phenols within the undetermined specimens.

**(D) Total flavonoid content:**

The total flavonoid content (mg/mL) was determined using the aluminium chloride ($AlCl_3$) method [26, 27]. The assay mixture consisting of 0.5 mL of the plant extract, 0.5 mL distilled water, and 0.3 mL of 5% $NaNO_2$ was incubated for 5 min at 25°C. This was followed by the addition of 0.3 mL of 10% $AlCl_3$ immediately. Two milliliters of 1 M NaOH were then added to the reaction mixture, and the absorbance was measured at 510 nm. Quercetin was used as a standard.

**2.4 Antioxidant testing**

**(A) The DPPH Radical Scavenging Activity Assay**

The DPPH activity of the hydro-alcoholic extracts was quantified in terms of hydrogen-donating or radical scavenging ability by the utilization of the DPPH technique as described by Braca et al., 2001, with a slight modification [17]. Then, 1 mL of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) solution (0.25 mM) was incubated with two mL of hydro-alcoholic extracts at different concentrations (10-250 µg/mL). The reaction mixture was shaken vigorously and incubated in the dark for 30 min at room temperature. The control was prepared as above without an extract. The absorbance of the solution was then quantified at 518 nm against a blank. The free radical scavenging activity was once quantified as a minimum in the absorbance of DPPH and was calculated the utilization of the following equation:

Scavenging effect (%) = [(OD Control – OD test)/O.D. Control] x100

Ascorbic acid was used as a reference standard.

**2.5 Anticancer activity**

**(A) Cell culture condition**

The cell proliferation inhibition induced by the Hydro-alcoholic seed extract of Ingudi (*Balanites aegyptiaca* Linn. Delile) was determined by the thiazolyl blue tetrazolium bromide (MTT) assay. The culture of cells for preparation and collection at the lag phase was achieved by culturing the cells in a T-25 tissue culture flask in a $CO_2$ incubator at 37°C with 95% air and $CO_2$. In this experiment, cell lines MCF-7, MDA-MB-231, FaDu, and human PBMCs (2×103) were seeded per well in 96-well plates and allowed to adhere overnight at 37°C in a $CO_2$ incubator. For this study, the team obtained Ethical Committee permission from Tezpur University, Tezpur (Protocol no. DoRD/TUEC/10-14/4361 dated 28.03.2014) before the study. Then, the effect of plant extract on human peripheral blood mononuclear cells (PBMCs) was evaluated using human blood, which was obtained from the volunteers with informed consent. The blood was centrifuged to get peripheral blood mononuclear cells (PBMCs), followed by PBMCs isolation from the blood by density gradient centrifugation, and seeded in RPMI-1640 media supplemented with 10% FBS. The cancer cell lines, such as MCF-7 cells, were cultured in MEM medium containing 10% fetal bovine serum, and MDA-MB-231 cells were cultured in Leibovitz-15 (L-15 medium containing 10% FBS, 100 U/mL penicillin, and 100mg/L streptomycin [18].

**(B)In Vitro Cytotoxicity Assay**

To examine the cytotoxicity of the extract, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed [19]. Briefly, MCF-7, MDA-MB-231, FaDu, and PBMCs were harvested, counted, and seeded at 3 × 103 cells per well in 96-well plates for 24 h. The next day, the cells were treated with different concentrations of plant extract and incubated at 37 °C for 24 h and 48 h, with 5% $CO_2$. After completion of incubation, 100µl of 0.5 mg/mL MTT was added to each well and again incubated for 4 h in the dark at 37 °C with 5% $CO_2$. Then, the solution was drained, and

150 µL of dimethyl sulfoxide (DMSO) was added to each well. Finally, the plate was read at 575 nm by an Infinite M200 Pro microplate reader.

### 2.6 Enzyme -Linked Immunosorbent Assay (ELISA)

Using kits from GenoTechnology, Inc., India, the quantities of caspase-8 (IT7172) and caspase-3 (KHO1091) were measured [20]. A kit from G-Biosciences, USA, was used to assay p53 (IT4417). We purchased the BAX (EEL030) and Bcl-2 (BMS244-3) kits from Thermo Fisher Scientific. Following the manufacturer's instructions, an ELISA kit was used to investigate the breast cancer cell line MCF-7 [21].

