Advances in Bioresearch
Adv. Biores., Vol 15 (2) March 2024: 81-92

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.2.8192

Advances in Bioresearch

REVIEW ARTICLE

Adeptness of Andrographolide and Its Analogs in Colorectal Cancer: A Review

Arigela Srikar Babu[1], Shaik Karimulla[2], Akkiraju Sudheer [1]*, Kanala Somasekhar Reddy[1]
[1]Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research (RIPER)-Autonomous, Anantapur, Andhra Pradesh, India-515721.
[2]Department of Pharmaceutical Analysis, Raghavendra Institute of Pharmaceutical Education and Research (RIPER)-Autonomous, Anantapur, Andhra Pradesh, India-515721.
Address for correspondence: Email: sudheercology@gmail.com

ABSTRACT
Colorectal cancer (CRC) is the world's second most lethal malignancy and the third most common sarcoma. The vast majority of CRCs develop gradually from adenomatous cysts or benign cysts. CRC is the major reason for cancer-related deaths globally, accounting for approximately 10 percent of total cancer diagnoses annually. Furthermore, while the prevalence and lethality of CRC had reduced as a consequence of the evolution of more effective medicines and improved diagnostic tests, the burden remains, and new CRC prevention strategies are necessary. Traditional therapies like surgery, chemotherapy, and radiation have been used for decades. Yet, in addition to its benefits, chemotherapy has significant downsides. In this context, a range of plant-derived bioactive compounds is employed in ethnomedicine to replace these conventional therapies. Andrographolide (AG), a diterpenoid that is the main component of Andrographis paniculata, has piqued the interest of various academic researchers due to its broad spectrum of pharmacological activities. Because of the AG phytochemical template's responsiveness to numerous synthetic transformations, several analogs with robust biological activities in vitro and in vivo have been developed. This review aims to depict the efficacy of andrographolide and its analogs in the fight against CRC and provide a quick overview of conventional therapies used in CRC.
KEYWORDS: *Andrographolide, Colorectal cancer, Phytochemical, Conventional therapies.*

Received 24.12.2023 Revised 01.01.2024 Accepted 11.02.2024

How to cite this article:
Arigela Srikar B, Shaik K, Akkiraju S, Kanala Somasekhar R. Adeptness of Andrographolide and its Analogs in Colorectal Cancer: A Review. Adv. Biores., Vol 15 (2) March 2024: 81-92.

INTRODUCTION

Colorectal cancer (CRC) is the world's second-leading lethal malignancy and is also the third most prevalent sarcoma [1]. Most CRCs develop gradually from benign or adenomatous cysts [2]. Globally, CRC is liable for around 10% of all cancer diagnoses and cancer-related deaths [1]. It is the second most prevalent kind of cancer in women and the third most common type in men. Women have a 25% lower rate of occurrence and lethality than males do. In 2035, it is anticipated that there would be 2–5 million newly diagnosed cases of malignancy worldwide as a result of continued development in emerging countries [3, 4]. CRC presently has an annual incidence rate of 38.7 per lakh of individuals and 13.9 deaths [5]. Older age, male sexuality, familial, ecological, socioeconomic, dietary, physical exercise, tobacco, and lifestyle variables may increase the chance of CRC [6, 7]. Typically, 71% of CRC tumors are found in the colon and 29% in the rectum[8]. However, the acceptable risk of the population accounts for the majority (around 70%) of CRC cases (sporadic), individuals who have a family history of the disease may account for up to 25% of occurrences and inherited genetic colorectal cancer syndromes account for around 10% of cases [9]. The incidence and death of CRC have decreased as a result of the advancement of more efficient therapies and improved diagnostic testing, but the burden still exists and new CRC preventative measures are required [10]. Chemotherapy and surgery are typically used to treat CRC. Chemotherapies can kill cancer cells by damaging their DNA or activating several different signaling pathways. The cell cycle, translation, and DNA repair are a few of these signaling cascades [11]. However,

the sort of cancer that CRC patients have affects the efficacy of anticancer medications. Past research, particularly MPE investigations, has demonstrated this. Cytotoxicity, tolerance to therapy, and patient suffering are the three primary concerns with chemotherapy [12]. A range of plant-derived bioactive compounds is used in phytotherapy as an alternative to chemotherapy, radiation therapy, immunotherapy, targeted treatment, and surgery because of their anti-tumor and chemoprotective activity and low risk of side effects whilst being employed to treat colon cancer [13]. In this regard, Andrographolide (AG), the main diterpenoid component of *Andrographis paniculata*, has drawn considerable interest from several academic researchers for its broad range of pharmacological actions. Given its structure's adaptability to several synthetic transformations, many analogs with strong biological functions both *in vitro* and *in vivo* have been produced using the AG phytochemical template [14]. Also as per numerous investigations, AG has been widely used as a tool in the battle against cancer [15]. On account of this, the present review discusses Conventional therapies and the effectiveness of andrographolide and its derivatives in the treatment of CRC.

ETIOPATHOGENESIS

The mechanism by which a benign polyp spreads and turns into a deadly adenocarcinoma from a healthy, normal colon epithelium is discussed in detail. Fearon and Vogelstein claim that several epigenetic and genetic aberrations have accumulated in important genes involved in silencing tumor suppressor genes (TSGs) and activating oncogenes, leading to CRC. In this context, two main routes for CRC development were identified. One route includes suppressing the expression of TSGs and adenomatous polyposis coli (APC). This is mutated in FAP patients' germlines and accounts for 85% of all CRC. The second route, which is responsible for roughly 15% of total rare instances and HNPCC syndrome, involves the mutational silencing of MMR-related proteins (MSH2, MLH1, and PMS2) [16-18]. Three other main mechanisms, including CIN, MSI, and CpG island methylator phenotype (CIMP), are linked to the etiopathogenesis of CRC [19]. Of now, the American Joint Committee on Cancer (AJCC) recommended the tumor-nodes-metastasis (TNM) paradigm as the main foundation for CRC staging [20]. Fig. 1, Created with BioRender.com

***Chromosomal instability* (CIN):**

The most frequent genetic instability in CRC is chromosomal instability, defined as a substantial rise in the addition or deletion of either the whole or major sections of chromosomes. Almost 85% of adenocarcinoma transitions [21, 22] have CIN, which is depicted by oncogene activation (KRAS and BRAF), TSG silencing (APC and TP53), and loss of heterozygosity for the long arm of chromosome 18 (18q LOH) shows the presence of many TSGs, including SMAD2, SMAD4, and Deleted in Colorectal Carcinoma (DCC), which encourages the development of CRC lesions [21, 23, 24]. Fearon and Vogelstein presented a multi-phase genomic paradigm where the primary stage is the suppression of APC, followed by KRAS genetic alterations mostly in the adenoma phase, and the last stage is the deletion of chromosome 18q and inhibition of TP53 on chromosome 17p upon escalation to carcinoma. [16, 24, 25]. Moreover, it has recently been discovered that the adenocarcinoma sequence model includes genetic abnormalities in TGF-R and PI3KCA [26, 27]. Moreover, both familial and sporadic CRCs have a strong APC/β-catenin/Wnt-Tcf pathway expression signature [28].

