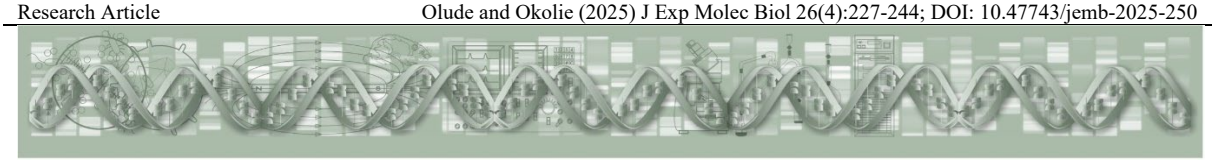

FICUS EXASPERATA SUPPRESSES EARLY EVENTS IN COLORECTAL CARCINOGENESIS BY DOWNREGULATING THE EXPRESSION OF BETA-CATENIN AND ENHANCING ADENOMATOUS POLYPOSIS COLI GENE EXPRESSION

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Abstract

Wingless (Wnt) signalling is an important evolutionarily conserved signalling pathway in animals that regulates biological processes such as development, cell growth, and differentiation. Abnormalities in the components of the Wnt/β-catenin signalling pathway have been a major cause of cancer, especially in colorectal cancer (CRC). This study investigates the effect of the methanol leaf extract of *Ficus exasperata* (MEFE) on the wnt signalling pathway, considering beta-catenin and adenomatous polyposis coli (APC) as key markers. This study further delves into the quantitative and qualitative phytochemicals present in the leaf extract. Malondialdehyde (MDA), reduced glutathione (GSH) level and some haematological indices were also assayed for, in the test animals. A total of forty-eight Wistar rats, grouped into 8 cages, were used for this study. The control group was the first group; group 2 was treated with extract alone (500 mg/kg body weight); group 3 rats were injected subcutaneously with 40 mg/kg b.w. of 1,2- dimethylhydrazine (DMH) twice a week; group 4 was treated with both the leaf extract (500 mg/kg b.w.) and DMH; group 5 was treated with the leaves extract (750 mg/kg b.w.) and DMH; group 6 was pretreated with the leaf extract (500 mg/kg b.w.) before the administration of DMH; group 7 (post-treated) was given DMH for some weeks before the commencement of treatment with the leaf extract (500 mg/kg b.w.); group 8 was given the carcinogen and treated with a standard drug (12.5 mg/kg b.w. of 5-fluorouracil} simultaneously. Appreciable amount of phenol, flavonoid, tannin and anthocyanidin were present in the plant extract. Alkaloids, terpenoids, phytosterols, saponins and anthraquinones were also found in the plant. The immune system of the rats was strengthened by the extract. The haemoglobin and red blood cell levels of rats treated with the plant extract were within the normal range compared to the control ($p<0.05$). Reduced glutathione level and adenomatous polyposis gene were reduced while, malondialdehyde level and beta-catenin gene expression were statistically significantly increased in carcinogen-only treated groups compared to other groups ($p < 0.05$). The plant was able to inhibit oxidative stress and also suppress the expression of β-catenin while enhancing the expression of adenomatous polyposis coli. These potentials might be a s a result of the phytochemicals present in the plant extract.

Keywords: *Ficus exasperata*, colorectal carcinogenesis, beta-catenin, Adenomatous polyposis coli

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Introduction

Colorectal cancer (CRC) arises from either the rectum or colon. The epithelial cells of the large intestine are known for the dynamics of consistent cell renewal, which makes it prone to proliferation disorders. Formation of polyps is one of the disadvantages of the high cell renewal dynamics, though it's benign, but under certain circumstances it can transit into the formation of malignant tumours and hence cancer (Kowalska et al. 2025). Initiation to metastasis in CRC is influenced by genetics, epigenetics, molecular alterations, mutation in oncogenes, mutation in tumour suppressor genes, and alterations in signalling pathways that control apoptosis, cell proliferation, cell survival and cell differentiation (Papavassiliou et al. 2024, Delle-cave 2025). Colorectal cancer (CRC) remains a threat to human health and the need to provide a lasting cure to this ailment is crucial. CRC is a solid tumor associated with the disorder of the Wnt/β-catenin signalling pathway. Wnt/βcatenin or the canonical Wnt signalling pathway, the planar cell polarity (Wnt-PCP) pathway and the Wnt-Ca2+ signaling pathway are classes of the Wnt signalling cascade (Nusse and Clevers 2017). The Wnt/β-catenin signalling pathway is associated with physiological processes, but dysregulation of this pathway has been linked to solid tumors and haematological disorders (Clevers and Nusse 2012, Cheng et al. 2019).

Normal physiological environment entails the transcription factor β-catenin, an important molecule in the Wnt/βcatenin or the canonical Wnt signaling pathway, being degraded by a complex called the β-catenin destruction complex. The destruction complex consists mainly of two kinases: glycogen synthase kinase 3β (GSK3β), casein kinase I (CK I) and two scaffolds: adenomatous polyposis (APC) and axis inhibition (Axin) (Kim et al. 2009; Mantilla et al. 2015). Casein kinase I and GSK3β phosphorylate β-catenin. CK 1 phosphorylates β-catenin at serine 45, Ser33, and Ser37 while GSK3β phosphorylates at threonine 41 (Thr41) (Koni et al. 2020). The phosphorylation of β-catenin makes it susceptible and recognised by E3 ubiquitin ligase (β-transducin repeat-containing protein: β-TrCP) for ubiquitination and proteasomal degradation (Zhu and Li 2023). The degradation inhibits the translocation of β-catenin into the nucleus, while allowing histone deacetylation, chromatin compaction and Groucho-mediated promoter repression. Hence, transcription is halted (MacDonald et al. 2009, Jackstadt et al. 2020). Phosphorylation, the frizzled (FZD) family receptors, and the low-density-lipoprotein-related protein 5/6 (LRP5/LRP6) co-receptors are required for the activation of the canonical Wnt signals (Gajos-Michniewicz et al. 2020). The binding of the Wnt ligands to its receptors results in dishevelled phosphorylation and release of β-catenin from the destruction complex. The release prevents β-catenin ubiquitination and degradation. Increase and accumulation of β-catenin aids its nuclear translocation. In the nucleus, the Groucho repressor undergoes displacement, allowing β-catenin to interact with the T-cell factor/lymphoid enhancer factor (TCF/LEF), chromatin remodelling and transcription of genes such as c-myc and cyclin D1 (Zhan et al. 2017). Anastas and Moon (2013), in their review, reported that the role of the Wnt in mouse models of mammary cancer and in human and mouse colon cancer was first described over 30 years ago. Nusse et al. (1982) and Nusse et al. (1984) also reported spontaneous mammary hyperplasia and tumours in mice induced by a proviral insertion at the Wnt1 locus, leading to aberrant overexpression of WNT1. Wnt1 transgenic mice similarly develop mammary tumours, suggesting a causative role for WNT1 in mammary tumorigenesis (Tsukamoto et al. 1988). Koni et al. (2020) reported that Wnt/β-catenin signalling pathways play a crucial role in carcinogenesis of all ovarian cancer subtypes (Jeong et al. 2009), glioma (Zhao et al. 2020), prostate cancer (Situ et al. 2020), osteosarcoma (Nomura et al. 2019), melanoma (Muralidhar et al. 2019), and pancreatic cancer (Chen et al. 2020). Hyperactive Wnt/β-catenin and elevated intracellular β-catenin have also been implicated in breast tumours (Khramtsov et al. 2010, Sormunen et al. 1999). Over 90% of non-metastasising fibromatosis

and metaplastic carcinomas have been associated with the high level of β-catenin expression (Lacroix-Triki et al. 2010).