### 2.7 Statistical analysis

All the data were expressed as means ± SEM, n=3, from three independent sets of experiments. Statistical analysis was performed using ANOVA followed by Tukey's multiple comparisons test. Three levels of significance were considered, and the sets as ap≤0.001, bp≤0.01, and cp≤0.05 (PRISM software, USA).

### 2.8 Molecular Docking Studies

To enhance the comprehension of the molecular interaction between ligands and proteins like Balanitin-6 and epidermal growth factor receptor (EGFR) (PDB id: 6DUK), molecular docking investigations were carried out using AutoDock 4.2 (https://doi.org/10.1002/jcc.21256). Gefitinib, an approved EGFR inhibitor, was used as a standard for comparison of binding. The crystal structures of these proteins were sourced from the Protein Data Bank (https://www.rcsb.org/). To ensure accurate protonation states for the residues, the pdb2pqr web server was utilized. Subsequently, water molecules, ligands, and ions were excluded from the analysis. Non-polar hydrogen atoms were also removed, and polar hydrogen atoms and Gasteiger charges were incorporated using M.G.L. Tools 1.5.6. For the computation of maps, AutoDock employed AutoGrid4.

The active site of the protein was identified with the help of the PLIP web server (https://projects.biotec.tu-dresden.de/plip-web/plip) (10.1093/nar/gkv315). The docking study was conducted using the Lamarckian Genetic Algorithm (LGA) with specific parameters: 100 runs, 150 population sizes, 27,000 generations, and 2,500,000 energy evaluations. During the docking simulation, a 'semi-empirical free energy force field' was utilized to assess conformations. BIOVIA Discovery Studio 2021 was used to visualize the docked poses and study the interactions (https://doi.org/10.1080/07391102.2020.1848630). Following the successful completion of the docking simulation, the best conformation was chosen based on the most favourable binding energy within the largest cluster, with a threshold of 2.0 Å.

## RESULTS AND DISCUSSION

### 3.1 Phytochemical and Antioxidant Analysis

Extraction of BA was done using four different organic solvents, i.e., heptane, hydro-alcohol, acetone, and ethyl acetate. The qualitative analysis of the extracts from all solvents is shown in Table 1. Saponins show antioxidant, antimicrobial, and anti-inflammatory properties [22]. Flavonoids exhibit antioxidant and anti-inflammatory properties, and tannins exhibit antimicrobial and free radical scavenging activity [23, 24]. Steroids show anti-inflammatory properties [25]. It was already documented that the major classes of antimicrobial compounds from plants include tannins, saponins, flavonoids, and glycosides [26].

**Table 1. Phytochemical screening of the extract of different solvents of *Balanites aegyptiaca*. Note: '+' shows low; '++' shows moderate, and '+++' shows high, while '-' shows absent.**

| Qualitative Phytochemical Screening | | hydro alcoholic extract of B. Aegyptiaca | Ethyl acetate extract of B. aegyptiaca | Heptane extract of B. aegyptiaca | Acetone extract of B. aegyptiaca |
|---|---|---|---|---|---|
| Alkaloids | Mayer's test: | ++ | +++ | - | + |
| | Wagner's Tests: | | | | |
| | Dragendroff's test: | | | | |
| Flavonoids | Ferric chloride test: | +++ | ++ | - | - |
| Steroids | Salkowski Tests: | - | ++ | +++ | - |
| | Lieberman Burchardt tests: | | | | |
| Phenol | Ferric chloride test: | ++ | - | - | - |
| Tannins | Ferric chloride test: | - | - | +++ | - |
| Glycosides | Kellar Killani's test: | +++ | ++ | - | - |
| Saponins | Foam test: | ++ | + | - | - |

### 3.2 Cell viability

The cytotoxic potential of the plant extract was assessed in MCF-7, MDA-MB-231, FaDu, and PBMCs using the MTT assay. A significant, dose-dependent reduction in cell viability was observed in breast cancer cell lines (MCF-7 and MDA-MB-231) and head and neck cancer cells (FaDu) at both 24 and 48 hours, whereas only minimal effects were noted on PBMCs (Figure 3). The observed anticancer activity may be attributed to the presence of flavonoids and phenolic constituents, as indicated in the preliminary phytochemical analysis (Table 2). Moreover, our previous NMR-based investigation of Balanites aegyptiaca fruits (fresh and dry) (Kumar et al., 2023) identified bioactive compounds such as oleic acid, linoleic acid, and linolenic acid, all of which have been reported to possess anti-tumorigenic properties [27, 28].