Microsatellite instability (MSI):

Another form of genomic instability is defined by tandem repetitions in repetitive DNA sequences of 1 to 5 base pairs. Around 15–25% of Sporadic CRC cases and 95% of Lynch syndrome cases are caused by MSI [29]. Moreover, genetic variations in one of the MMR genes (MLH1, MSH2, MSH6, and PMS2) usually cause MSI CRCs in patients with Lynch syndrome; whilst defects in the MLH1 or MSH2 genes raise the odds of getting cancer (70-80%), MSH6 or PMS2 genetic abnormalities have a relatively modest risk (25-60%) [30]. Nevertheless, sporadic MSI CRCs commonly exhibit failure of MMR function as a result of aberrant DNA methylation that silences MLH1 [31, 32]. The TGF-Receptor II (TGF-RII) has mutations in more than 80% of MSI-CRC cases [33]. TGF-RII mutations are a frequent source of neoplastic development in the late and metastatic stages of MSI-High CRCs and are identified in adenomas that either have severe dysplasia or advance to malignancy [34]. Moreover, MSI-high CRCs frequently include mutations in the TGF- pathway's Smad2 and Smad4 genes [34]. Another target in the MSI-high CRC pathway is the pro-apoptotic protein TSG BAX [35]. Despite early adenoma mutations, BAX gene variations like TGF-RII aberrations might appear in oncogenic advancements [36]. Additional genes, which are seen in MSI-high CRC at a low-frequency range (roughly 20%), include alterations in the MMR genes hMSH3 and hMSH6, Insulin Growth Factor Type 2 Receptor (IGF-IIR), BLM gene, PIK3CA, G protein-coupled receptor of Prostaglandin-endoperoxide synthase 2 (PTGS2), and Cyclin D1 [37].

CpG island methylator phenotype (CIMP):

CpG island methylator phenotype (CIMP) is the 3rd avenue via which CRC develops [37]. It is now understood that transcriptional deactivation caused by DNA hypermethylation at TSG promoter CpG islands, which results in gene silence, is a key factor in the development of tumor formation [38]. Depending on the amount of methylation indicators, the CIMP phenotype may also be divided into CIMP-high and CIMP-low groups [39]. Shen and colleagues examined the genetic as well as epigenetic aberrations in 97 original CRC specimens and discovered that CIMP-high tumors are linked with MSI progress (80%) and BRAF alteration (53%); CIMP-low tumors are related to KRAS alterations (92%); and CIMP- tumors have a greater incidence of p53 mutation (71%) [40].

CONVENTIONAL TREATMENTS OF COLORECTAL CANCER

Depending on the stage, respectability, biology, comorbidity, and patient's condition, a bunch of treatment interventions, including surgical procedures, radiotherapy, and chemotherapy, have been employed clinically for treating cancer. These treatment options may be used alone, in combo, or gradually [41].

Endoscopic Procedure:

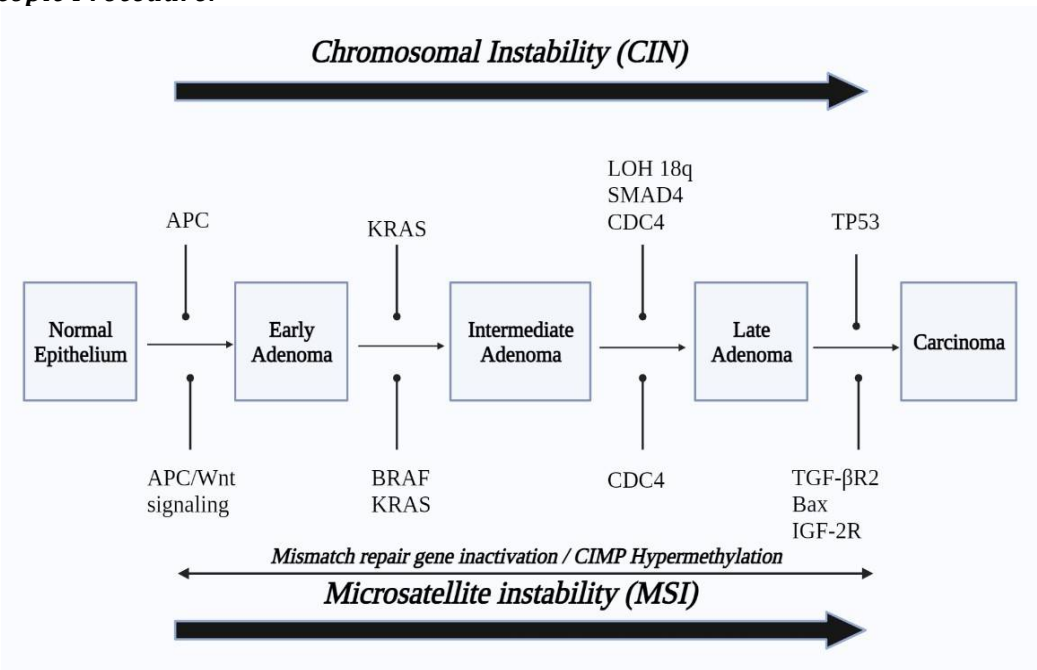

Figure 1: Mechanism of CRC progression

New flexible

endoscope technology and other endoscopic tools have raised a demand for less invasive procedures and broadened their applications [42]. En-bloc endoscopic mucosal resection, endoscopic submucosal dissection, and endoscopic full-thickness resection are suitable endoscopic resection methods for T1 tumors, depending on the tumor size [4]. When carried out by skilled endoscopists, endoscopic excision is both safe and more affordable than surgical procedures [43].

Surgery:

Although chemotherapy, radiation therapy, and immunotherapy have developed quickly, surgery remains the mainstay of potential treatment. Surgical resection is the sole treatment option for CRC that has progressed to this stage. Hence, it is crucial to enhance surgical alternatives for treating advanced CRC [4, 44].

Radiotherapy:

To shrink the tumor and protect the anal sphincter, radiation may be given to patients with loco-regional rectal cancer either as an adjunctive treatment after surgery to avoid relapse or prior to operation. Hospice care is available for people with problematic tumors and advanced or metastatic CRC to help them feel better and live longer [45].

Chemotherapy:

Chemotherapy for CRC is generally categorized as a treatment for unresectable or recurring instances, postoperative chemotherapy, and preoperative chemotherapy [45]. The following are examples of frequently used anticancer medications that the Japanese National Health Insurance will pay for and that have been licensed for the treatment of CRC:

Cytotoxic medications include capecitabine, irinotecan hydrochloride hydrate, oxaliplatin (OX), trifluridine/tipiracil hydrochloride (FTD/ TPI), fluorouracil (5-FU), 5-FU + levofolinate calcium (l-LV), tegafur uracil (UFT), tegafur gimeracil oteracil potassium (S-1), UFT + calcium foli Bevacizumab (BEV), ramucirumab (RAM), aflibercept beta (AFL), cetuximab (CET), panitumumab (PANI), regorafenib hydrate (REG), encorafenib, and binimetinib are examples of molecularly targeted medications.
Pembrolizumab is an inhibitor of immune checkpoints (Pembro).
Based on a meta-analysis, neoadjuvant chemotherapy may prolong survival in patients suffering from locally advanced colon cancer compared to adjuvant chemotherapy without worsening surgical morbidity [46]. Additionally, it has been suggested that total neoadjuvant chemotherapy (TNT), also known as preoperative chemotherapy combined with chemoradiation, offers similar advantages [47]. Fluoropyrimidine-based chemotherapy increases survival in stage III tumors that have been surgically removed and in a fraction of high-risk stage II colon cancers. Adjuvant chemotherapy spanning six months has been the norm for years. However, the IDEA team discovered that for patients with stage III colon tumors with at least modest risk, limiting drug treatment to three months could lessen toxicity (like decreased progressive neuropathy) while not affecting therapeutic efficacy [48].