A major hallmark of colorectal cancer is the hyperactivation of this canonical Wnt signalling associated with a mutation in the tumour suppressor gene, APC (Tewari et al. 2021). Loss of a functional adenomatous polyposis coli (APC) is one of the initiating factors of colorectal cancer (Noe et al. 2021). The APC gene, located on chromosome 5q regulates the β-catenin /WNT pathway, which in turn facilitates differentiation and growth of cells (Rubinfeld et al. 1993). Mutation of the APC gene inhibits the formation of the complex necessary for β-catenin degradation (Sakanaka et al. 1998). The absence of this destruction complex enables and promotes β-catenin accumulation, leading to gastrointestinal epithelial stem cell carcinogenic proliferation (Noe et al. 2021). This signalling cascade remains crucial and important in many biological physiological processes, including embryonic development, cell cycle regulation, apoptosis, inflammation, and cancer (Tai et al. 2015). This cascade determines cell survival, cell fate, cell differentiation, and survival (Yamamoto et al. 2022). The Wnt pathway components have been identified as reliable biomarkers and potential targets for cancer treatment (Zhao et al. 2022).

This study aims to investigate the effect of *Ficus exasperata* on beta-catenin and adenomatous polyposis coli expression in colorectal carcinogenesis. The phytochemicals present in the plants were also assayed, as well as the effect of the plant on some haematological parameters and oxidative stress in CRC.

Materials and Methods

Extraction of Plant

In a local farm situated in Benin City, Edo State, Nigeria, *Ficus exasperata* leaves were harvested and taken to the Department of Plant Biology and Biotechnology in the University of Benin for identification by Prof Henry Akinnibosun. The leaves were properly air dried for three weeks, they were ground, weighed, and macerated for 72hours in methanol. This was stirred at intervals. After 72 hours, it was decanted and freeze-dried using a freeze-dryer. The result was a powdered extract kept in a container and preserved at 4 ºC.

Chemicals and reagents

1,2-Dimethylhydrazine (DMH) was obtained from Sigma Aldrich, Germany. All reagents used were of analytical grade and had the highest purity.

Preparation of the Carcinogen Used

Colorectal cancer was induced in experimental rats using a potent carcinogen named 1,2-dimethyl hydrazine (DMH). DMH was administered subcutaneously (40 mg/kg body weight) two times in a week. It was prepared according to the method of Chari et al. (2018).

Animal grouping and Sacrifice

The experiment lasted for 12 weeks. Wistar rats weighing more than 150 g were used in this study. A total of 8 groups comprising 6 animals each were allowed to acclimatized for two weeks. The first group was the control group, the second group was the positive control group (500mg/kg body weight of extract only), and the negative control was group 3 (40 mg/kg body weight of DMH only), the fourth and fifth group received DMH and the extract together but at different concentrations (500 and 750 mg /kg body weight of extract, respectively). The sixth group was administered the leaf extract before carcinogen treatment, while the seventh group was treated with carcinogen before administering the extract. The eighth group was concomitantly treated with both the carcinogen and the standard drug (12.5 mg/kg body weight of 5-fluorouracil intraperitoneally). At the end of the stipulated weeks, the animals were fasted overnight and sacrificed. Blood samples were collected, and the colons of the animals were also excised for heamatological and biochemical assays.

Tissue homogenate preparations were done using a weighed portion of the excised organs. The organs (1 g) were homogenised in normal saline solution (10 ml), centrifuged, and the supernatant obtained was labelled according to the groupings. This was used for reduced glutathione (GSH) and lipid peroxidation assay marker (malondialdehyde) assays.

Biochemical Assays

Malondialdehyde (MDA) levels were estimated by the method of Burge and Aust (1978). It is one of the products of lipid peroxidation which forms a pink colour when reacted with 2-thiobarbituric acid. The reaction result is read at 535nm wavelength.

Reduced glutathione was estimated according to the method of Ellman (1959). the reaction between GSH and 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB), also known as Ellman's reagent, produces the TNB chromophore and reads spectrophotometrically at 412 nm.

A Gene expression study of beta-catenin and adenomatous polyposis: This was done according to the method described by Elekofehinti et al. (2020). The Quick-RNA MiniPrep™ Kit (Zymo Research) was used to extract the total RNA from the tissue samples, and DNAse I treatment (NEB, Cat: M0303S), employed for the elimination of DNA contaminants. One microgramme (1 µg) of DNA-free RNA underwent a reverse transcriptase reaction with a cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs). The reaction occurred in three steps: 65 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min (Elekofehinti et al. 2020). Polymerase chain reaction (PCR) for gene amplification utilized OneTaqR2X Master Mix (NEB) with specific primers (Inqaba Biotec, Hatfield, South Africa). The amplified molecules were resolved on one percent agarose gel (1.0%) followed by the quantification of the gene expressed using the GAPDH gene and "ImageJ" software (Elekofehinti et al. 2020).

Hematological Analysis

The automated haemolyzer was employed to determine the full blood count. Whole blood was used for the determination of hematology. white blood cell, red blood cell, hemoglobin, percentage WBC count (neutrophils, eosinophils and basophils), were analyzed with an automated analyzer (SYSMEX K-21N: SYSMEX CORPORATION, JAPAN).

Phytochemical Screening

The qualitative phytochemical screening followed the methodologies of Harborne (1998), Sofowora (1993), and Trease and Evans (1998), with all samples analysed in triplicate.

Total phenolic compounds: The method of Folin and Ciocalteau (1927) was adopted in the determination of total phenolic content.

Total flavonoid contents: This was determined using the method described by Ayoola et al. (2008).

Proanthocyanidin determination: It was carried out according to the method of Sun et al. (1998).

Data Analysis

Data was statistically analysed using ANOVA, and GraphPad Prism was used in plotting the graphs. Values were presented as mean ± standard error of mean. Data was considered to be statistically significant at $p < 0.05$.

Results

Phytochemical results reveal the presence of tannins, phenols, flavonoids, terpenoids, alkaloids, phytosterols, saponins, anthraquinones and carbohydrates. The spectrophotometric readings reveal that an appreciable amount of total tannins, total flavonoids, proanthocyanidin and total phenolic content was present in the leaf extract of *Ficus exasperata* and shown in Tables 1 and 2.

Table 1. Qualitative phytochemical screening of plant extract

Phytochemicals	
Tannins	Present
Phenol	Present
Flavonoids	Present
Terpenoids	Present
Alkaloids	Present
Reducing sugar	Absent
Phytosterol	Present
Saponins	Present
Catechin	Absent
Anthraquinones	Present
Xanthoproteins	Absent
Carbohydrates	Present

Table 2. Quantitative phytochemical screening of *Ficus exasperata* leaves

Phytochemical	Amount
Total phenolic content (mg GAE/g extract)	25 ± 0.015
Total Flavonoid content (mg QE/g extract)	117 ± 0.029
Proanthocyanidin content (mg AAE/g extract)	235 ± 0.005
Total tannin (mg TAE/g extract)	167.6 ± 0.04

GAE: Gallic acid equivalent; AAE: Ascorbic acid equivalent; QE: Quercetin equivalent; TAE: Tannic acid equivalent. Values are expressed as mean ± SEM, n=3/group

Effect of the Extract on Reduced Glutathione and MDA levels: Figure 1 shows the reduced glutathione (GSH) level of the various groups. The GSH level of groups 3 to 8 was statistically significantly different compared to group 2. The malondialdehyde (MDA) level of the DMH-only group was increased statistically compared to other groups, as displayed in Figure 2.

