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Original Research

Phytochemical Screening and Anticancer Evaluation of Ethanolic Root Extract of *Asparagus gonoclados* Baker: Insight for Colorectal Cancer Treatment

Mishiriya Vallikkaparambil[1], Hibasherin Kalakkandan[1], Mohammed Saifudheen Koomankottil[1], Silmy Shareef[1], Musliyarakath Nishida[1], Jibin Joy[2], Pattilthodika Suhail[1]*

[1]Department of Pharmacology, Al Shifa College of Pharmacy, Affiliated to KUHS, Perinthalmanna, Kerala, India 679325

[2]Department of Pharmaceutical Chemistry, Al Shifa College of Pharmacy, Affiliated to KUHS, Perinthalmanna, Kerala, India 679325

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Abstract

Herbs are useful because of their therapeutic characteristics and efficacy in a variety of conditions, including cancer, when allopathic medications are ineffective. This study investigates the phytochemical composition and anticancer potential of the ethanolic root extract of *Asparagus gonoclados* Baker for colorectal cancer treatment. Phytochemical screening was conducted through preliminary phytochemical analysis and Liquid Chromatography-Mass Spectrometry (LC/MS) techniques. Molecular docking studies were performed to evaluate the interactions of the identified phytoconstituents with key molecular targets, including nuclear factor- κB (NF-κB) and phosphodiesterase which play critical roles in cancer progression. Additionally, the cytotoxic effects of the compounds were assessed using the MTT assay on the HCT-116 colorectal cancer cell line. Phytochemical screening revealed the presence of bioactive compounds, including glycosides, flavonoids, and alkaloids, which are known for their therapeutic properties. LC/MS analysis confirmed the presence of phytoconstituents longistylin, nicofetamide, and vomicine, which have varied biological consequences. The docking analysis demonstrated strong binding affinities, with docking scores of -6.62, -6.14 and -4.84 kcal/mol for NF-κB and -7.31, -9.89 and -5.52 kcal/mol for phosphodiesterase, indicating potential inhibitory effects on these targets. *In vitro* cytotoxicity was assessed using the MTT assay on colorectal cancer cell lines (HCT-116), which demonstrated significant reduction in cell viability with an IC_{50} value of 144.1 ± 0.045 µg/mL. Microscopic analysis further confirmed morphological changes such as shrinkage, separation, membrane blebbing, and evident changes in cell shape. These findings highlight the potential of *A. gonoclados* root extract as a natural source of anticancer agents, offering insights into its mechanisms and supporting its further development as a complementary therapy for colorectal cancer.

Keywords: *Asparagus gonoclados* Baker; Colorectal cancer; LC/MS analysis; Molecular docking; *In vitro* anticancer activity

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*Corresponding Author: Pattilthodika Suhail
Department of Pharmacology, Al Shifa College of Pharmacy, Affiliated to KUHS, Perinthalmanna, Kerala, INDIA 679325
Email: ptsuhl@gmail.com

Introduction

Cancer is one of the most significant global health challenges, responsible for approximately 10 million deaths in 2020 [1]. This disease can impact multiple organs and systems in the body, with metastasis being a primary driver of its progression [2]. Among the most prevalent types are breast, lung, colorectal, and prostate cancers [3]. Risk factors such as tobacco use, high body mass index (BMI), excessive alcohol consumption, inadequate intake of fruits and vegetables, and sedentary lifestyles contribute significantly to its development. However, early diagnosis and timely intervention can lead to successful treatment and cure in many cases [3]. It contains both inherited and environmental components, originating from a mix of genetic abnormalities and extrinsic stimuli. Somatic DNA mutations and carcinogen exposure are often the starting points for the illness, which causes cellular alterations that lead to malignancy [4]. These mutations usually entail gene translocations and amplification of certain genes, leading in altered cellular behaviour and the appearance of "reformed genes" [5,6]. These altered genes, known as proto-oncogenes, are crucial in cancer formation [7,8]. Over time, repeated cell division cycles may consolidate these genetic changes, rendering them permanent and continuing the illness [9].

Colorectal cancer (CRC) ranks as the third most commonly diagnosed cancer globally, with over half of the fatalities associated with the disease occurring in more industrialized nations [10]. CRC symptoms vary depending on the tumor's location in the colon and its migration to other parts of the body. Symptoms are classified as local, constitutional, and metastatic [11]. To treat advanced CRC, new techniques are needed to supplement or replace existing therapy [12-14]. Transcription factor targeting is becoming more popular [14,15]. The transcription factor nuclear factor-κB (NF-κB) induces genes for cytokines and enzymes, which play crucial functions in different cell types [16]. Phosphodiesterases (PDEs) may have a role in the initiation and spread of colorectal cancer, according to research [17]. Research indicates that by modifying epithelial homeostasis, PDE-5 inhibitors that target cyclic guanosine monophosphate may lower the risk of intestinal cancer [18].