Andrographis paniculata

Andrographis paniculata (Burm. f) Nees is an annual plant that belongs to the Acanthaceae family and is colloquially referred to as the "king of bitters". It is found throughout tropical and subtropical Asia, as well as in South-eastern Asia and India. A. paniculata is also known as Kalmegh in India, Chuan-Xin-Lian in China, Fah Tha Lai in Thailand, *Hempedu bumi* in Malaysia, Senshinren in Japan, and "green chiretta" in Scandinavian countries [49-52]. This plant is acknowledged as a popular remedy for several disorders, including liver problems, according to both Indian pharmacopoeia and Ayurvedic medicine. It is available in over 26 unique polyherbal formulations [49, 53].

PHYTOCHEMICAL CONSTITUENTS OF Andrographis paniculata

Andrographis paniculata includes a substantial number of flavonoids, labdane diterpenoids, stigmasterols, xanthones, quinic acids, noriridoids, and polyphenols, as per empirical evidence on its phytochemistry [49-52]. Diterpene lactones (deoxy andrographolide, andrographolide, neoandrographolide, and 14-deoxy-11, 12-didehydroandrographolide), diterpene glucosides (deoxyandrographolide19β-d-glucoside), and flavonoids (5,7,2´,3´-tetramethoxy flavanone and 5-hydroxy-7,2´,3´-trimethoxy flavone) are among the bioactive constituents found in leaves of A. paniculata [54]. More importantly, Labdane diterpenoids, which may be found in free and glycoside forms, are the primary ingredients of A. paniculata. The labdane diterpenoids include andrographolide and andrographiside, neoandrographolide,6 acetyl-neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, 14-deoxy-11,12-didehydroandrographiside, 14-deoxy-andrographolide, 14-deoxyandrographiside, andrographanin andropanoside, isoandrographolide andrographatoside, andropanolide and bis-andrographolides A, B, C, and D[6] [50].

ANDROGRAPHOLIDE AS A KEY PHYTOCHEMICAL

Ent-labdane andrographolide (AG) is extracted from the annual plant *Andrographis paniculata* (Burm. f.) Wall ex Nees is one intriguing natural substance that has garnered substantial attention in recent years [55]. The positive benefits of the plant extracts are thought to be caused by andrographolide, which makes up 1.38 to 3.12% of A. paniculata in dried forms [56]. Andrographolide has been the object of study interest due to its broad range of medicinal prospects ever since its initial discovery in 1951 [57]. Due to the plant's well-known intense bitterness, it is frequently referred to as the "King of Bitters" [58]. It is also known by its chemical name, 3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylene-1-napthalenyl] ethylidene] dihydro-4-hydroxy-2(3H)-furanone]. It demonstrates a staggering variety of biological processes [58]. As per assertions, the mere existence of α-alkylidene γ-butyrolactone, the D12 (13) double bond, the C-14 hydroxyl, and the D8 (17) double bond, as well as other compounds, is what gives AG its vicious impact on various cancer cell types [59]. Remarkably, the only strategy to halt the tumor cells from multiplying was to extract A. paniculata from methanol and then into a dichloromethane fraction. The methanolic extracts were further fractionated into AG, which was the most efficient method of combating cancer [60].

BIOACTIVE AGENT ANDROGRAPHOLIDE

AG has been associated with a wide range of pharmacological effects as a bioactive component, and its treatment toolkit is always broadening. Ayurveda, Unani, Siddha, and traditional Chinese medicine have all noted the medicinal benefits of this substance. It has properties that are hepatoprotective, anti-HIV, antigenotoxic, pro-apoptotic, cardioprotective, anti-obesity, antioxidant, antipyretic, anti-diarrheal, anti-

leishmanial, anti-fertility, choleretic, anti-allergic, antibacterial, antifungal, and antiviral. It additionally possesses properties that are anticancer, anti-HIV, antigenotoxic, and immune response [61-63]. Relying on toxicity studies conducted using experimental models, it is also proven as a safe chemical. To realize their full potential in the pharmaceutical industry, research is still being done on evaluating phytochemicals for further therapeutic properties and drug action mechanisms [52, 64, 65].

ANTI-CANCER PROPERTIES OF KING OF BITTERS

Globally, the rising number of cancer-related deaths is a severe problem. Proliferation, resistance to growth-inhibitory signals, insensitivity to apoptosis, progression of angiogenesis, invasion, and metastasis are the six traits of cancer that make cancer cells immune-resistant. The growth and development of cancer are significantly influenced by the tumor microenvironment [66]. Phytoconstituents are important anticancer agents that regulate cancer-related pathways that cause cancer [67]. As demonstrated by a variety of investigations, AG affects the signaling pathways that regulate the cell cycle, apoptosis, and adhesion of cells [68]. As a result, AG can be utilized to treat malignancies, including CRC, by acting as an anticancer agent [69].

ROLE OF ANDROGRAPHOLIDE & ITS ANALOGS IN COLORECTAL CANCER

A gruelling study carried out in the past on the phytochemical AG gives us in-depth insights into how efficient it is in treating multiple medical conditions. It also inspires us to look more into AG and its derivatives in the hopes of advancing the research. The significance of AG and its analogs in colorectal cancer is primarily addressed in this section. In a *Somrudee reabroi et al*. study, the HT29 colon cancer cells were subjected to a silyl andrographolide analog **3A.1**'s (19-tert-butyldiphenylsilyl-8, 17-epoxy andrographolide) anti-cancer potential. The drug inhibited Wnt/β-catenin signaling using a GSK-3-dependent route. It lowered the expression of Wnt genes of interest implicated in carcinogenesis and cell cycle progression, resulting in decreased cell survival and growth and, ultimately, apoptosis. [70]. SRS07, one of the AG derivatives, showed potential in vitro anticancer properties in cancerous cells such as HCT116 cells by producing G1 phase cell cycle arrest that ultimately led to apoptotic death via triggering caspase 8 and Bid [71]. The finest derivative was SRS07, exhibiting GI_{50} values between 0.8 to 1.7 μM. The first-generation AG derivatives' primary anticancer molecule was SRJ09 [3,19-(2-bromobenzylidene) andrographolide], whereas SRS07 was SRJ09's acetylated product. After being treated with SRJ09 and SRJ23 for 48 hours, a sizable number of HCT-116 cells displayed severe nuclear consolidation and breakage, which are indications of apoptotic cell death. HCT-116 cells that were treated with 3,19- (3-chloro-4-fluorobenzylidene) andrographolide (SRJ23) displayed G1 phase arrest and induction of the sub-G1 population, which are signs of apoptotic cells. Consequently, phase-specific cell cycle inhibitors and effective apoptosis inducers may be created by including a benzylidene pharmacophore at 3,19 locations in the andrographolide scaffold [72]. AG derivatives 3,19-(2-bromobenzylidene) and 3,19-(3-chloro-4-fluorobenzylidene) exhibited greater cytotoxicity and had growth-inhibiting effects on MCF-7 and HCT-116 cell lines. In xenografts, the 3,19-(2-bromobenzylidene) andrographolide stimulated p21 expression and decreased Cdk-4 expression without impacting Cyclin D1 to cause G1 arrest at concentrations of 100–400 mg/kg (i.p.) in HCT-116 and MCF-7 cancer cells [72]. Moreover, in vitro, studies have demonstrated that SRJ09 can sparsely penetrate the DLD-1 colon cancer multicell layer (MCL) and induce significant cytotoxic effects (IC_{50} = 41 μM, which is four times lesser than that of AG) [73]. In a work by Wang and associates [74], it was demonstrated that AG directly binds and stabilizes Bax by not affecting its mRNA level. Treatment with AG (10 mM) dramatically raised Bax expression and concurrently improved 5-FU-induced cell death in a 5-FU-resistant HCT116 cell line (HCT116/5-FUR). Other processes, such as increased expression of the Bax: Bcl-2 protein ratio, caspase induction, and greater interaction of death ligand with receptors that promote cytochrome c release might potentially contribute to the synergistic impact [75].By suppressing Notch signaling, the growth of SW-480 cell lines is halted by AG. Upregulation of ROS causes SW-480 cells to become stuck in the G0/G1 stage of the cell cycle. Besides, B-cell lymphoma-2 (Bcl-2) expression is suppressed, whilst the Bax is increased [76]. AG, either alone or in conjunction with cisplatin, promoted apoptosis in CRC Lovo cells by increasing Bax, Bcl-2 expression, and Fas/FasL interaction, which boosted cytochrome c release and activated caspases [75]. Another research by Hsueh-Ping Chao et al. examined MMP2 expression and action in CT26 and HT29 cell lines following AG treatment (0.3–3 μM). The results demonstrated that AG exposure reduced MMP2 activity in a dose-dependent approach, with the largest impact being attained at 3 μM in both without changing the expression of the MMP2 protein. Similar to what was shown with CT26 cells, AG inhibited the HT29 cells' capacity for invasion [77]. Sumit Kumar Dey et al. tested the cytotoxicity of halogenated Di-spiropyrrolizidino oxindole (viz. proline series) analogs of andrographolide (CY2, CY14, and CY15) in research by employing an array of six lineages of human cancer cells from various origins, that involves HCT116 cell lines. Their most powerful derivative was CY2, with a GI_{50} on HCT116 of 10.5 M.