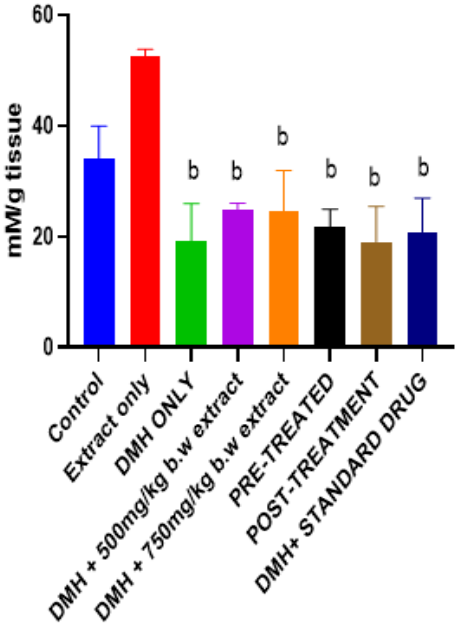

Figure 1. Colon reduced glutathione (GSH) level. Sample size = 6 per group, and values are expressed as mean ± standard error of mean. The alphabet 'b' represents that there was a statistically significant difference ($P < 0.05$) compared to the extract-only group. Reduced

glutathione levels showed no significant difference between the MEFE (cotreated, pretreated and post-treatment) groups and group 8 compared to group 3. All these aforementioned groups differ significantly from group 2. The group that received the carcinogen alone has the lowest level of reduced glutathione.

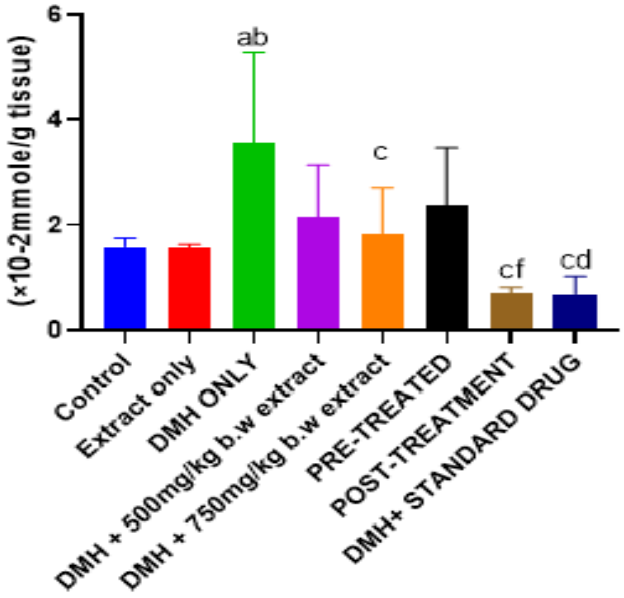

Figure 2. Colon malondialdehyde (MDA) level. Values are expressed as mean ± standard error of mean with a total of six animals in each group. The alphabet represents significant differences at $P < 0.05$. The alphabet 'a' denotes there was a statistically significant difference compared to the control; 'b' denotes there was a statistically significant difference compared to the extract-only group; 'c' denotes there was a statistically significant difference compared to the DMH group; 'd' denotes there was a statistically significant difference compared to group DMH+500 mg/kg b.w.; 'f' denotes there was a statically significant difference compared to the pre-treated group. The group that received DMH only expressed a high level of MDA ($P < 0.05$), which differs from MEFE-treated groups. The MDA level of group 7 that received post-treatment with MEFE was not statistically different from the group treated with standard drugs.

Effect of the Extract on the Expression of beta-Catenin and Adenomatous polyposis coli (APC) gene: Relative expression of the beta-catenin gene displayed in Figure 3 shows that the gene was highly expressed in the DMH-only group. The lowest level of this gene expression was seen in the group treated with the standard drugs, which is closer to the level of the pretreatment group. Adenomatous polyposis relative gene expression is shown in Figure 4. In this result, the pretreated group has the highest relative gene expression of APC, which was statistically different from the remaining groups.

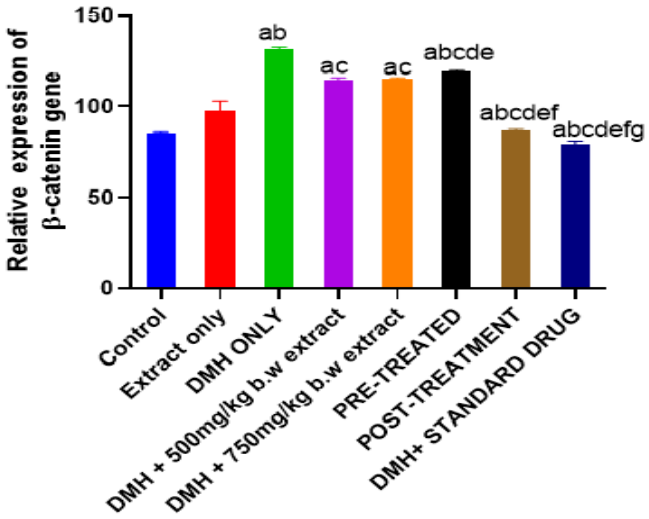

Figure 3. Relative expression of the β-catenin gene. Data are expressed as mean ± standard error with a total of six animals per group. Significant differences ($P < 0.05$) between the groups were represented by lowercase letters. The alphabet 'a' denotes there was a statistically significant difference compared to the control; 'b' denotes there was a statistically significant difference compared to extract-only group; 'c' denotes there was a statistically significant difference compared to the DMH group; 'd' denotes there was a statistically significant difference compared to the group treated with DMH + 500 mg/kg b.w. of extract; f denotes there was a statistically significant difference compared to the pre-treated group, and 'g' denotes there was a statistically significant difference compared to the post-treated group. Each group was statistically different from the others. The post-treated group's beta-catenin gene expression was close to that of groups 1 and 8. There was a high accumulation of beta-catenin in the carcinogen-only treated group.

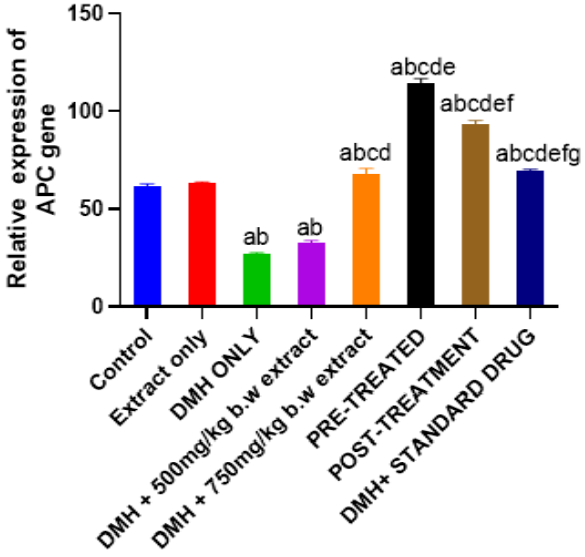

Figure 4. Relative expression of adenomatous polyposis coli (APC) gene. Sample size = 6 per group. Data are expressed as mean ± standard error. Significant differences ($P < 0.05$) between the groups were represented by lowercase letters. 'a' denotes there was a statistically significant difference compared to control; 'b' denotes there was a statistically significant difference compared to extract-only; 'c' denotes there was a statistically significant difference compared to the DMH group; 'd' denotes there was a statistically significant difference compared to the group treated with DMH + 500 mg/kg b.w.; 'f' denotes there was a statistically significant

difference compared to the pre-treated group, and 'g' denotes there was a statistically significant difference compared to the post-treated group. The APC gene was greatly expressed in the pretreated and the post-treated groups compared to other groups.