Medicinal plants offer diverse bioactive molecules that can target cancer pathways with high specificity and minimal toxicity. Ethnobotanical wisdom accelerates the discovery of novel chemotypes, enabling resistance mitigation and synergistic therapies. Combining traditional herbal knowledge with modern robust screening techniques fosters effective, sustainable colorectal cancer interventions while conserving ecological heritage. In several herbal preparations, *Asparagus gonoclados* Baker (*A. gonoclados*) has been utilized as a replacement for *Asparagus racemosus* wild, a unique source of Shatavari, an Ayurvedic medicinal that has been employed in ancient medical traditions including Ayurveda, Unani, and Siddha [19]. The root tubers of *A. gonoclados*, a branching sub-candent shrub with arms, have been used traditionally as an antidiabetic, diuretic, antioxidant, galactagogue, antiulcer, and antipyretic [20]. This investigation involves the identification of phytoconstituents and evaluation of anticancer activity of the ethanolic root extract of *A. gonoclados.*

Materials and Methods

Root collection and authentication

Roots of *A. gonoclados* were collected from Malappuram, Kerala, India and identified by scientist from CMPR, Kotakkal, Malappuram Kerala. A voucher specimen (Ref No: CMPR/AIF/PHG/476) was kept in the Herbarium of CMPR. The collected roots were shade dried and powdered by grinding.

Preparation of root extract

Approximately 100 g of powdered material was extracted using 500 mL of ethanol using a cold maceration procedure that was allowed to sit at room temperature for 72 hours with periodic shaking before being filtered. Using a Superfit-Rotary flash evaporator set at 40°C and 50 rpm, the solvent was removed from the filtrate. After that, the solid residue was put in a desiccator (Borosil-100 mm Flenge) for later usage. The extract was stored at 4°C and its percentage yield was 12.32 ± 0.6% w/v.

Identification of phytochemicals

Following the preliminary phytochemical tests, Liquid Chromatography Mass Spectroscopy (LC/MS) was used to identify the chemical components of Ethanolic extract of *Asparagus gonoclados* Roots (EAGR). The Agilent 6530C Q-TOF LC/MS system with HPLC 1260 Infinity II Prime LC series and a Quadrupole Time of Flight (Q-TOF) mass analyzer with an Electron Spray Ionization (ESI) source the total High-Performance Liquid Chromatography instrument was used for the LC/MS analysis. The METLIN PCDL database was used in the research to identify compounds based on mass spectrometry. At a source temperature of 140℃, full-scan mode was used from m/z 100 to 1200. With a mobile phase consisting of acetonitrile and 0.1% formic acid in water in a gradient elution, molecules are separated using a unique column C-18 reverse phased (Agilent poroshell 120 EC-C8 µm, 3.0 x 150 mm) that aids in separating molecules according to their characteristics [21]. A 40-psi nebulizer, a 325℃-source temperature, a 10 L/min nitrogen flow, and a 345°C jet stream sheath temperature were the optimized settings for the negative mode of the ESI

ionization process. Agilent Mass Hunter software (v. 10) was used to process the total ion chromatogram, which improved the analysis's accuracy [22-24].

In silico studies of phytoconstituents

Using molecular docking studies, the *in silico* anticancer activity of EAGR was examined in a virtual setting. It would be challenging to analyze large-scale processes using only experimental methods, but computational tools make this possible. Nuclear proteins, NF-κB (PDB ID; 1ZKA) and phosphodiesterase (PDB ID; 6L6E), sequences were acquired from Protein Data Bank (PDB) (https://www.rcsb.org). Target protein structures were selected based on structural quality and relevance to colorectal cancer pathways. For NF-κB, the structure 1ZKA represents the RelB dimerization domain, which is a critical component of the NF-κB family that forms homodimers and heterodimers essential for DNA binding and transcriptional regulation [25]. RelB is particularly relevant in colorectal cancer as it mediates inflammatory responses and cancer cell survival [26]. The phosphodiesterase structure (PDB ID; 6L6E) represents PDE10A, which is overexpressed in colon tumors and serves as a potential therapeutic target [27]. All structures were validated for docking reliability by reproducing known inhibitor binding modes before conducting the study. Compounds' chemical structures were sourced from PubChem (https://pubchem.ncbi.nlm.nih.gov). The protein preparation wizard carefully honed the protein structure using Prime (Schrodinger-Maestro 12.8) and Epik version 3.4. This included protonation, general minimization, H-bond optimization, ionization, and addressing loop and side chain gaps.

Target selection was based on established roles of these proteins in colorectal cancer pathogenesis and documented interactions with herbal derived compounds. NF-κB is constitutively activated in 60-80% of colorectal cancers, promoting cell proliferation and chemotherapy resistance [28]. Phosphodiesterases, particularly PDE10, are elevated in colon tumor cells and regulate cGMP/PKG signaling pathways that control β-catenin-dependent transcription [29]. The selection of these targets was further supported by literature evidence showing that phytoconstituents structurally similar to those identified in *A. gonoclados* (alkaloids, flavonoids, and stilbenes) have demonstrated binding affinity to these proteins [30,31].