In addition, it was discovered that after 36 hours of exposure to CY2 at increasing concentrations in HCT116 cells, the MMP level had decreased. MMP disruption was not very severe in cells treated with AG. This work shows that the caspase-mediated mitochondrial pathway is the mechanism through which both andrographolide and CY2 cause apoptosis in HCT116 cells. CY2 disrupted MMP to a greater extent than andrographolide [78]. AG promotes ER stress and death in colon cancer cell lines (T84, HCT116, and COLO 205), thus inhibiting the uncontrolled development of neoplasia, according to research by Banerjee et al. [79]. The antiproliferative effects of AG on the colon cancer SW620 cell line were described by Zhang et al. AG, a potential treatment for CRC, suppresses the signaling pathways for TLR4, MyD88, NF-κB-p65, and MMP-9 [80]. According to Henhena *et al*., AG's antioxidant properties drove it to suppress the activity and expression of the flag genes for CRC progression. The effectiveness of AGP as an epigenetic as well as genetic modulator was demonstrated. The chemopreventive efficacy of AG on CRC in fighting the carcinogen azoxymethane was also observed [81]. Similarly, AG has been studied for efficacy in treating CRC, either separately or in association with capecitabine, due to its considerable pharmacological properties and encouraging preclinical study findings (NCT01993472). The inclusion of AG in clinical studies unambiguously supported its significant contribution [82]. In intense mechanistic research by Kandanur *et al*., compound **3g** (3,19-diacetyl-12-phenylthio-14-deoxy-andrographolide), a powerful cytotoxic agent towards the HCT-116 cell line indicates that the molecule is a powerful cell cycle suppressor and apoptosis activator [83]. Additionally, **3c** (3,19-diacetyl-12-(2-methylthio) phenyl-amino)-14-deoxy-andrographolide), an effective cytotoxic compound with an equivalent mechanism, was discovered through testing the newly synthesized 3,19-diacetyl-C-12-substituted-14-deoxy-andrographolide derivative products **3a-f** against the HCT-116 cell line [84]. Besides, the substances namely AG, neoandrographolide, 14-deoxyandrographolide, and 14-deoxy-12-hydroxy andrographolide showed the maximum cytotoxicity on the colon cancer cell lines HT-29 and HCT-116 as well as notable and substantial antiproliferative effect on HCT-116 [85]. According to Miaomiao Yuan *et. al*, by inhibiting NADPH oxidase, ROS, Erk1/2, P38 MAPK, NF-κB, and AP-1 upregulation in (Human Colorectal cancer) HCT-116 cells, AG successfully reduced IL-8 production as well as angiogenesis in the tumor microenvironment [86]. Imran Khan et al. described the mechanism of action of AG concerning its effects on the erratic Hh signaling pathway in HCT-116 cells. These studies demonstrate that AG inhibits colon cancer cell growth and induces apoptosis via producing ROS, destabilizing the membrane of the mitochondria, activating caspases, and altering the appearance of Bcl2 family genes. The two primary mechanisms at work are cell cycle halt and Hh signal suppression. Moreover, AG promoted apoptosis via both intrinsic and extrinsic intracellular mechanisms. In addition, it exhibits a significant decline in cell survival, migration, and cytotoxicity in HCT-116 cells in a dose and time-dependent fashion [87].

Table 1: An overview of current studies looking at the effects of andrographolide and its analogs on CRC cell lines

Author/ References	CRC Cell Lines	Effects/ Features
[70]	HT 29	Downregulated Wnt/β-catenin signaling via GSK-3 dependent pathway, Decreased Cell viability & proliferation, Apoptosis.
[71]	HCT 116	G1 stage cell cycle halt triggers caspase 8 and bid, which causes apoptotic cell death.
[72]	HCT 116	Enhanced P21 and reduced Cdk4 expression leads to G1 phase cell cycle arrest and apoptosis.
[74]	HCT 116/ 5-FUR	Upregulates Bax expression, Enhanced 5-FU- induced apoptosis.
[73]	DLD -1	Cytotoxicity
[76]	SW- 480	Downregulating Notch signaling pathway, Generation of ROS, arrest at G0/G1.
[75]	LOVO	Promotes apoptosis by upregulating Bax, Bcl2, Fas/FasL interaction,
[77]	CT26, HT29	Decreased MMP2 activity.
[78]	HCT 116	Decreased MMP2 level, Caspase-mediated apoptosis.
[79]	T 84, HCT 116, COLO 205	Increase in ER stress and cell death
[80]	SW- 620	Suppressing the TLR4/NF-KB/MMP-9 signaling axis has an anti-proliferative impact
[83]	HCT 116	Cell cycle suppressor & apoptosis activator
[85]	HCT 116, HT 29	Anti-proliferative effect & Cytotoxicity

[86]	HCT 116	Decreased IL-8 expression, Angiogenesis by inhibiting NADPH oxidase and stimulating ROS, Erk ½, P38 MAPK, NF-KB, AP-1 upregulation.
[87]	HCT 116	Inhibiting Hh signaling pathway, Cell cycle arrest, anti-proliferative effect, apoptosis, cytotoxicity, reduction in cell survival & migration.