Effect of the Extract on Haematological Parameters: The haematological result is shown in Figures 5 to 10. The white blood cells, a true picture of the immune system, were statistically reduced in the group treated with the standard drug compared to the pretreatment group ($p<0.05$). Neutrophil levels were high in the DMH-only group, though not statistically significant compared to other groups. The basophil level of DMH+500 mg/kg body weight was reduced compared to other groups, while the group that took the carcinogen with a higher dose of the extract had an increased basophil level that was statistically significant compared to group 4. Red blood cell level showed no statistically significant difference across the groups, though it was a bit reduced in the DMH-only group ($p>0.05$), and the haemoglobin level of group 7 was statistically significantly different from the control ($p<0.05$).

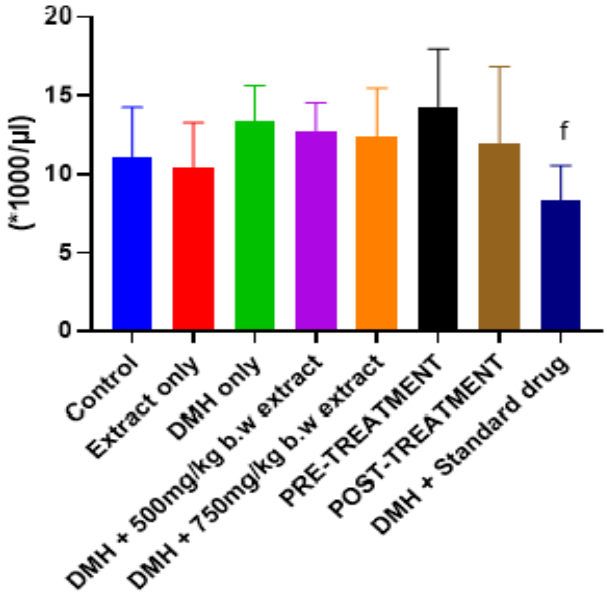

Figure 5. White blood cell count. A sample size of 6 rats per group, and data are expressed as mean ± standard error of means. Lowercase letter represents a significant difference at $P < 0.05$. The alphabet 'f' denotes there was a statistically significant difference compared to the pre-treated group. White blood cell levels showed no significant difference between the groups except for group 8, which differs statistically from group 6.

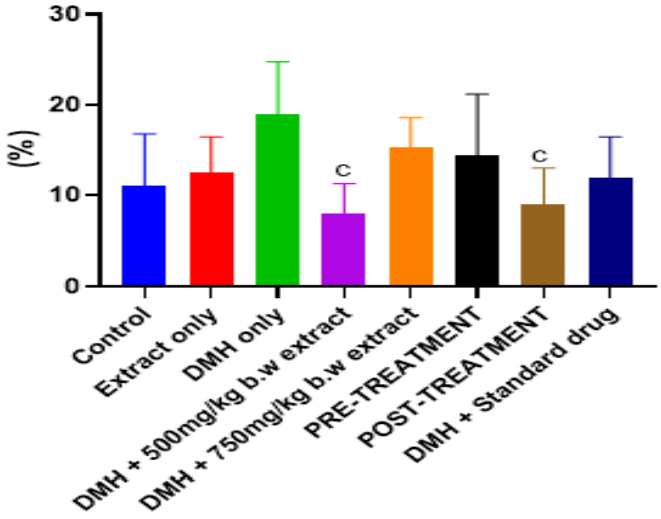

Figure 6. Percentage of neutrophils of rats in different groups. A sample size of 6 rats per group, and data are expressed as mean ± standard error of mean. Lowercase letters 'c' represent a statistically significant difference at $P < 0.05$ compared to the DMH-only group. The neutrophil level of the DMH-only group was greatly increased compared to other groups. Groups 4 and 7 had the lowest level of neutrophil count.

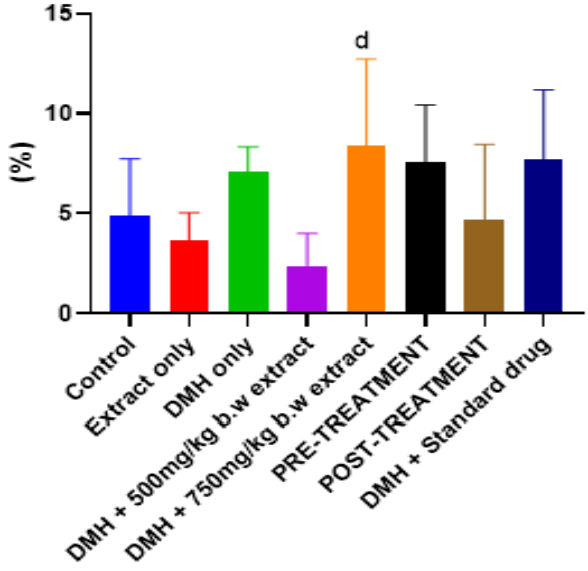

Figure 7. Percentage of basophils in rats of different groups. A sample size of 6 rats per group, and data expressed as mean ± standard error of mean. The alphabet 'd' represents a statistically significant difference at $P < 0.05$ compared to the cotreated group that received DMH + 500 mg/kg b.w. of the extract. The data were statistically insignificant except for group 5, which was significantly higher compared to group 4.

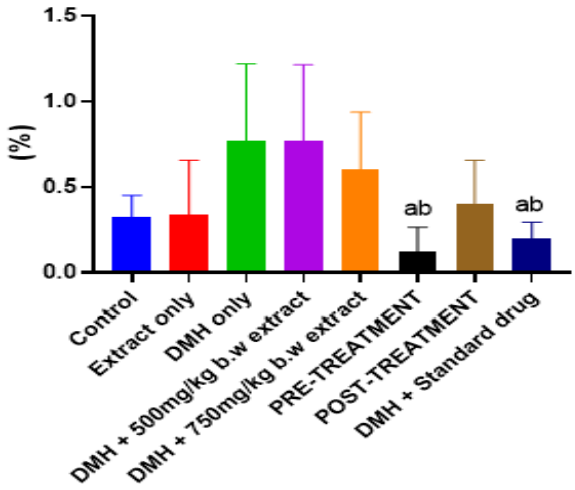

Figure 8. Percentage of eosinophils in rats in different groups. The alphabet 'a' denotes a significant difference from the control, and 'b' denotes a significant difference from the extract-only group. A sample size of 6 rats per group, and data are expressed as mean ± standard error of mean. Groups 1 and 2 were not statistically different from most of the groups except groups 6 and 8. Groups 6 and 8 had the lowest eosinophil count compared to the other groups.