In vitro anticancer activity

The MTT Assay was used carefully to examine the substances' cytotoxic effects on HCT-116 cell lines, which were acquired from NCCS Pune. Dulbecco's Modified Eagle Medium, a nourishing medium supplemented with 10% FBS (fetal bovine serum) and antibiotics, was used to cultivate cells on a 96-well plate in a sterile environment (37°C with 5% CO_2). They were exposed to different sample concentrations after a day. An MTT solution was added after treatment to evaluate cell viability; for precision, controls and blanks were used. Following the matrix's dissolution in DMSO, measurements were made using an ELISA plate reader in triplicate at 540 and 660 nm to analyze the data [32]. Using Graph Pad Prism 6 software, IC_{50} values were accurately determined, and an Olympus inverted microscope was used to take detailed pictures. The following formula was used to calculate the

$$\%\ viable\ cell = \left(\frac{Absorbance\ of\ test}{Absorbance\ of\ Control}\right) \times 100$$

percentage of viable cells:

Pharmacokinetic prediction

The Maestro program's QikProp module is a tool for determining distinct ADME (Absorption Distribution, Metabolism and Elimination) relevant descriptions [33]. It provides a fresh method for enhancing medicinal substances' pharmacokinetic profiles. ADME evaluations have been included into drug design processes earlier because of the recognition of the significance of favorable pharmacokinetic properties in successful drug development. To create relationships between 3D molecule structures and physicochemical and pharmacokinetic characteristics, QikProp was used in this investigation. To determine their minimum energy conformations, the top three molecules underwent energy minimization. These conformations were then used to calculate ADME properties.

Result and discussion

Identification of phytochemicals

The preliminary phytochemical investigation of EAGR showed the presence of glycosides, flavonoids, and alkaloids. The LC/MS phytochemical investigation identified a variety of chemical compounds with various biological implications (Table 1). Vomicine (C_{22} H_{24} N_2 O_4, m/z = 380.1742) (Figure 1), a natural alkaloid having potential anticancer activity in preclinical studies [34]. Its mechanism involves inducing apoptosis and inhibiting cancer cell proliferation [35]. Studies suggest that vomicine has the ability to influence essential signaling pathways, including PI3K/AKT and MAPK, which play a vital role in the survival and proliferation of cancer cells. Furthermore, it has been shown to induce cell cycle arrest, specifically at the G2/M phase, thereby impeding the advancement of tumors [35].

EAGR showed the presence of longistylin ($C_{20}H_{22}O$, m/z = 278.1668) (Figure 1), a natural stilbenoid molecule found in some plants and showed strong anticancer activity in preclinical trials [36]. Its anticancer

efficacy is principally due to its ability to induce apoptosis, decrease cell proliferation, and prevent tumor angiogenesis [37]. Longistylin inhibits PI3K/AKT and NF-κB signaling, which is crucial for cancer cell survival and proliferation [38]. Furthermore, it causes oxidative stress in cancer cells, which leads to DNA damage and cell death [39]. Research has demonstrated its efficacy against multiple cancer types, such as breast, lung, and colon cancer [38]. Additionally, longistylin exhibits anti-metastatic properties by suppressing the epithelial-to-mesenchymal transition, a critical process for cancer cell invasion and metastasis [36-38].

Nicofetamide ($C_{20}H_{18}N_2O$, m/z-302.1415) (Figure 1) has showed promise anticancer efficacy by inhibiting critical cellular processes involved in cancer cell survival and growth [39]. It has been shown to cause apoptosis by activating intrinsic mechanisms such as the release of cytochrome-c and caspase-3 [40]. It also changes mitochondrial membrane potential, which causes increased oxidative stress and DNA damage in cancer cells [40]. According to research, nicofetamide may block angiogenesis, the process by which tumors form new blood vessels to support their development [41]. It also inhibits the production of pro-angiogenic molecules such as VEGF, which slows tumor development [40,41]. The LC/MS analysis also showed the presence of acadesine (m/z–258.096), theasaponin B1 (m/z-1306.6003, assamsaponin A (m/z-1172.5622 and Soyasaponin A1 (m/z-1268.6035).

In silico anticancer evaluation

Longistylin had a higher molecular docking score (1ZKA -6.62 and 6L6E -7.31 kcal/mol) when it came to binding to NF-κB and PDE. It produces hydrogen bonds and hydrophobic interactions with amino acids PHE 384, TYR 211, ASP 362 and HIP 212 of 1ZKA; TYR 211, PHE 384, HIP 212, and ASP 362 of 6L6E protein (figure 2). PDEs could serve as promising targets for inhibiting tumor cell growth and inducing apoptosis, supported by several key observations [42]. Firstly, the modulation of cyclic nucleotide signaling is considered one of the multiple pathways that play a role in tumor cell dissemination and functionality. Secondly, different PDE isozymes are implicated in various types of tumor tissues. Lastly, non-selective PDE inhibitors, such as theophylline and aminophylline, have been shown to influence the growth of numerous cancer cell lines [42].