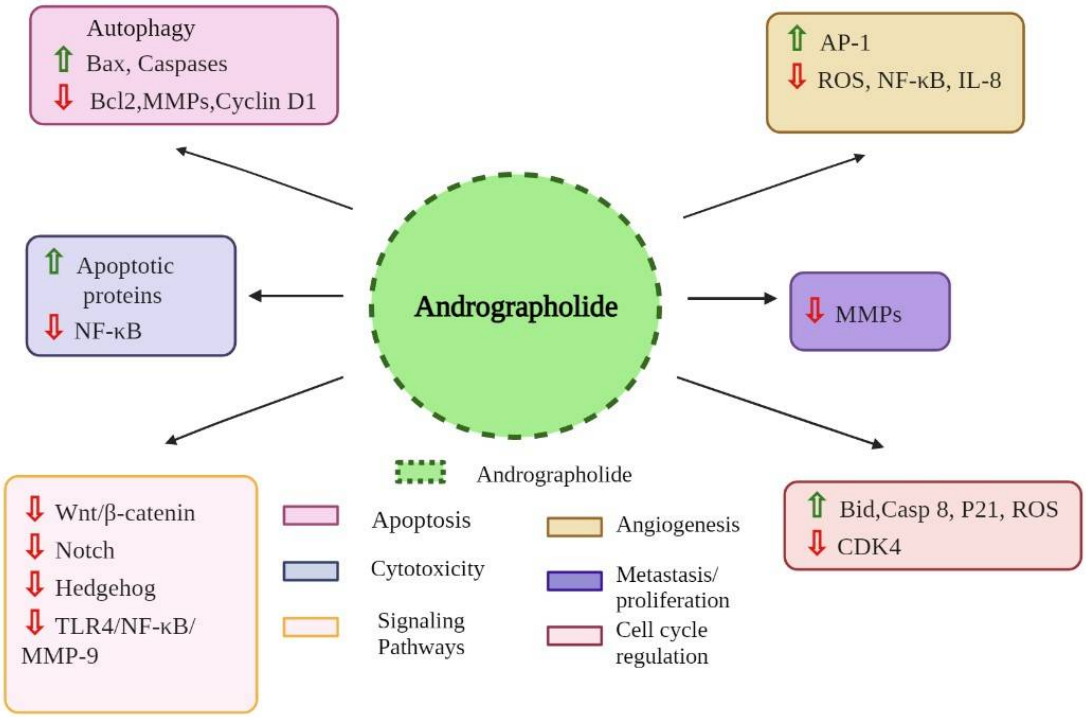

Figure 2: Various Mechanisms Acted by Phytochemical Andrographolide

We can illustrate the different processes by which the aforementioned studies on the natural weapon known as AG may work on the exposed malignant cells in a substantial way feasible. **Fig. 2** Created with BioRender.com

ANDROGRAPHOLIDE LIMITATIONS

While having excellent biological activity, AG's primary drawback is its low water solubility, which makes it difficult to formulate it for therapeutic use. The chemical has poor solubility in lipids as well. To find a better lead, several semi-synthetic analogs are being developed and tested. [52]. Its limited bioavailability, which is mostly due to its quick metabolism and clearance from the body, is a significant barrier to its clinical development [88]. Yet what is certain about AG is that several in vivo investigations, including several clinical trials, have shown it to be effective in treating a wide range of illness conditions, even at modest dosages [89].

APPLICATION STRATEGIES TO OVERCOME AG PHYTOCHEMICAL DRAWBACKS

Owing to the aforementioned restrictions, different application techniques must be developed to achieve improved therapeutic outcomes and bioavailability. The solvent's characteristics greatly impact the amount of material that can be absorbed optimally. The effectiveness of phytochemical treatments is improved by pharmaceutical dosage forms with nanostructures that employ phytomedicine as a carrier of drugs. The operation of these dosage forms is highly reliant on the solvent's ability for absorption [90]. In order to be used therapeutically, AG has been created as micro- or nanoparticles. Vesicles, polymeric nanoparticles, solid lipid nanoparticles, gold nanoparticles, nanocrystals, microemulsions, nanoemulsions, and nanosuspensions are some of the types of nanoparticles. Alginic acid, glucan derivatives, and polylactic-glycolic acid are among the microparticles. [91]. As compared to the sole suspension, the formulation of the nanoparticles enhanced the bioavailability of AG by 241%. These formulations assist in overcoming AG's low solubility in water [92].

Table 2: Application strategies to bypass andrographolide limitations

References	Formulation Type	Features/ Delivery method
[93]	Polymeric Nanoparticles (PNPs)	Delivers drugs by nanospheres and nanocapsules.
[94]	Polymeric Micelles (PM)	AG is wrapped in a micelle's composition.
[95]	Vesicles	AG has been delivered to the tumor location using particles called liposomes.
[58]	Solid Lipid Nanoparticles	Enhancing AG's absorption and effectiveness at the tumor location by encasing it in SLNs.
[96]	Nanoemulsions Microemulsions	Good water solubility and improves uptake of AG.
[97]	Mesoporous nanoparticles	Targeted medication distribution to a specific location.
[97]	Gold nanoparticles	Increasing AG's tumor-specific cytotoxicity through targeted drug delivery.
[98]	Nanocrystals and Nanosuspensions	Intended to improve AG's dissolution.

CONCLUSION

In sum, *Andrographis paniculata* (Burm. f) Nees, an herbaceous plant with several common names, is the source of the bioactive chemical known as AG. It is renowned as a well-liked phytochemical for several medicinal values comprising immunological response, antigenotoxic, pro-apoptotic, cardioprotective, anti-obesity, antioxidant, antipyretic, anti-diarrheal, hepatoprotective, and anticancer activities. According to claims, the mere existence of α- alkylidene γ-butyrolactone, the D12 (13) double bond, the C-14 hydroxyl, and the D8 (17) double bond, among others, is what gives AG its lethal effect on different sorts of cancer cells. Regardless of their low bioavailability and poor solubility, many semi-synthetic analogs are being developed and tested in search of a better lead. In this context, AG was tested against a multitude of malignant colorectal or colon cancer cells. The anti-cancer effectiveness of AG over cancer cells is compelling. Moreover, it has low to no toxicity in contrast to other CRC treatment options including chemotherapy. The fact that several in vivo studies, including multiple clinical trials, have demonstrated AG's efficacy in treating a variety of sickness conditions, even at low dosages, makes it definite about it. On this premise, AG and its semi-synthetic analogs are intriguing candidates that may serve as a starting point for synthesizing newer anti-cancer drugs. Together, this compound and its analogs have the potential to establish themselves as front-runners in the creation of an entirely novel class of chemotherapeutic medications for the treatment of colorectal cancer.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this review**.**

ABBREVIATIONS

AG	Andrographolide
APC	Adenomatous polyposis coli
AP-1	Activator protein 1
BAX	Bcl-2-associated X
BCL2	B-cell lymphoma 2
BRAF	v-raf murine sarcoma viral oncogene homolog B1
BLM	Bloom syndrome
CRC	Colorectal cancer
CIN	Chromosomal instability
CIMP	CpG island methylator phenotype
CDK-4	Cyclin-dependent kinases 4
DCC	Deleted in Colorectal Cancer gene
ERK	Extracellular-signal-regulated kinase
ER	Endoplasmic reticulum
FAP	Familial adenomatous polyposis
HNPCC	Hereditary non-polyposis colorectal cancer
Hh	Hedgehog signaling
IGF-IIR	Insulin Growth Factor Type 2 Receptor
IL-8	Interleukin 8
KRAS	Kirsten rat sarcoma
LOH	Loss of Heterozygosity