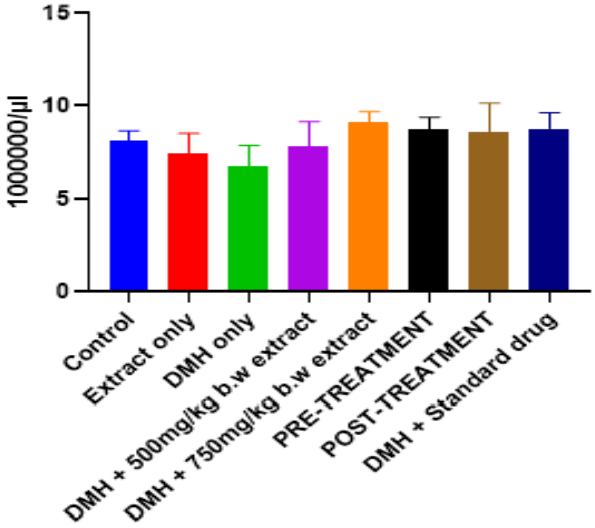

Figure 9. Red blood cell levels of rats in different groups. Values are expressed as mean ± SEM, n=6/group. Values were considered statistically significant at $P < 0.05$. This data also showed no significant difference in red blood cell levels. However, the exposed animals that were treated with MEFE recorded a higher volume of RBC.

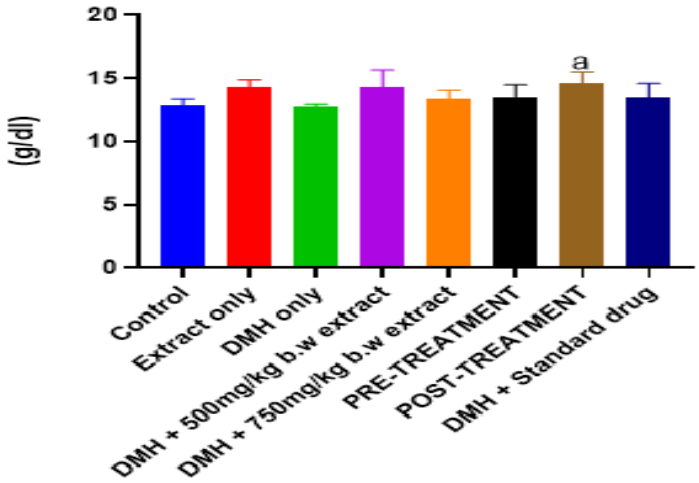

Figure 10. Haemoglobin level of rats in different groups. The letter 'a' signifies a statistically significant difference from the control group. Data are expressed as mean ± standard error of mean. MEFE increased the haemoglobin of treated animals; this was evident in group 2 animals and the animals that received post-treatment.

Discussions

Adenomatous polyposis coli (APC) is one of the components of the Wnt signaling pathway. Derangement in this pathway is a key event in the initiation of colorectal cancer. Mutation of APC, a tumor suppressor gene, predisposes individuals to colon cancer. It is believed that the main function of APC is the regulation of free β-catenin in concert with glycogen synthase kinase 3β (GSK-3β) (Perse and Cerar 2011). It has been found that half of the human colon tumors with intact APC protein have a mutation in the β-catenin gene (Tanaka et al. 2017).

Studies with the DMH rat model have detected mutations in the APC gene in colorectal epithelial lesions. In DMH/AOM-treated rats, up to 33% of colon tumors harbour APC mutations, but β-catenin mutations are more frequent than APC mutations (Takahashi et al. 2000, Bordonaro et al. 2016). When APC or β-catenin is mutated, β-catenin cannot be degraded but accumulates in the cytoplasm and translocates into the nucleus, where it binds to T-cell factor (TCF) and activates the Wnt target genes (Jin et al. 2008, Perse and Cerar 2011, Jin et al. 2022). In our study, in line with the previous findings (Yu et al. 2018, Salim et al. 2023), the relative expression of the APC gene in DMH-treated rats (grp3) was significantly decreased compared to the groups that were either cotreated, pretreated, or post-treated with *F.exasperata.* The high level of β-catenin that was expressed in group 3 might be a result of a decreased level of APC, which activates β-catenin phosphorylation, hence inhibiting its degradation (Shojaei-Zarghani et al. 2020). However, the level of expression of the β-catenin gene was lowered in the post-treated group. The results obtained in this study indicate that *F. exasperata* may be an effective chemopreventive agent for colon cancer through down-regulation of the GSk3β/β-catenin pathway.

The ability of *Ficus exasperata* leaf extract to suppress beta-catenin and uphold the APC gene may be a result of the phytochemicals present in this plant. Phytochemical screening reveals the presence of saponins, terpenoids, anthraquinones, flavonoids, alkaloids, carbohydrates, steroids, phenols, and tannins (Alli-Smith et al. 2018, Anigboro et al. 2019). These diverse phytochemicals contribute to the plant's anticarcinogenic properties. Anthraquinones have demonstrated anti-inflammatory, immunoregulatory, anti-hyperlipidemic, and anticancer effects in pharmacological studies (Lin et al. 2015, Cui et al. 2016, Abu et al. 2018, Yang et al. 2019, Wang et al. 2021). Flavonoids, abundant in the extract, exhibit antioxidant properties and

have demonstrated anticancer activities (Yahfoufi et al. 2018, Abotaleb et al. 2018, Chirumbolo et al. 2018, Rodriguez-Garcia et al. 2019). Saponins possess antimicrobial, antiviral, anti-inflammatory, anticancer, antioxidant, and immunomodulatory effects (Barbosa et al. 2014, Juang and Liang 2020). Terpenoids, known for their antitumor, anti-inflammatory, antibacterial, and antiviral properties, were also identified (Yang et al. 2020, Jahangeer et al. 2021). Plant alkaloids in the extract may contribute to its anti-inflammatory, antioxidant, and anti-mutagenic characteristics (Biribi 2018, Louis et al. 2019).

Lipid peroxidation of membranes is a process that plays an important role in initiating and mediating various health challenges, ranging from inflammation, cancer, and cardiovascular diseases, among others (Friedmann et al. 2019). In lipid peroxidation, carbon-carbon double bonds in lipids such as polyunsaturated fatty acids, phospholipids, glycolipids, and cholesterol undergo hydrogen abstraction and oxygen insertion in the allylic position, resulting in lipid peroxyl radicals and hydroperoxides (Stoyanovsky et al. 2019). The products of this lipid peroxidation are deleterious to DNA and other biological macromolecules (Olude and Omoregie 2024), which are considered oxidative stress biomarkers to specify the extent of cell injury (Valaei et al. 2022). High lipid peroxidation level in the colon tissue is directly related to the severity of DMH-induced lesions. (Jeong et al. 2025). Lipid peroxidation has been linked to one of the causes of colon cancer, which leads to the production of malondialdehyde. This product damages bio-cellular components, such as mutating DNA, signifying tumorigenicity (Yang et al. 2020). Administration of DMH elevated the level of lipid peroxidative product (MDA). MDA levels were significantly increased in group 3 compared to the other groups. A reduction in this lipid peroxidation marker was observed in groups treated with the plant extract. The post-treated and standard drug-treated groups had the lowest level of MDA. The first line of defense against oxidative stress is antioxidants. They break down free radicals into less reactive and harmless molecules (Balaji et al. 2015). Reduced glutathione level was reduced in group 3 compared to other groups, though not statistically significant compared to other treated groups. The decrease in the level might be due to its function in catalyzing and trapping free radicals.