Nicofetamide also displayed greater affinity towards NF-κB and PDE enzyme (1ZKA -6.14 and 6L6E -9.89 kcal/mol) (figure 3), which suggests potential anticancer activity through inhibition of the enzymes [43]. By inhibiting PDE activity, Nicofetamide leads to an accumulation of the cyclic nucleotides, thereby disrupting signaling pathways that promote cancer cell survival and growth [44].

Vomicine also displayed affinity with proteins (Docking score; 1ZKA -4.84 and 6L6E -5.52 kcal/mol) (figure 4), indicating its anticancer activity [45]. All of the three compounds exhibited greater interaction with NF-κB and PDE proteins compared to the standard drug Sildenafil citrate (1ZKA -3.67 and 6L6E -6.38 kcal/mol). Overall, longistylin, nicofetamide and vomicine demonstrated the most favorable binding affinities, particularly with PDE, which is crucial for DNA replication and cell division in cancer cells. Table 2 displays the molecular interactions of identified phytochemicals with target proteins.

In silico ADMET prediction

Lipinski's rule of five is a guideline that predicts the effectiveness of a chemical compound as an oral drug in humans. It outlines five key chemical and physical criteria related to ADME properties, absorption, distribution, metabolism, and excretion. This rule is crucial for understanding drug behavior in the human body, making ADME modelling a valuable tool in drug development. The Qikprop tool was used to evaluate the properties of compounds tested for oral therapeutics. The partition coefficient, which measures the interaction between a drug and the body, was used to assess the properties of the compounds. The results aligned with Lipinski's rule of five, indicating promising properties for further development (Table 3). This approach accelerates drug discovery and prioritizes the most viable candidates for future research.

In vitro anticancer activity

Metastasis is a key difficulty in cancer therapy since it entails the spread of tumor cells throughout the body [46]. To fight this, effective medicines must target and diminish the invasiveness of cancer cells. The sample demonstrated cytotoxic activity against the colon cancer cell line HCT-116, showing an IC_{50} value of 144.1 ± 0.045 µg/mL (Figure 5). The comparison of the IC_{50} values with the standard drug was done with the previous studies (IC_{50} of cisplatin 30 µg/mL) [47]. Microscopic images (100×) revealed a notable reduction in cell viability following treatment with the extract compared to the untreated control group (Figure 6). The MTT assay, a well-established method for assessing cell viability, relies on the conversion of yellow tetrazolium dye (MTT) into purple, insoluble formazan crystals by metabolically active cells with functional mitochondria [48]. The results revealed that cell viability decreased as extract concentrations rose. The control group's cells seemed healthy, with prominent purple formazan crystals. This progressive decline in formazan formation demonstrates the extract's efficacy in reducing cancer cell viability.