MPE	Molecular pathophysiology epidemiology
MSH2	MutS homolog 2
MLH1	MutL homolog 1
MMR	Mismatch Repair
MSI	Instability of microsatellite DNA regions
MAPK	Mitogen-activated protein kinase
MMP	Matrix metalloproteinases
MyD88	Myeloid differentiation primary response gene 88
NF-KB	Nuclear factor kappa B
NADPH	Nicotinamide adenine dinucleotide phosphate
PMS2	PMS1 Homolog 2
PI3KCA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PTGS2	G protein-coupled receptor of Prostaglandin-endoperoxide synthase 2
ROS	Reactive oxygen species
SMAD2	Suppressor of Mothers against Decapentaplegic 2
SMAD4	Suppressor of Mothers against Decapentaplegic 4
TLR4	Toll-like receptor 4
TSGs	Tumor suppressor genes
Tp53	Tumor protein p53
Wnt	Wingless-related integration site

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. ;68(6):394-424.
2. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. (1988). Genetic alterations during colorectal-tumor development. New England Journal of Medicine. 1988;319(9):525-32.
3. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. (2017). Global patterns and trends in colorectal cancer incidence and mortality. Gut. 66(4):683-91.
4. Dekker E, Tanis P, Vleugels J. A.; Kasi, PM; Wallace, (2019). MB Colorectal cancer. Lancet.394:1467-80.
5. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. (2020). Colorectal cancer statistics, CA: a cancer journal for clinicians. 70(3):145-64.
6. Thanikachalam K, Khan G. (2019). Colorectal cancer and nutrition. Nutrients. 11(1):164.
7. Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB, Maisonneuve P. (2008). Smoking and colorectal cancer: a meta-analysis. Jama.300(23):2765-78.
8. Cronin KA, Lake AJ, Scott S, Sherman RL, Noone AM, Howlader N, et al. (2018). Annual Report to the Nation on the Status of Cancer, part I: National cancer statistics. Cancer. 124(13):2785-800.
9. Hadjipetrou A, Anyfantakis D, Galanakis CG, Kastanakis M, Kastanakis S. (2017). Colorectal cancer, screening and primary care: a mini literature review. World journal of gastroenterology. 23(33):6049.
10. Katona BW, Weiss JM. (2020). Chemoprevention of colorectal cancer. Gastroenterology. 158(2):368-88.
11. Smith DL, Woodman B, Mahal A, Sathasivam K, Ghazi-Noori S, Lowden PA, et al. (2003). Minocycline and doxycycline are not beneficial in a model of Huntington's disease. Annals of neurology. 54(2):186-96.
12. Islam MR, Akash S, Rahman MM, Nowrin FT, Akter T, Shohag S, et al. (2022). Colon cancer and colorectal cancer: Prevention and treatment by potential natural products. Chemico-Biological Interactions. 110170.
13. Sharma R, Jain S.(2014). Cancer tretment: an overview of herbal medicines. World Journal of Pharmacy and Pharmaceutical Sciences. 3(8):222-30.
14. Kandanur SGS, Tamang N, Golakoti NR, Nanduri S. (2019). Andrographolide: A natural product template for the generation of structurally and biologically diverse diterpenes. European Journal of Medicinal Chemistry. 176:513-33.
15. Mishra SK, Tripathi S, Shukla A, Oh SH, Kim HM. (2015). Andrographolide and analogues in cancer prevention. Frontiers in Bioscience-Elite. 7(2):292-304.
16. Fearon ER, Vogelstein B. (1990). A genetic model for colorectal tumorigenesis. cell. 61(5):759-67.
17. Grady W. (2005). Epigenetic events in the colorectum and in colon cancer. Biochemical Society Transactions. ;33(4):684-8.
18. Grady WM, Markowitz SD. (2002). Genetic and epigenetic alterations in colon cancer. Annual review of genomics and human genetics. 3(1):101-28.
19. Tejpar S, Van Cutsem E. (2002). Molecular and genetic defects in colorectal tumorigenesis. Best Practice & Research Clinical Gastroenterology. 16(2):171-85.
20. O'Connell JB, Maggard MA, Ko CY. (2004). Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. Journal of the National Cancer Institute. 96(19):1420-5.
21. Markowitz SD, Bertagnolli MM. (2009). Molecular basis of colorectal cancer. New England journal of medicine. ;361(25):2449-60.

22. Tsang AH-F, Cheng K-H, Wong AS-P, Ng SS-M, Ma BB-Y, Chan CM-L, et al. (2014). Current and future molecular diagnostics in colorectal cancer and colorectal adenoma. World Journal of Gastroenterology: WJG. ;20(14):3847.
23. Pino MS, Chung DC. (2010). The chromosomal instability pathway in colon cancer. Gastroenterology. ;138(6):2059-72.
24. Thiagalingam S, Lengauer C, Leach FS, Schutte M, Hahn SA, Overhauser J, et al. (1996). Evaluation of candidate tumour suppressor genes on chromosome 18 in colorectal cancers. Nature genetics.13(3):343-6.
25. Baker SJ, Fearon ER, Nigro JM, Hamilton SR, Preisinger AC, Jessup JM, et al.(1989). Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. Science. 244(4901):217-21.
26. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, et al. (1995). Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. Science. 268(5215):1336-8.
27. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. (2004). High frequency of mutations of the PIK3CA gene in human cancers. Science. 304(5670):554-.
28. Christie M, Jorissen R, Mouradov D, Sakthianandeswaren A, Li S, Day F, et al. (2013). Different APC genotypes in proximal and distal sporadic colorectal cancers suggest distinct WNT/β-catenin signalling thresholds for tumourigenesis. Oncogene.32(39):4675-82.
29. Abdel-Rahman W, Peltomäki P. (2004). Molecular basis and diagnostics of hereditary colorectal cancers. Annals of medicine. 36(5):379-88.
30. Meyer LA, Broaddus RR, Lu KH. (2009). Endometrial cancer and Lynch syndrome: clinical and pathologic considerations. Cancer Control.16(1):14-22.
31. Kaiser JC, Meckbach R, Jacob P. (2014). Genomic instability and radiation risk in molecular pathways to colon cancer. PloS one. 9(10):e111024.
32. Chen H, Ye D, Xie X, Lu W, Zhu C, Chen X. (2005). Mismatch repair gene promoter methylation and expression in hydatidiform moles. Archives of Gynecology and Obstetrics.;272:35-9.
33. Takayama T, Miyanishi K, Hayashi T, Sato Y, Niitsu Y. (2006). Colorectal cancer: genetics of development and metastasis. Journal of gastroenterology. 41:185-92.
34. Grady WM, Rajput A, Myeroff L, Liu DF, Kwon K, Willis J, et al. (1998). Mutation of the type II transforming growth factor-β receptor is coincident with the transformation of human colon adenomas to malignant carcinomas. Cancer research.58(14):3101-4.
35. Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, et al. (1997). Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science. 275(5302):967-9.
36. Yagi OK, Akiyama Y, Nomizu T, Iwama T, Endo M, Yuasa Y.(1988). Proapoptotic gene BAX is frequently mutated in hereditary nonpolyposis colorectal cancers but not in adenomas. Gastroenterology. 114(2):268-74.
37. Colussi D, Brandi G, Bazzoli F, Ricciardiello L. (2013). Molecular pathways involved in colorectal cancer: implications for disease behavior and prevention. International journal of molecular sciences.14(8):16365-85.
38. Nguyen HT, Duong HQ. (2018). The molecular characteristics of colorectal cancer: Implications for diagnosis and therapy. Oncology letters. 16(1):9-18.
39. Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Meyerhardt JA, Loda M, et al. (2009). CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut. 58(1):90-6.
40. Shen L, Toyota M, Kondo Y, Lin E, Zhang L, Guo Y, et al. (2007). Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer. Proceedings of the National Academy of Sciences. ;104(47):18654-9.
41. Min H-Y, Lee H-Y. (2022). Molecular targeted therapy for anticancer treatment. Experimental & Molecular Medicine. 54(10):1670-94.
42. Goto O, Koizumi E, Higuchi K, Noda H, Onda T, Omori J, et al. (2021). Cutting-edge technologies for gastrointestinal therapeutic endoscopy. Journal of Nippon Medical School. 88(1):17-24.
43. Law R, Das A, Gregory D, Komanduri S, Muthusamy R, Rastogi A, et al.(2016). Endoscopic resection is cost-effective compared with laparoscopic resection in the management of complex colon polyps: an economic analysis. Gastrointestinal endoscopy. 83(6):1248-57.
44. Poston GJ, Figueras J, Giuliante F, Nuzzo G, Sobrero AF, Gigot J-F, et al.(2008). Urgent need for a new staging system in advanced colorectal cancer. Journal of clinical oncology.26(29):4828-33.
45. Hashiguchi Y, Muro K, Saito Y, Ito Y, Ajioka Y, Hamaguchi T, et al.(2020). Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2019 for the treatment of colorectal cancer. International journal of clinical oncology. 25:1-42.
46. Cheong CK, Nistala KRY, Ng CH, Syn N, Chang HSY, Sundar R, et al.(2020). Neoadjuvant therapy in locally advanced colon cancer: a meta-analysis and systematic review. Journal of gastrointestinal oncology. 11(5):847.
47. Cercek A, Roxburgh CS, Strombom P, Smith JJ, Temple LK, Nash GM, et al. (2018). Adoption of total neoadjuvant therapy for locally advanced rectal cancer. JAMA oncology. 4(6):e180071-e.
48. Grothey A, Sobrero AF, Shields AF, Yoshino T, Paul J, Taieb J, et al. (2018). Duration of adjuvant chemotherapy for stage III colon cancer. New England Journal of Medicine.378(13):1177-88.
49. Kumar RA, Sridevi K, Kumar NV, Nanduri S, Rajagopal S.(2004). Anticancer and immunostimulatory compounds from *Andrographis paniculata*. Journal of ethnopharmacology.92(2-3):291-5.
50. Lim JCW, Chan TK, Ng DS, Sagineedu SR, Stanslas J, Wong WF. (2012). Andrographolide and its analogues: versatile bioactive molecules for combating inflammation and cancer. Clinical and Experimental Pharmacology and Physiology. 39(3):300-10.