Alterations and aberrations in haematological profiles by 1,2-dimethylhydrazine have been reported in previous studies. Most of the studies are unanimous on the fact that DMH suppresses haemoglobin level (Hb), as obtained in this study (Mishra et al. 2022, Salehi et al. 2022). Reduction in the level of heamoglobin (Hb) is a pointer to anaemia. RBCs are prone to the deleterious effects of radicals and carbonium ions that are produced during 1,2-dimethylhydrazine metabolism. These products formed from the activation of DMH from its proactive to its active state are released into biological systems, which elicits oxidative stress, DNA damage, and ultimately cell death. Thus, explaining the reduction of Hb in the DMH-treated group (Akinwunmi et al. 2023). The groups treated with the methanol extract of *Ficus exasperata* had a normal level of Hb and RBC, suggesting the plant extract may possess some anti-anaemic compounds. In the present report, WBC was also elevated after DMH exposure, as reported in a recent study (Mishra et al. 2022). The increase in the level of white blood cells recorded in this study might be as a result of inflammation, tumor production, and metastasis in DMH-induced rats, which provoked the immune system to produce a large amount of white blood cells (WBCs). However, the WBC differential pattern did not agree with previous studies. For instance, Mishra et al. (2022) reported a drastic decrease in neutrophils and lymphocytes, while Salehi et al. (2022) reported an increase in neutrophils. Neutrophil permeation has been described in human sporadic premalignant colonic adenomas (Mclean et al. 2011). Herein, it was demonstrated that DMH administration in the DMH-only group resulted in a significant increase in neutrophils, and eosinophils, and a slight increase in basophils. Simultaneous treatment with the extract enhances the immune system, with post-treatment being more beneficial. Methanol extract of *F. exasperata* may be beneficial in protecting blood cells against

anaemia and white blood cell pathologies during colon cancer proliferation. Molecular docking is recommended for further studies.

Conclusions

The study shows clearly that the plant extract is rich in numerous phytochemicals, which would have greatly contributed to one of the mechanisms through which this plant was able to suppress beta-catenin and uphold the expression of the tumor suppressor gene APC. In addition, this plant contains haematinic components and also boosts the immune system.

References

Abotaleb M, Samuel SM, Varghese E, Varghese S, Kubatka P, Liskova A, Busselberg DM. 2019. Flavonoids in Cancer and Apoptosis. Cancers. 11(1): 28. doi:10.3390/cancers11010028.

Abu N, Zamberi NR, Yeap SK, Nordin N, Mohamad NE, Romli MF, Rasol NE, Subramani T, Ismail NH, Alitheen NB. 2018. Subchronic toxicity, immunoregulation and anti-breast tumor effect of Nordamnacantal, an anthraquinone extracted from the stems of *Morinda citrifolia* L. BMC Complementary and Alternative Medicine. 18: 31. doi:10.1186/s12906- 018-2102-3.

Akinwumi KA. 2023. Gross histological and hematological profile of β-caryophyllene and mifepristone in 1, 2-dimethylhydrazine-induced rat model of colon cancer. Proceedings of the 4th International Conference, The Federal Polytechnic, Ilaro, Nigeria in Collaboration with Takoradi Technical University, Takoradi, Ghana 3 rd – 7 th September, 2023. University Auditorium, Takoradi Technical University, Takorad.

Alli-Smith YR, Aluko BT, Faleye FJ. 2018. Antioxidant activity and inhibitory effect of ethanolic extract of *Ficus exasperata* leaves on pro- oxidant induced hepatic and cerebral lipid peroxidation in albino rats in vitro. International Journal of Herbal Medicine. 6(3): 20-24.

Anigboro AA, Avwioroko OJ, Ohwokevwo OA, Pessu B. 2019. Bioactive components of *Ficus exasperata, Moringa oleifera* and *Jatropha tanjorensis* leaf extracts and evaluation of their antioxidant properties. EurAsian Journal of BioSciences. 13: 1763-1769.

Ayoola GA, Folawewo AD, Adesegun SA, Abioro OO, Adepoju-Bello AA, Coker HAB. 2008. Phytochemical and antioxidant screening of some plants of Apocynaceae from South West Nigeria. African Journal of Plant Science. 2(9): 124-128.

Balaji C, Muthukumaran J, Nalini N. 2015. Effect of sinapic acid on 1,2 dimethylhydrazine induced aberrant crypt foci, biotransforming bacterial enzymes and circulatory oxidative stress status in experimental rat colon carcinogenesis. Bratisl Lek Listy. 116: 560-6.

Bordonaro M, Shirasawa S, Lazarova DL. 2016. In hyperthermia increased ERK and WNT signaling suppress colorectal cancer cell growth. Cancers. 8: 49; doi:10.3390/cancers8050049.

Bribi N. 2018. Pharmacological activity of Alkaloids: A Review. Asian Journal of Botany. Volume 1. doi:10.63019/ajb.v1i2.467.

Chari KY, Polu PR, Shenoy RR. 2018. An appraisal of pumpkin seed extract in 1, 2-dimethylhydrazine induced colon cancer in wistar rats. Journal of Toxicology. Article ID 6086490. https://doi.org/10.1155/2018/6086490.

Chen T, Lei S, Zeng Z, Zhang J, Xue Y, Sun Y, Lan J, Xu S, Mao D, Guo B. 2020. Linc00261 inhibits metastasis and the WNT signaling pathway of pancreatic cancer by regulating a miR-552-5p/FOXO3 axis. Oncology Reports. 43: 930-942.

Cheng X, Xu X, Chen D, Zhao F, Wang W. (2019). Therapeutic potential of targeting the wnt/beta-catenin signaling pathway in colorectal cancer. Biomedicine and Pharmacotherapy. 110: 473-481.