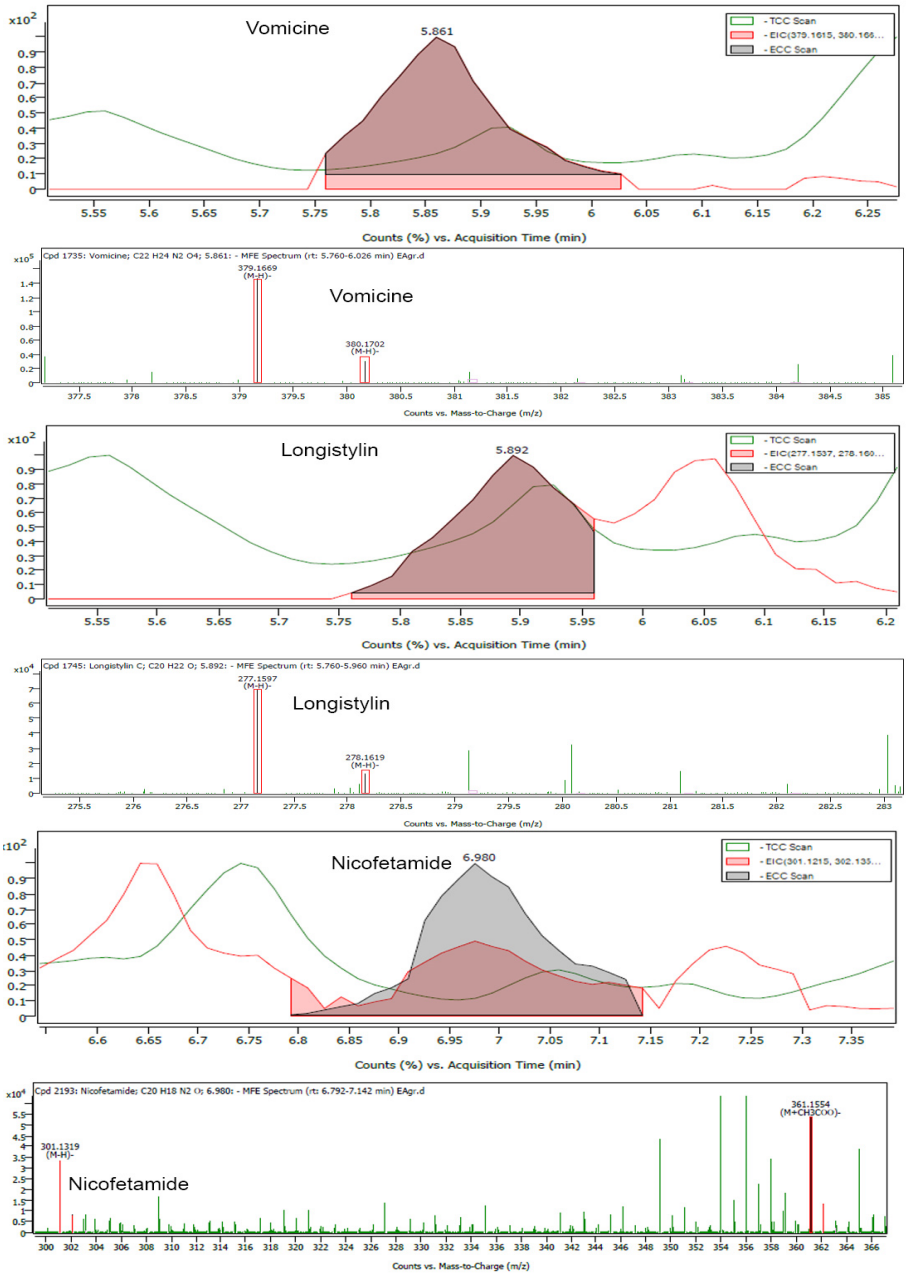

Figure 1. LC/MS chromatogram of Vomicine, Longistylin, Nicofetamide with retention time and m/z.

Table 1. Identified phytochemicals from EAGR through LC/MS analysis.

Identified Phytochemical having anticancer activity	Molecular Formula	Retention time (min)	m/z
Vomicine	$C_{22}H_{24}N_2O_4$	5.861	380.1742
Longistylin	$C_{20}H_{22}O$	5.892	278.1668
Nicofetamide	$C_{20}H_{18}N_2O$	6.98	302.1415
Acadesine	$C_9H_{14}N_4O_5$	6.574	258.096
Theasaponin B1	$C_{65}H_{94}O_{27}$	7.441	1306.6003
Assamsaponin A	$C_{57}H_{88}O_{25}$	8.878	1172.5622
Soyasaponin A1	$C_{59}H_{96}O_{27}$	9.176	1268.6035

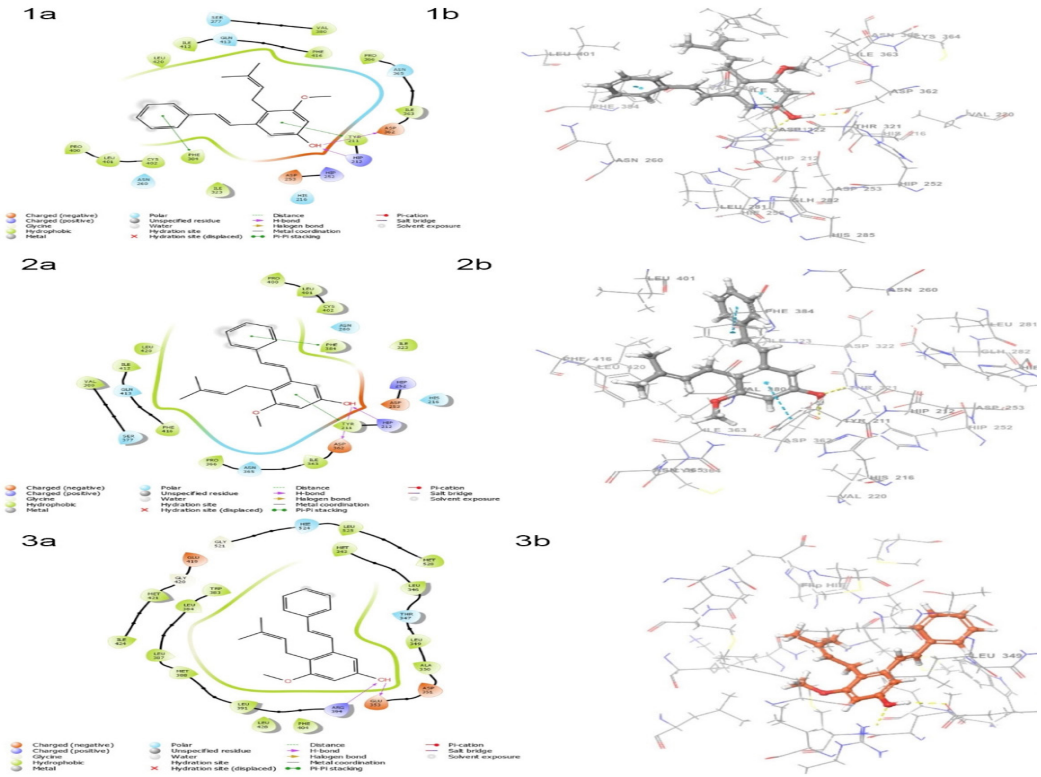

Figure 2. 2D and 3D molecular interactions of Longistylin with NF-κB (PDB ID; 1ZKA, docking score -6.62 kcal/mol) and PDE (PDB ID; 6L6E, docking score -7.31 kcal/mol) proteins.