**Table 2. Interactions of the EGFR receptor with Balanitin-6 and Gefitinib.**

| Compound | Binding Energy (kcal/mol) | Ligand Efficiency (kcal/mol) | Ligand Interactions (PDB ID: 6DUK) |
|---|---|---|---|
| Gefitinib | -9.590 | -0.309 | VAL A:726 (Pi-Sigma), MET A:766 (Pi-Sulfur), MET A:790 (Pi-Sulfur), ASP A:855 (H-Bond) |
| Balanitin 6 | -10.438 | -0.168 | LYS A:745 (H-Bond), ASP A:837 (H-Bond), ASP A:855 (H-Bond), PHE A:856 (H-Bond) |

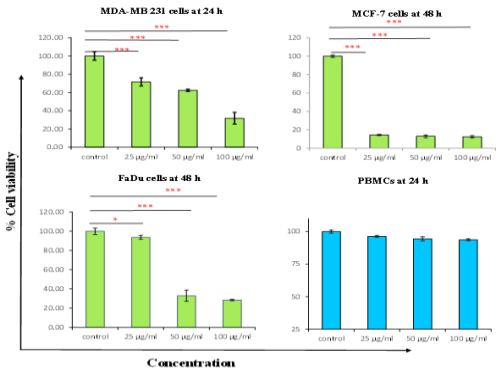



**Figure 3: The effect of plant extract on cell viability of cancer cell lines (MCF-7, MDA-MB-231, and FaDU) and PBMCs at 24 and 48 h, measured by the MTT-based method. Values were expressed as mean ± sem; n=3; values were significant when ***p≤0.001, **p≤0.01, and *p≤0.05**

### 3.3 BA extract treatment decreases cell Migration.

The effect of BA extract on the migratory potential of breast cancer cells was evaluated using a scratch assay. The results revealed that the mean scratch distance increased significantly with higher concentrations of the extract (Figure 4), as quantified using ImageJ software (Figure 2). This inhibition of cell migration is likely attributable to the phytochemicals present in the extract. Comparable findings were reported by Hsu et al. (2015), where treatment with increasing doses of betulin also resulted in a significantly larger wound area in breast cancer cells compared to the control [29].