Chirumbolo S, Bjorklund G, Lysiuk R, Vella A, Lenchyk L, Upyr T. 2018. Targeting cancer with phytochemicals via their fine tuning of the cell survival signaling pathways. International Journal of Molecular Science. doi:10.3390/ijms19113568
Clevers H, Nusse R. 2012. Wnt/beta-catenin signaling and disease. Cell. 149: 1192-1205.
Cui Y, Lu P, Song G, Liu Q, Zhu D, Liu X. 2016. Involvement of PI3K/ Akt, ERK and p38 signaling pathways in emodin-mediated extrinsic and intrinsic human hepatoblastoma cell apoptosis. Food and Chemical Toxicology. 92: 26-37.
Delle-Cave D. 2025. Advances in molecular mechanisms and therapeutic strategies in colorectal cancer: a new era of precision medicine. International Journal of Molecular Science. 26: 346. https://doi.org/10.3390/ ijms26010346.
Ellman GL. 1959. Tissue Sufhydryl Groups. Archives of Biochemistry and Biophysics. 82: 70-77.
Fang DC, Luo YH, Yang SM. 2002. Mutation analysis of APC gene in gastric cancer with microsatellite instability. World Journal of Gastroenterology. 8: 787-91.
Folin O, Ciocalteau V. 1927 Tyrosine and tryptophane in proteins. Journal of Biological Chemistry. 73(2): 627-648.
Friedmann AJP, Krysko DV, Conrad M. 2019. Ferroptosis at the crossroads of cancer-acquired drug resistance and immune evasion. Nature Reviews Cancer. 19(7): 405-414.
Gajos-Michniewicz A, Czyz M. 2020. WNT Signaling in Melanoma. International Journal of Molecular Sciences. 21(14): 4852. doi:10.3390/ijms21144852
Harborne JB. 1998. Phytochemical methods: A guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall. p. 54-84.
Jackstadt R, Hodder MC, Sansom OJ. 2020. WNT and β-Catenin in Cancer: Genes and therapy. Annual Review of Cancer Biology. 4: 177-196.
Jahangeer M, Fatima R, Ashiq M, Basharat A, Qamar SA, Bilal M, Hafiz MNI. 2021. Therapeutic and Biomedical Potentialities of Terpenoids - A Review. Journal of Pure and Applied Microbiology. 15(2): 471-483.
Anastas JN, Moon RT. 2013. WNT signalling pathways as therapeutic targets in cancer. Cancer. 13: 11-26. doi:10.1038/nrc3419.
Jeong HJ, Min S, Villalon LA, Jeong K, Chung JK. 2025. Lipid-oxidative enzymes and Fenton-like reactions are synergistic in promoting membrane lipid peroxidation. Journal of the American Chemical Society. 5(9): 4337-4345. doi: 10.1021/jacsau.5c00696.
Jeong JW, Lee HS, Franco HL, Broaddus RR, Taketo MM, Tsai SY, Lydon JP, DeMayo FJ. 2009. B-Catenin Mediates Glandular Formation and Dysregulation of B-Catenin Induces Hyperplasia Formation in the Murine Uterus. Oncogene. 28: 31-40.
Ji Y, Lv J, Sun D, Huang Y. 2022. Therapeutic strategies targeting Wnt/β-catenin signaling for colorectal cancer (Review). International Journal of Molecular Medicine. 49: 1. DOI: 10.3892/ijmm.2021.5056.
Jin T, Fantus G, Sun J. 2008. Wnt and beyond Wnt: Multiple mechanisms control the transcriptional property of β-catenin. Cellular Signalling. 20: 1697-1704.
Khramtsov AI, Khramtsova GF, Tretiakova M, Huo D, Olopade OI, Goss KH. 2010. Wnt/β-catenin pathway activation is enriched in basal-like breast cancers and predicts poor outcome. The American. Journal of Pathology. 176: 2911-2920.
Kim NG, Xu C, Gumbiner BM. 2009. Identification of targets of the wnt pathway destruction complex in addition to beta-catenin. Proceedings of the National Academy of Sciences USA. 106: 5165-5170.
Koni M, Pinnaro V, Brizzi MF. 2020. The Wnt Signalling Pathway: A Tailored Target in Cancer. International Journal of Molecular Science. 21: 7697. doi:10.3390/ijms21207697.
Kowalska MK, El-Mallul A, Lubojanska JE, Hudecka W, Orłowska SM, Lubojanski PJ, Bednarczyk Ł. 2025. Molecular Basis, Diagnostic Approaches, and Therapeutic Strategies in

Colorectal Cancer – Comprehensive Review. International Journal of Molecular Sciences. 26: 9520. https://doi.org/10.3390/ ijms26199520.
Lacroix-Triki M, Geyer FC, Lambros MB, Savage K, Ellis IO, Lee AHS, Reis-Filho JS. 2010. β-Catenin/Wnt signalling pathway in fibromatosis, metaplastic carcinomas and phyllodes tumours of the breast. Modern Pathology. 23:1438-1448.
Li VSW, Ng SS, Boersema PJ, Low TY, Karthaus WR, Gerlach JP, Mohammed S, Heck AJR, Maurice MM, Mahmoudi T. 2012. Wnt signaling through inhibition of beta-catenin degradation in an intact axin1 complex. Cell. 149: 1245-1256.
Lin L, Ni B, Lin H, Zhang M, Li X, Yin X, Qu C, Ni J. 2015. Traditional usages, botany, phytochemistry, pharmacology and toxicology of *Polygonum multiflorum* Thunb.: a review. Journal of Ethnopharmacology. 159: 158-183.
Louis H, Adejoke HT, Amusan OO, Apebende G. 2019. A Review on Classes, Extraction, Purification and Pharmaceutical Importance of Plants Alkaloid. Journal of Medicinal and Chemical Sciences. 2: 130-139.
MacDonald BT, Tamai K, He X. 2009. Wnt/β-Catenin signaling: components, mechanisms, and diseases. Developmental Cell. 17: 9-26.
Mantilla C, Mellado S, Jaramillo DA, Navas MC. 2015. β-catenin signaling mechanisms and its role in carcinogenesis. CES Medicina. 29: 109-127.
McLean MH, Murray GI, Stewart KN, Norrie G, Mayer C. 2011. The inflammatory microenvironment in colorectal neoplasia. PLoS ONE. 6(1): e15366. doi:10.1371/journal.pone.0015366
Mishra S, Alhodieb FS, Mohd A, Hassan Z, Abul-Barkat H, Ali R, Alam P, Alam O. 2022. Antitumor and hepatoprotective effect of *Cuscuta reflexa* Roxb. in a murine model of colon cancer. Journal of ethnopharmacology. https://doi.org/10.1016/j.jep.2021.114597.
Muralidhar S, Filia A, Nsengimana J, Pozniak J, O'Shea SJ, Diaz JM, Harland M, Randerson-Moor JA, Reichrath J, Laye JP, van der Weyden L, Adams DJ, Bishop DT, Newton-Bishop J. 2019. Vitamin D-VDR signaling inhibits Wnt/β-Catenin-mediated melanoma progression and promotes antitumor immunity. Cancer Research. 79(23): 5986-5998. doi: 10.1158/0008-5472.CAN-18-3927.
Noe O, Filipiak L, Royfman R, Campbell A, Lin L, Hamouda D, Stanbery L, Nemunaitis J. 2021. Adenomatous polyposis coli in cancer and therapeutic implications. Oncology Reviews. 15: 534 doi:10.4081/oncol.2021.534.
Nomura M, Rainusso N, Lee YC, Dawson B, Coarfa C, Han R, Larson JL, Shuck R, Kurenbekova L, Yustein JT. 2019. Tegavivint and the β-Catenin/ALDH Axis in chemotherapy-resistant and metastatic osteosarcoma. Journal of the National Cancer Institute. 111: 1216-1227.
Nusse R, Clevers H. 2017. Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. Cell. 169(6): 985-99.
Nusse R, Varmus HE. 1982. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell. 31: 99-109.
Nusse R, Van Ooyen A, Cox D, Fung YK, Varmus H. 1984. Mode of proviral activation of a putative mammary oncogene (int-1) on mouse chromosome 15. Nature. 307: 131-136.
Olude OM, Omoregie FO. 2024. Comparative study for response to *Cycas circinalis*-induced toxicity in liver, kidney and colon of rats and guinea pigs. African Journal of Health, Safety and Environment. 5(1): 23-34.
Papavassiliou AG, Delle Cave D. 2024. Novel Therapeutic Approaches for Colorectal Cancer Treatment. International Journal of Molecular Science. 25(4): 2228. doi: 10.3390/ijms25042228.
Perse M, Cerar A. 2011. Morphological and molecular alterations in 1,2 dimethylhydrazine and azoxymethane induced colon carcinogenesis in rats. Journal of Biomedicine and Biotechnology. doi:10.1155/2011/473964.