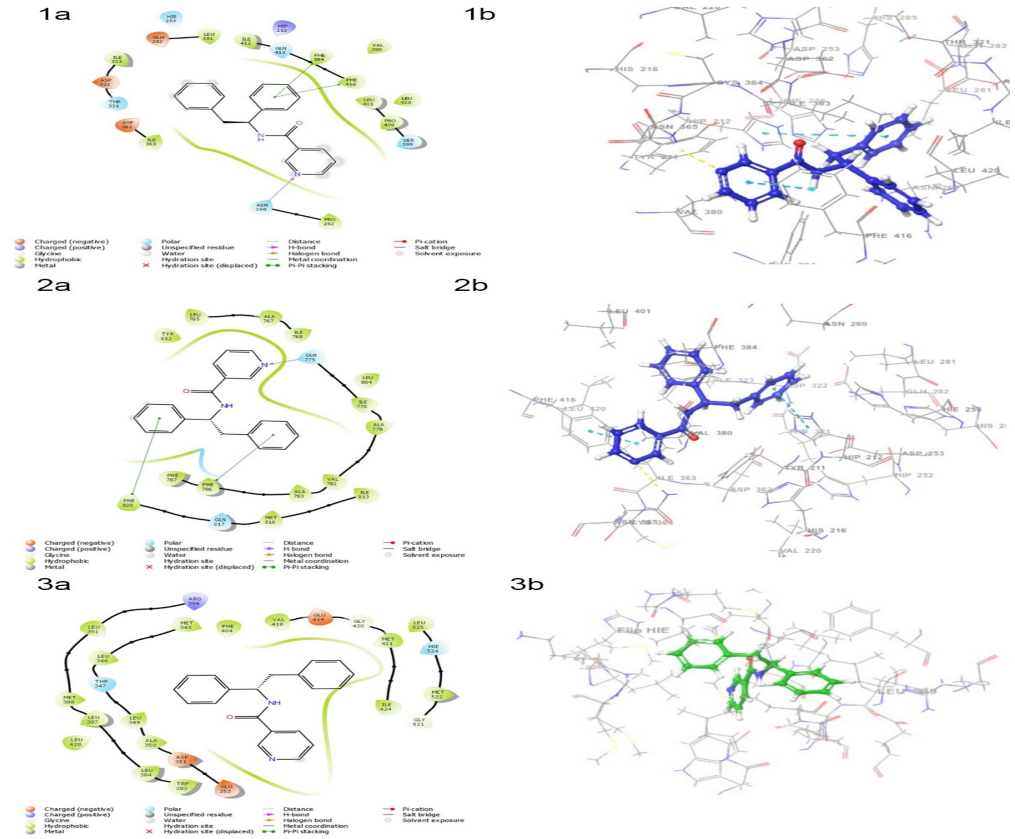

Figure 3. 2D and 3D molecular interactions of Nicofetamide with (PDB ID; 1ZKA, docking score -6.14 kcal/mol) and PDE (PDB ID; 6L6E, docking score -9.89 kcal/mol) proteins.