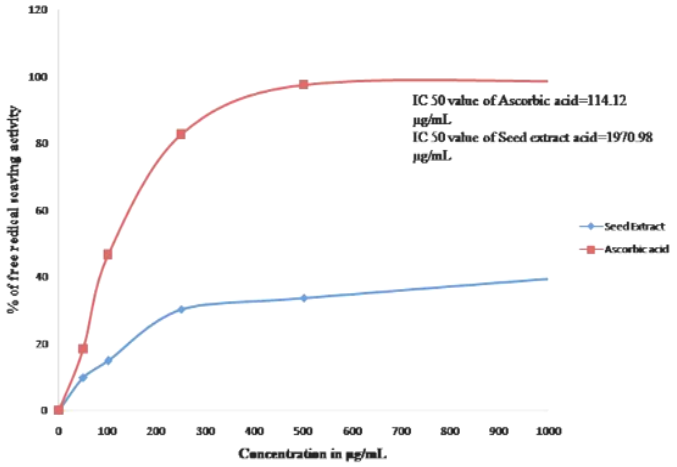



**Figure 2. DPPH Free radical scavenging activity of plant extracts of *Balanites aegyptiaca***

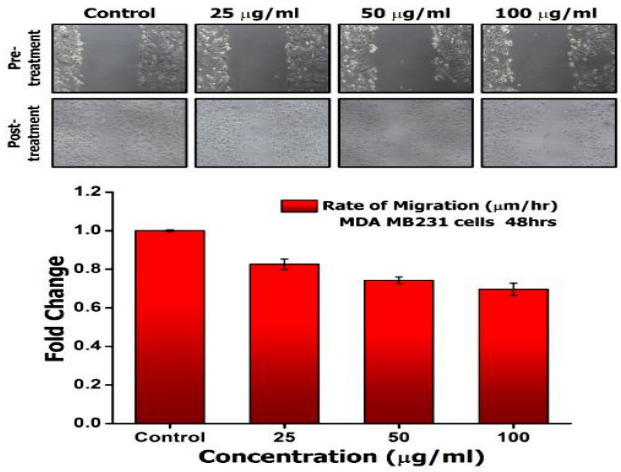



**Figure 4. Effects of Balanites seed extracts on cell migration of MDA-MB-231. Photomicrographs were acquired at 48 h. The applied concentrations of extracts were 25, 50, and 100 µg/mL.**

### 3.4 Molecular Docking

Molecular docking studies were conducted to evaluate the binding affinity of Balanitin-6 towards the EGFR receptor (PDB ID: 6DUK), in comparison with the standard drug Gefitinib. As shown in Table 2, Balanitin-6 exhibited a more favorable binding energy (−10.438 kcal/mol) than Gefitinib (−9.590 kcal/mol), indicating stronger overall binding. Balanitin-6 demonstrated a rich hydrogen-bonding network involving LYS745, ASP837, ASP855, and PHE856. The presence of multiple hydrogen bonds suggests enhanced stability of the ligand–receptor complex. These results suggest that Balanitin-6 has a stronger and potentially more stable binding interaction with EGFR compared to Gefitinib, warranting further investigation as a potential EGFR inhibitor. Since EGFR blockade is known to promote apoptosis via activation of downstream caspases, such as caspase-3 and caspase-9, it is plausible that Balanitin-6 may induce caspase-dependent apoptotic responses, like EGFR inhibitors.

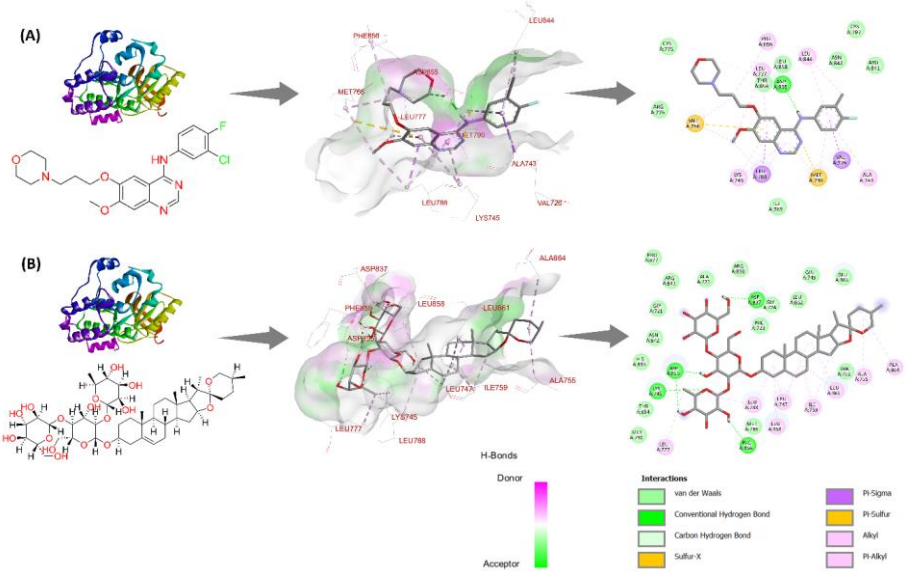



**Figure 5. The protein-ligand interaction diagram of compounds A) Gefitinib and B) l against the EGFR receptor protein (PDB ID: 6DUK).**