51. Malik Z, Parveen R, Parveen B, Zahiruddin S, Khan MA, Khan A, et al. (2021). Anticancer potential of andrographolide from *Andrographis paniculata* (Burm. f.) Nees and its mechanisms of action. Journal of Ethnopharmacology. 272:113936.
52. Sharma V, Sharma T, Kaul S, Kapoor KK, Dhar MK. (2017). Anticancer potential of labdane diterpenoid lactone "andrographolide" and its derivatives: a semi-synthetic approach. Phytochemistry Reviews. 16:513-26.
53. Khare CP. (2008). Indian medicinal plants: an illustrated dictionary: Springer Science & Business Media; 2008.
54. Akbar S. (2011). *Andrographis paniculata*: a review of pharmacological activities and clinical effects. Alternative Medicine Review. 16(1):66-77.
55. Hossain M, Urbi Z, Sule A, Rahman K. (2014). *Andrographis paniculata* (Burm. f.) Wall. ex Nees: a review of ethnobotany, phytochemistry, and pharmacology. The Scientific World Journal. 20:67-75.
56. Pandey A, Gulati S, Gupta A, Tripathi Y. (2019). Variation in andrographolide content among different accessions of *Andrographis paniculata*. The Pharma Innovation Journal.8(4):140-4.
57. Chakravarti R, Chakravarti MD.(1951). Andrographolide, the Active Constituent of *Andrographis paniculata* Nees. A Preliminary Communication. The Indian medical gazette. 86(3):96.
58. Parveen R, Ahmad F, Iqbal Z, Samim M, Ahmad S. (2014). Solid lipid nanoparticles of anticancer drug andrographolide: formulation, in vitro and in vivo studies. Drug development and industrial pharmacy. ;40(9):1206-12.
59. Sirion U, Kasemsook S, Suksen K, Piyachaturawat P, Suksamrarn A, Saeeng R.(2012). New substituted C-19-andrographolide analogues with potent cytotoxic activities. Bioorganic & medicinal chemistry letters. ;22(1):49-52.
60. Chao W-W, Lin B-F. (2010). Isolation and identification of bioactive compounds in *Andrographis paniculata* (Chuanxinlian). Chinese medicine. 5:1-15.
61. Li M, Yang X, Guan C, Wen T, Duan Y, Zhang W, et al.(2018). Andrographolide sulfonate reduces mortality in Enterovirus 71 infected mice by modulating immunity. International immunopharmacology. ;55:142-50.
62. Chao W-W, Lin B-F. (2012). Hepatoprotective diterpenoids isolated from *Andrographis paniculata*. Biochem Pharmacol, 6;46(1):182-5.doi: 10.1016/0006-2952(93)90364-3.
63. Aromdee C. (2014). Andrographolide: progression in its modifications and applications–a patent review (2012–2014). Expert Opinion on Therapeutic Patents. 24(10):1129-38.
64. Kumar P, Naik R, Karra N. (2013). Experimental and clinical evidence of *Andrographis paniculata* (Roxb.) Wall. Ex Nees (Bhunimba)—a review. Int J Pharm Biol Sci Arch.4(6):1086-93.
65. Thakur AK, Chatterjee SS, Kumar V. (2015). Adaptogenic potential of andrographolide: An active principle of the king of bitters (*Andrographis paniculata*). Journal of Traditional and Complementary Medicine. 5(1):42-50.
66. Weinberg R, Hanahan D. (2000). The hallmarks of cancer. Cell. 100(1):57-70.
67. Newman DJ, Cragg GM. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of natural products. 75(3):311-35.
68. Lai Y-H, Yu S-L, Chen H-Y, Wang C-C, Chen H-W, Chen JJ. (2013). The HLJ1-targeting drug screening identified Chinese herb andrographolide that can suppress tumour growth and invasion in non-small-cell lung cancer. Carcinogenesis. 34(5):1069-80.
69. Paul S, Roy D, Pati S, Sa G.(2021). The adroitness of andrographolide as a natural weapon against colorectal cancer. Frontiers in Pharmacology. 2818.
70. Reabroi S, Chairoungdua A, Saeeng R, Kasemsuk T, Saengsawang W, Zhu W, et al. (2018).A silyl andrographolide analogue suppresses Wnt/β-catenin signaling pathway in colon cancer. Biomedicine & Pharmacotherapy. ;101:414-21.
71. Wong CC, Sagineedu SR, Sumon SH, Sidik SM, Phillips R, Lajis NH, et al. (2014). NCI in vitro and in silico anticancer screen, cell cycle pertubation and apoptosis-inducing potential of new acylated, benzylidene and isopropylidene derivatives of andrographolide. Environmental toxicology and pharmacology. 38(2):489-501.
72. Jada SR, Matthews C, Saad MS, Hamzah AS, Lajis N, Stevens M, et al. (2008). Benzylidene derivatives of andrographolide inhibit growth of breast and colon cancer cells in vitro by inducing G1 arrest and apoptosis. British journal of pharmacology. 155(5):641-54.
73. Wong CC, Periasamy N, Sagineedu SR, Sidik S, Sumon SH, Loadman P, et al.(2014). *In vitro* 3D colon tumor penetrability of SRJ09, a new anti-cancer andrographolide analog. Investigational new drugs. ;32:806-14.
74. Wang W, Guo W, Li L, Fu Z, Liu W, Gao J, et al. (2016). Andrographolide reversed 5-FU resistance in human colorectal cancer by elevating BAX expression. Biochemical pharmacology. 121:8-17.
75. Lin H-H, Shi M-D, Tseng H-C, Chen J-H. (2014). Andrographolide sensitizes the cytotoxicity of human colorectal carcinoma cells toward cisplatin via enhancing apoptosis pathways in vitro and in vivo. Toxicological Sciences. ;139(1):108-20.
76. Khan I, Mahfooz S, Saeed M, Ahmad I, Ansari IA. (2021). Andrographolide inhibits proliferation of colon cancer SW-480 cells via downregulating notch signaling pathway. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 21(4):487-97.
77. Chao H-P, Kuo C-D, Chiu J-H, Fu S-L.(2010). Andrographolide exhibits anti-invasive activity against colon cancer cells via inhibition of MMP2 activity. Planta Medica. 76(16):1827-33.
78. Dey SK, Bose D, Hazra A, Naskar S, Nandy A, Munda RN, et al. (2013). Cytotoxic activity and apoptosis-inducing potential of di-spiropyrrolidino and di-spiropyrrolizidino oxindole andrographolide derivatives. PLoS One. ;8(3):e58055.