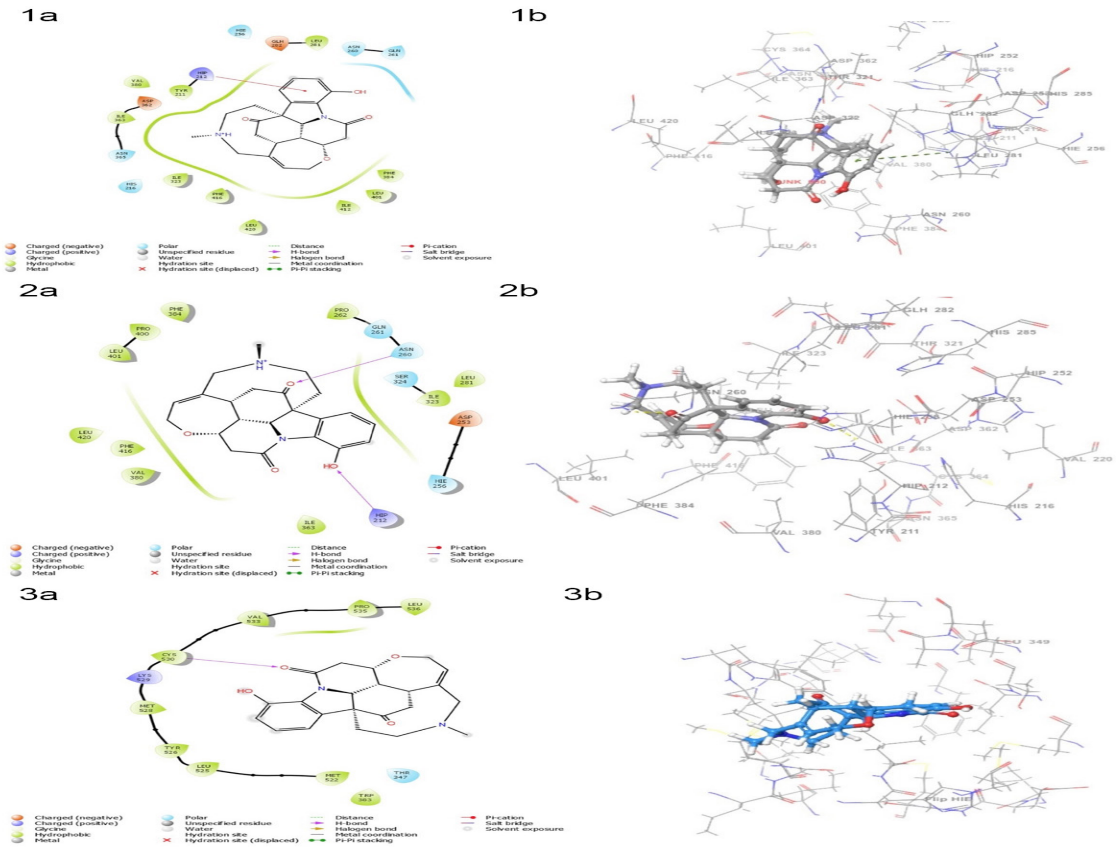

Figure 4. 2D and 3D molecular interactions of Vomicine with NF-κB ((PDB ID; 1ZKA, docking score -4.84 kcal/mol) and PDE (PDB ID; 6L6E, docking score -5.52 kcal/mol) proteins.

Table 2. Docking scores and molecular interactions of identified phytochemicals with NF-κB (1ZKA) and PDE (6L6E) proteins.

Compounds	Docking score (kcal/mol)		Residues participating in intermolecular hydrogen bonds with ligand		Residues participating in hydrophobic interaction with ligands	
	1ZKA	6L6E	1ZKA	6L6E	1ZKA	6L6E
Longistylin	-6.62	-7.31	PHE 384, TYR 211, ASP 362, HIP 212	TYR 211, PHE 384, HIP 212, ASP 362	PHE 384	PHE 384
Nicofetamide	-6.14	-9.89	PHE 384, PHE 416, ASN 260	PHE 786, PHE 820, GLN 775	PHE 384, PHE 416, ASN 260	PHE 820, GLN 775
Vomicine	-4.84	-5.52	-	ASN 260, HIP 212	-	ASN 260
Sildinafil Citrate	-3.67	-6.38	PHE 416, GLN 261, TYR 211	PHE 416, TYR 211, GLN 261	GLN 261	-

Table 3. Physicochemical properties of compounds in EAGR.

Compound	QPlogPO/W[1]*	QPlog Pw*	QPlog Kp*	QPlogBB[5]*	Donor HB*	Accpt HB*	Percent Human Oral Absorption*
Longistylin	-0.871	24.379	-6.293	-4.123	5.41	15.7	3.39
Nicofetamide	1.067	7.991	-5.182	-1.465	1.65	4.25	68.81
Vomicine	-0.019	8.423	-4.066	-0.626	1.03	5.34	72.963
Sildinafil Citrate	-0.54	26.865	-0.844	-5.175	7.26	11.95	5.54

*The phytochemical properties include octanol/water partition coefficient (QPlogPO/W), water/gas partition coefficient (QPlog Pw), brain/blood partition coefficient (QPlog Kp), donor hydrogen bond count (Donor HB), acceptor hydrogen bond count (Accpt HB), and percentage of human oral absorption.