### 3.5 ELISA of Apoptotic Markers

Apoptotic mediators (caspase-3, caspase-8, and p53) were quantified in MCF-7 cells using ELISA to evaluate the impact of BAL-6 (25, 50, and 100 µg/mL) on apoptosis-related anticancer pathways (Figure 6A-B). In the carcinogen control group, these apoptotic markers were suppressed by nearly 50%. Among the tested concentrations, BAL-6 at 100 µg/mL was the most effective in restoring caspase and p53 levels, compared to BAL-6 at 25 or 50 µg/mL. In addition, the expression of Bcl family proteins (Bcl-2 and BAX) was examined (Figure 6C-D). The carcinogen control markedly elevated Bcl-2 while reducing BAX, reflecting cancer-associated, Bcl-dependent apoptotic dysregulation. Treatment with BAL-6 (25, 50, and 100 µg/mL) normalized BAX and Bcl-2 levels, with BAL-6 at 100 µg/mL showing the strongest restorative effect. Overall, while all tested doses of BAL-6 reinstated caspase, p53, Bcl-2, and BAX expression, the 100 µg/mL concentration demonstrated the most pronounced efficacy.

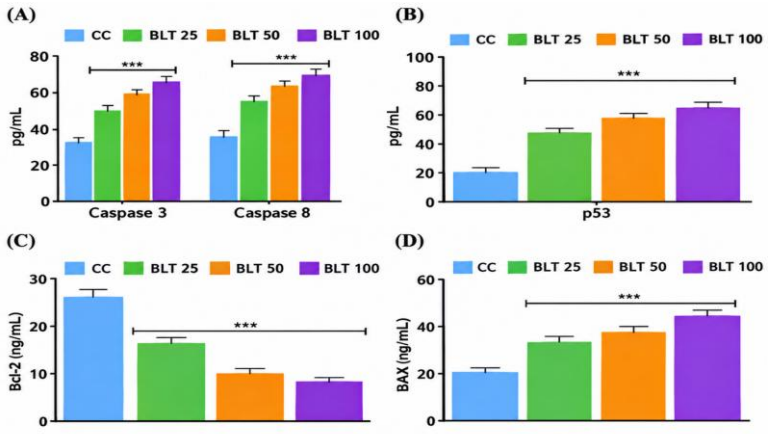



**Figure 6. ELISA study of antiproliferative biomarkers: (A) caspase-3 and caspase-8; (B) p53; (C) Bcl-2; and (D) BAD; activities in MCF-7 Cells after treatment with BLT 25, BLT 50, and BLT 100.**

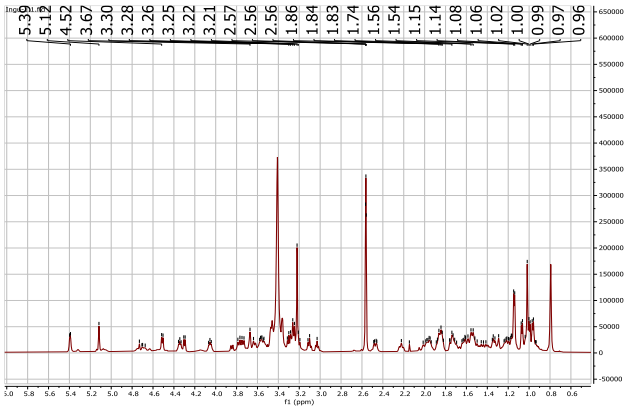


**Fig S2. 1H NMR spectrum of Balanitin 6**

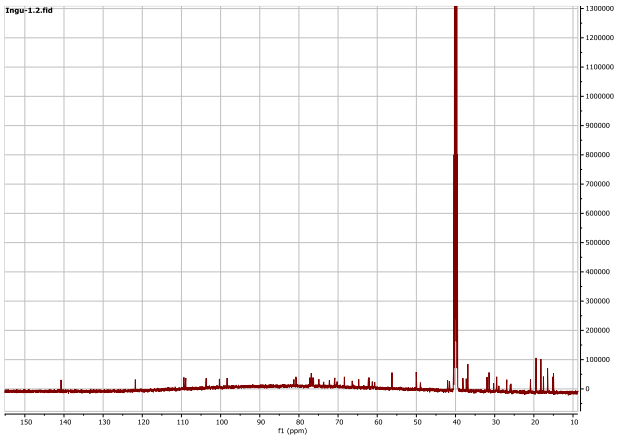
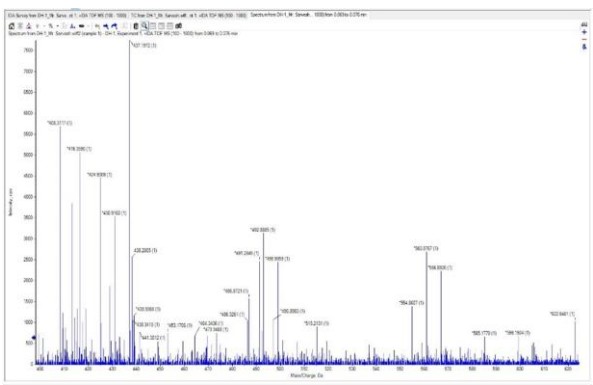