Rodriguez-Garcia C, Sanchez-Quesada C, Gaforio JJ. 2019. Dietary flavonoids as cancer chemopreventive agents: an updated review of human studies. Antioxidants. 8: 137. doi:10.3390/antiox8050137.
Rubinfeld B, Souza B, Albert I, Müller O, Chamberlain SH, Masiarz FR, Munemitsu S, Polakis P. 1993. Association of the APC gene product with beta-catenin. Science. 262(5140): 1731-4. doi: 10.1126/science.8259518.
Sakanaka C, Weiss JB, Williams LT. 1998. Bridging of β-catenin and glycogen synthase kinase-3β by axin and inhibition of βcatenin-mediated transcription. Proceedings of the National Academy of Sciences. 95: 3020-3.
Salehi A, Hosseini SM, Kazemi S. 2022. Antioxidant and anticarcinogenic potentials of propolis for dimethylhydrazine-induced colorectal cancer in wistar rats. BioMed Research International. https://doi.org/10.1155/2022/8497562.
Salim MNH, Mussa A, Ahmed N, Ahmad S, Yean C, Hassan R, Uskokovic V, Mohamud R, Jalil CNA. 2023. The immunosuppressive effect of tnfr2 expression in the colorectal cancer microenvironment. Biomedicines. 11: 173. https://doi.org/10.3390/ biomedicines11010173.
Shojaei-Zarghani S, Rafraf M, Khosroushahi AY, Sheikh-Najaf S. 2020. Effectiveness of theobromine on inhibition of 1,2-dimethylhydrazine-induced rat colon cancer by suppression of the Akt/GSK3β/β-catenin signaling pathway. Journal of Functional Foods. 75: 104293. https://doi.org/10.1016/j.jff.2020.104293.
Situ J, Zhang H, Jin Z, Li K, Mao Y, Huang W. 2020. MicroRNA-939 directly targets HDGF to inhibit the aggressiveness of prostate cancer via deactivation of the WNT/β-catenin pathway. OncoTargets and Therapy. 13: 4257-4270.
Sofowora A. 1993. Medicinal plants and traditional medicine in Africa. John Wiley and Sons Ltd. 8: 256.
Sormunen RT, Leong ASY, Vaaraniemi JP, Fernando SSE, Eskelinen SM. 1999. Immunolocalization of the fodrin, E-cadherin, and β-catenin adhesion complex in infiltrating ductal carcinoma of the breast – Comparison with an in vitro model. Journal of Pathology. 187: 416-423.
Stoyanovsky DA, Tyurina YY, Shrivastava I, Bahar I, Tyurin VA, Protchenko O, Jadhav S, Bolevich SB, Kozlov AV, Vladimirov YA, Shvedova AA, Philpott CC, Bayir H, Kagan VE. 2019. Iron catalysis of lipid peroxidation in ferroptosis: Regulated enzymatic or random free radical reaction? Free Radical Biology and Medicine. 133: 153-161.
Sun B, Leandro C, Ricardo da Silva JM, Spranger I. 1998. Separation of grape and wine proanthocyanidins according to their degree of polymerization. Journal of Agriculture and Food Chemistry. 46: 1390-1396.
Zhan T, Rindtorff N, Boutros M. 2017. Review: Wnt signaling in cancer. Oncogenes. 36: 1461-1473. doi:10.1038/onc.2016.304.
Tai D, Wells K, Arcaroli J, Vanderbilt C, Aisner D, Messersmith W. 2015. Targeting the wnt signaling pathway in cancer therapeutics. Oncologist. 20(10): 1189-98.
Takahashi M, Nakatsugi S, Sugimura T, Wakabayashi K. 2000. Frequent mutations of the β-*catenin* gene in mouse colon tumors induced by azoxymethane. Carcinogenesis. 21(6): 1117-1120.
Tanaka N, Mashima T, Mizutani A, Sato A, Aoyama A, Gong B, Yoshida H, Muramatsu Y, Nakata K, Matsuura M, Katayama R, Nagayama R, Fujita N, Sugimoto Y, Seimiya H. 2017. APC mutations as a potential biomarker for sensitivity to tankyrase inhibitors in colorectal cancer. Molecular Cancer Therapeutics. 16(4). DOI: 10.1158/1535-7163.MCT-16-0578.
Tewari D, Bawari S, Sharma S, DeLiberto L, Bishayee A. 2021. Targeting the crosstalk between canonical Wnt/β-catenin and inflammatory signaling cascades: A novel strategy for cancer prevention and therapy. Pharmacology and Therapeutics. 227: 107876.

Trease GE, Evans WC. 1996. A textbook of Pharmacognosy. 14th ed. London: Bailliere Tindall Ltd.
Tsukamoto AS, Grosschedl R, Guzman RC, Parslow T, Varmus HE. 1988. Expression of the int-1 gene in transgenic mice is associated with mammary gland hyperplasia and adenocarcinomas in male and female mice. Cell. 55: 619-625.
Valaei A, Azadeh F, Niaki STM, Salehi A, Khoob MS, Mirebrahimi SHO, Kazemi S. 2022. Antioxidant and anticancer potentials of the olive and sesame mixture against dimethylhydrazine-induced colorectal cancer in Wistar rats. Biomed Research International. 5440773. https://doi.org/10.1155/2022/5440773.
Wang D, Wang XH, Yu X, Cao F, Cai X, Chen P, Li M, Feng Y, Li H, Wang X. 2021. Pharmacokinetics of anthraquinones from medicinal plants. Frontiers in Pharmacology. 12: 638993. doi: 10.3389/fphar.2021.638993.
Yahfoufi N, Alsadi N, Jambi M, Matar C. 2018. The immunomodulatory and anti-inflammatory role of polyphenols. Nutrients. 10: 1618. doi:10.3390/nu10111618.
Yamamoto D, Oshima H, Wang D, Takeda H, Kita K, Lei X. 2022. Characterization of RNF43 frameshift mutations that drive Wnt ligand- and R-spondin-dependent colon cancer. The Journal of Pathology. 257(1): 39-52.
Yang B, Li Y, Yang Z, Xue L, Zhang M, Chen G. 2020. Anti-inflammatory and anti-cell proliferative effects of dieckol in the prevention and treatment of colon cancer induced by 1,2-dimethyl hydrazine in experimental animals. Pharmacognosy Magazine. 16: 851-8.
Yang N, Li C, Li H, Liu M, Cai X, Cao F, Feng Y, Li M, Wang X. 2019. Emodin induced SREBP1-dependent and SREBP1-independent apoptosis in hepatocellular carcinoma cells. Frontiers in Pharmacology. 10: 709. doi:10.3389/fphar.2019.00709.
Yu J, Han Z, Sun Z, Wang Y, Zheng M, Song C. 2018. LncRNA SLCO4A1-AS1 facilitates growth and metastasis of colorectal cancer through β-catenin-dependent Wnt pathway. Journal of Experimental & Clinical Cancer Research. 37: 222. https://doi.org/10.1186/s13046-018-0896-y.
Zhao H, Ming T, Tang S, Ren S, Yang H, Liu M, Tao Q, Xu H. 2022. Wnt signaling in colorectal cancer: pathogenic role and therapeutic target. Molecular Cancer. 21: 144. https://doi.org/10.1186/s12943-022-01616-7.
Zhao JB, Xue JF, Zhang WZ, Ren YL, Yan DM. 2020. Long noncoding RNA FGD5-AS1 promotes glioma cell proliferation, migration and invasion by regulating wnt/β-catenin pathway. Cancer Management and Research. 12: 6187-6193.
Zhu Y, Li X. 2023. Review advances of wnt signalling pathway in colorectal cancer. Cells. 12: 447. https://doi.org/10.3390/cells12030447.