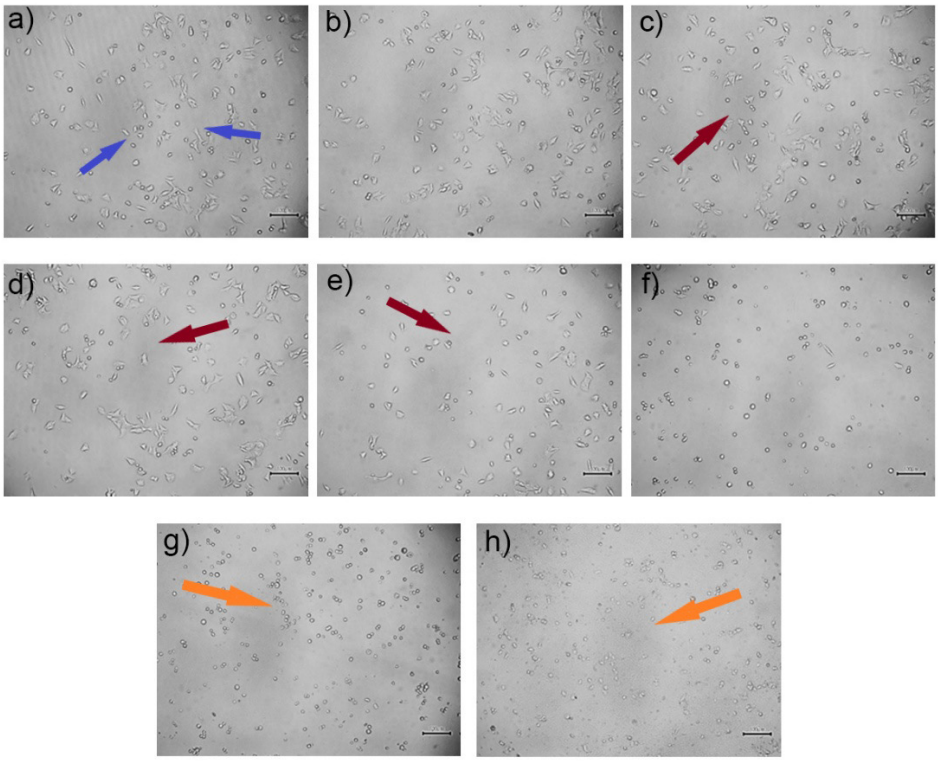

Figure 5. Cell viability of different concentrations of EAGR against HCT-116 cell lines. The IC_{50} value was found to be 144.1 ± 0.045 µg/ml. Untreated cells was kept as control. The values represented in mean ± SEM.

This preliminary screening was conducted using HCT-116 as the primary model system based on established scientific rationale. HCT-116 is widely recognized as a representative and well-characterized colorectal cancer cell line that exhibits many molecular features and genetic mutations typically observed in CRC patients, including microsatellite instability (MSI) and mutations in key oncogenes [49]. As an initial screening approach, the use of a single, well-validated cell line is consistent with standard practices in preliminary anticancer drug discovery, where HCT-116 serves as a benchmark model for colorectal cancer research [50,51]. This approach allows for controlled, reproducible assessment of cytotoxic potential before advancing to more comprehensive multi-cell line studies [52].

Conclusion

In summary, the phytochemical analysis and anticancer evaluation of the ethanolic extract from *A. gonoclados* roots revealed significant potential as an anticancer agent, particularly against the HCT-116 colorectal cancer cell line, with an IC_{50} value of 144.1 ± 0.045 µg/mL. Molecular docking studies highlighted strong binding interactions between key bioactive compounds, such as longistylin, nicofetamide and vomicine, and critical molecular targets associated with cancer progression. The presence of these naturally

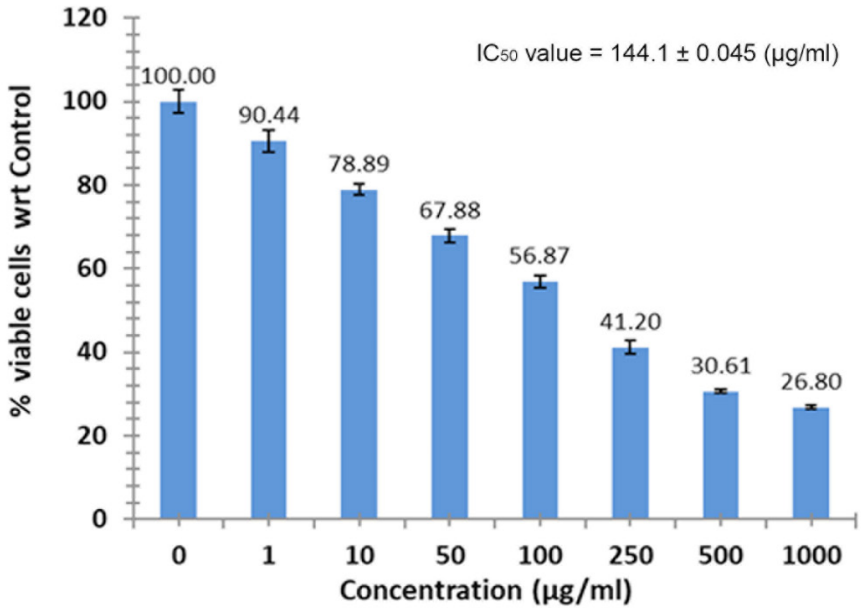

Figure 6. The photomicrograph depicts the morphological changes induced by EAGR therapy in HCT-116 cells. (a) Untreated cells, (b) 1 µg/mL, (c) 10 µg/mL, (d) 50 µg/mL, (e) 100 µg/mL, (f) 250 µg/mL, (g) 500 µg/mL, and (h) 1000 µg/mL for 24 hours. The image shows considerable changes produced by the extract treatment, such as shrinkage, separation, membrane blebbing, and evident changes in cell shape (red and orange arrows). In contrast, the control group retains its original structure (blue arrow), demonstrating the treatment's influence on cellular integrity and behavior. These visual contrasts highlight the extract's effects in an arresting and engaging way.

occurring compounds in *A. gonoclados* suggests a novel strategy for colorectal cancer treatment, underscoring the value of natural products in oncology. This approach could serve as a complementary or alternative therapy, potentially reducing side effects compared to conventional treatments. However, further research, especially *in vivo* studies, is essential to fully assess the therapeutic potential of these compounds and their role in enhancing current cancer treatments.

Conflict of Interests

None.

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