**Fig S3. 13C NMR spectrum of Balanitin 6.**



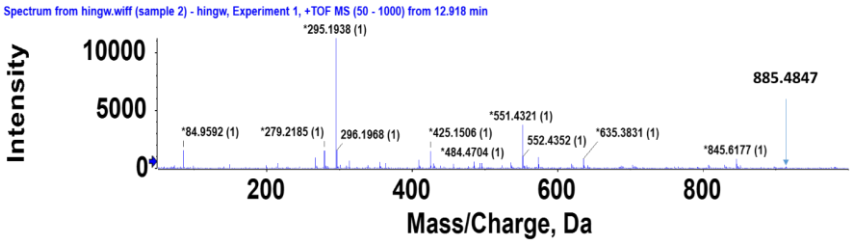



**Fig. S4. Mass spectrum of Balanitin 6**

## CONCLUSION

The findings of the present study suggest that the hydro alcoholic seed extract of *Balanites aegyptiaca* (BA) represents a promising source of bioactive constituents with antioxidant and anticancer potential. The extract demonstrated appreciable antioxidant activity and inhibited the migratory capacity of cancer cells, indicating its potential to modulate processes associated with tumour progression and metastasis. Furthermore, BA exhibited significant anticancer activity against MCF-7 and MDA-MB-231 breast cancer cell lines, as well as the FaDu cancer cell line.

The in-silico analysis further indicated that balanitin-6 (BAL-6), a bioactive constituent of BA, may exert anticancer effects through interactions associated with disruption of the actin cytoskeleton, a critical component of cellular architecture involved in cancer cell proliferation, motility, and migration. Consistent with this observation, BAL-6 treatment in MCF-7 cells was associated with the upregulation of pro-apoptotic markers, including caspase-3, caspase-8, Bax, and p53, accompanied by downregulation of the anti-apoptotic protein Bcl-2. These molecular alterations suggest the possible activation of caspase-mediated apoptotic signalling involving the p53 and Bcl-2 regulatory pathways.

Collectively, these findings indicate that BAL-6 may possess promising pro-apoptotic and antitumor potential, potentially mediated through the modulation of the caspase/p53/Bcl-2 signalling axis. The combined antioxidant, antimigratory, and apoptosis-associated effects of BA and its bioactive constituent BAL-6 provide a scientific rationale for further mechanistic investigations and preclinical evaluation of their therapeutic potential in cancer management.

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### Conflict of interest

All authors have read this manuscript and have no conflict of interest.

### Data Availability Statement:

Data from this study will be available upon reasonable request to the corresponding author.

## DECLARATIONS

### Ethical approval

The approval of this study was obtained from the Tezpur University Ethical Committee (TUEC), Tezpur University, Tezpur (Protocol no. DoRD/TUEC/10-14/4361 dated 28.03.2014) for human blood samples before starting the experiments. All experiments based on humans will be conducted according to the Ethical Guidelines of the committee.

### Consent to participate

Not applicable. This study did not involve human participants.

### Consent to publish

Not applicable. This study did not involve human participants or personal data requiring consent for publication.

### Author's contribution:

PP collected the raw drugs, formed the polyherbal formulation, drafting and reviewing the manuscript; AR, and AY was the drafting and reviewing the manuscript. SK was the principal investigator of the study and drafted the manuscript. SK and SKJ served as advisors to the study. A cytotoxicity study, ELISA of apoptotic markers, and molecular docking were performed by MD, PK, and BG, respectively. SK, AM, and SKJ contributed to the design and data analysis and assisted in the preparation of the final version of the manuscript. All authors read and approved the final version of the manuscript.

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