79. Banerjee A, Ahmed H, Yang P, Czinn SJ, Blanchard TG. (2016). Endoplasmic reticulum stress and IRE-1 signaling cause apoptosis in colon cancer cells in response to andrographolide treatment. Oncotarget.7(27):41432.
80. Zhang R, Zhao J, Xu J, Jiao DX, Wang J, Gong ZQ, et al.(2017). Andrographolide suppresses proliferation of human colon cancer SW620 cells through the TLR4/NF-κB/MMP-9 signaling pathway. Oncology letters. 2;14(4):4305-10.
81. Al-Henhena N, Ying RPY, Ismail S, Najm W, Khalifa SA, El-Seedi H, et al.(2014). Chemopreventive efficacy of *Andrographis paniculata* on azoxymethane-induced aberrant colon crypt foci in vivo. PloS one. ;9(11):e111118.
82. Farooqi AA, Attar R, Sabitaliyevich UY, Alaaeddine N, de Sousa DP, Xu B, et al.(2020). The prowess of andrographolide as a natural weapon in the war against cancer. Cancers.12(8):2159.
83. Kandanur SGS, Golakoti NR, Nanduri S. (2015). Synthesis and in vitro cytotoxicity of novel C-12 substituted-14-deoxy-andrographolide derivatives as potent anti-cancer agents. Bioorganic & Medicinal Chemistry Letters. 25(24):5781-6.
84. Kandanur SGS, Kundu S, Cadena C, Juan HS, Bajaj A, Guzman JD, et al. (2019). Design, synthesis, and biological evaluation of new 12-substituted-14-deoxy-andrographolide derivatives as apoptosis inducers. Chemical Papers. 73:1669-75.
85. Kumar S, Singh B, Bajpai V. (2021). *Andrographis paniculata* (Burm. f.) Nees: Traditional uses, phytochemistry, pharmacological properties and quality control/quality assurance. Journal of Ethnopharmacology. 275:114054.
86. Yuan M, Meng W, Liao W, Lian S. (2018). Andrographolide antagonizes TNF-α-induced IL-8 via inhibition of NADPH oxidase/ROS/NF-κB and Src/MAPKs/AP-1 axis in human colorectal cancer HCT116 cells. Journal of agricultural and food chemistry. 66(20):5139-48.
87. Khan I, Mahfooz S, Faisal M, Alatar AA, Ansari IA. (2021). Andrographolide induces apoptosis and cell cycle arrest through inhibition of aberrant hedgehog signaling pathway in colon cancer cells. Nutrition and Cancer.;73(11-12):2428-46.
88. Panossian A, Hovhannisyan A, Mamikonyan G, Abrahamian H, Hambardzumyan E, Gabrielian E, et al. (2007). Pharmacokinetic and oral bioavailability of andrographolide from *Andrographis paniculata* fixed combination Kan Jang in rats and human. Phytomedicine.7(5):351-64.
89. Tran QT, Tan WD, Wong WF, Chai CL. (2021). Polypharmacology of andrographolide: beyond one molecule one target. Natural Product Reports.38(4):682-92.
90. Butnariu M, Sarac I, Samfira I. (2020). Spectrophotometric and chromatographic strategies for exploring of the nanostructure pharmaceutical formulations which contains testosterone undecanoate. Scientific Reports. 10(1):3569.
91. Casamonti M, Risaliti L, Vanti G, Piazzini V, Bergonzi MC, Bilia AR. (2019). Andrographolide loaded in micro-and nano-formulations: Improved bioavailability, target-tissue distribution, and efficacy of the "king of bitters". Engineering. 5(1):69-75.
92. Yang T, Sheng H-H, Feng N-P, Wei H, Wang Z-T, Wang C-H. (2013). Preparation of andrographolide-loaded solid lipid nanoparticles and their in vitro and in vivo evaluations: characteristics, release, absorption, transports, pharmacokinetics, and antihyperlipidemic activity. Journal of pharmaceutical sciences.102(12):4414-25.
93. Bilia AR, Piazzini V, Guccione C, Risaliti L, Asprea M, Capecchi G, et al. (2017). Improving on nature: the role of nanomedicine in the development of clinical natural drugs. Planta Medica. 83(05):366-81.
94. Zhang J, Li Y, Gao W, Repka MA, Wang Y, Chen M. (2014). Andrographolide-loaded PLGA-PEG-PLGA micelles to improve its bioavailability and anticancer efficacy. Expert opinion on drug delivery.11(9):1367-80.
95. Bilia AR, Bergonzi MC, Guccione C, Manconi M, Fadda AM, Sinico C. (2016). Vesicles and micelles: Two versatile vectors for the delivery of natural products. Journal of Drug Delivery Science and Technology. 32:241-55.
96. McClements DJ. (2012). Nanoemulsions versus microemulsions: terminology, differences, and similarities. Soft matter. ;8(6):1719-29.
97. Das S, Halder A, Mandal S, Mazumder MAJ, Bera T, Mukherjee A, et al. (2018). Andrographolide engineered gold nanoparticle to overcome drug resistant visceral leishmaniasis. Artificial Cells, Nanomedicine, and Biotechnology. ;46(sup1):751-62.
98. Ma Y, Yang Y, Xie J, Xu J, Yue P, Yang M. (2018). Novel nanocrystal-based solid dispersion with high drug loading, enhanced dissolution, and bioavailability of andrographolide. International journal of nanomedicine.;13:3763.