ORIGINAL PAPER

JMS Journal of Medical Science

Isolation of Some Bioactive Compounds in the Methanol Extract of *Ficus exasperata* Leaves and the Effect of the Extract on Inflammatory Markers in 1,2 Dimethylhydrazine Induced Colorectal Cancer in Rats

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Received 2025-02-17
Accepted 2025-03-09
Published 2025-03-18

How to Cite: Okolie NP, Olude OM. Isolation of Some Bioactive Compounds in the Methanol Extract of Ficus exasperata Leaves and the Effect of the Extract on Inflammatory Markers in 1,2 Dimethylhydrazine Induced Colorectal Cancer in Rats. Journal of Medical Science. 2025 March;94(1);e1232. doi:10.20883/medical.e1232

doi: https://doi.org/10.20883/medical.e1232

Keywords: colorectal cancer, 1,2-dimethylhydrazine, inflammation, bioactive compounds, antioxidants

ABSTRACT

Background. Colorectal cancer remains the third dominant cancer and is one of the leading cancer-related deaths in the world. The current investigation explores the chemoprotective roles of *Ficus exasperata* against inflammation and oxidative stress in 1,2-dimethylhydrazine (DMH)-induced colorectal cancer in rats. Some bioactive compounds were also isolated.

Material and methods. Forty-eight Wistar rats were grouped in 8 cages; group 1; control, group 2 was treated with 500mg/kg body weight of extract, group 3 received DMH twice a week, group 4 was treated with both the extract (500mg/kg b.w) and DMH, group 5 was treated with the extract (750mg/kg b.w) and DMH, group 6 was pretreated with the extract before DMH administration, group 7 was given DMH before the commencement of extract and group 8 was given the carcinogen and treated with 12.5mg/kg b.w of 5-fluorouracil simultaneously. Using high-performance liquid chromatography some bioactive compounds were isolated from the leaves extract of *Ficus exasperata*.

Results and conclusions. The bioactive compounds present in high quantity include; alpha-caryophyllene, isoquinoline, quercetin, kaempferol and rutin. After the 12th week, the animals were sacrificed. Total protein, catalase, superoxide dismutase and glutathione peroxidase activities were statistically significantly lower in group 3 ($p < 0.05$) compared with other groups. Gene expression of the Interleukins and cyclooxygenase 2 were statistically reduced and significant in all the groups except group 3. The extract suppressed the inflammatory cascade and also boosted antioxidant activities. This might be a result of some anticancer compounds that were discovered during the isolation of the compounds present in the plant.

Introduction

Colon cancer remains a challenge to human health. Cancer of the colon is common in both developed and developing countries. This cancer is also a major cause of death in both males and females which affects both the old and young [1]. Genetics, epigenetics and environmental factors are the causative agents of colorectal cancer. The cancer of the colon is accompanied by inflammation, a product of the immune response, proinflammatory and anti-inflammatory cytokines play different roles in tumor progression [2]. Tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β, are proinflammatory cytokines that accelerate tumor progression [3]. Inflammation activates the Janus kinase/signal transducers and activators of transcription (JAK-STAT) and nuclear factor (NF)–κB signaling pathways hereby promoting the proliferation of cells, migration, and invasion. inflammatory pathway [4–5]. Inhibition of the inflammatory cascade slows cancer cell growth and delays tumor progression [6]. Oxidative stress; an imbalance between reactive oxygen species and antioxidants is another factor implicated in the development of colorectal cancer [7].

Despite innovations and advancements in technology toward the production of a permanent cure for colon cancer, there remain setbacks and drawbacks as a result of the adverse effects of synthetic chemicals released by the drugs used in the treatment of this ailment. Natural products, especially those derived from plants, have been used to help mankind sustain its health since the dawn of medicine [8]. Herbal and natural medicine with lower side effects is crucial for lowering the mortality and morbidity rate related to colorectal cancer. Plants' active ingredients serve as a promising treatment with little or no adverse effects. Reports have shown that plants contain numerous active compounds ranging from antimicrobial, anthelmintic, antidiabetic, antihypertensive and anticancer compounds [9–10]. The presence of these active biological molecules is responsible for the medicinal and therapeutic contribution of plants in the treatment of various diseases [11–12].

The current investigation isolated some bioactive compounds present in *Ficus exasperata* and also explored the chemoprotective roles of *Ficus exasperata* against inflammation and oxidative stress in 1,2-dimethylhydrazine (DMH)-induced colorectal cancer in rats. 1,2- dimethylhydrazine (DMH) is a specific colon procarcinogen [13]. It has been shown in animal studies that experimental colonic tumors induced by DMH were similar in histology, morphology and anatomy to human colonic neoplasms [14].

Material and methods

Plant Extract and Preparation

The *Ficus exasperata* leaves was obtained from a local garden in Benin City and were authenticated by Dr. Akinnibosun of the Department of Plant Biology and Biotechnology of the University of Benin. The voucher number was UBH-F319. The plucked leaves were dried under shade for some weeks, after which it was ready for pulverization. Extraction was carried out by soaking the pulverized *Ficus exasperata* leaves in methanol for 72 hours. Extracts were concentrated over a rotary evaporator, freeze-dried, and stored in an airtight container in the freezer.

Experimental Animals

A total of 48 male Wistar rats weighing above 150g were kept in different cages and given 14 days to acclimatize under typical laboratory circumstances. The 48 Wistar rats were grouped into 8 different cages of 6 rats each. Written approval for the study was obtained from the Research Ethics Committee Guideline Principles on Handling of Animals of the Faculty of Life Sciences, University of Benin, and was strictly adhered to.

- **Group 1:** Control group (feed only).
- **Group 2:** Leaf Extract only (500mg/kg b.w) for 12 weeks.
- **Group 3:** DMH only (40mg/kg b.w) for 12 weeks.
- **Group 4:** Leaf Extract (500mg/kg b.w) + DMH (40mg/kg b.w) for 12 weeks.
- **Group 5** Leaf Extract (750mg/kg b.w) + DMH (40mg/kg b.w) for 12 weeks.
- **Group 6:** Leaf extract for 4 weeks before DMH (500mg/kg and 40mg/kg respectively).
- **Group 7:** DMH for 8 weeks before leaf extract (40mg/kg and 500mg/kg respectively).
- **Group 8:** 5fluorouracil (12.5mg/kg b.w) + DMH (40mg/kg b.w).

The stock solution of the extract was prepared and kept refrigerated at 4°C. For the groups that received DMH, it was administered subcutaneous-

ly twice a week, while leaf extracts were administered orally. 5-fluorouracil was administered intraperitoneally. The experiment lasted for 12 weeks. The animals were fasted overnight and sacrificed. The colon was excised. A small portion was used for relative gene expression of cyclooxygenase-2 (COX-2) and tumor necrosis factor-alpha (TNF-α). The other portion was used for antioxidant assays. Blood samples were collected in plain bottles, allowed to stay for some hours at 4°C, and centrifuged at 3000rpm for 5 minutes. The serum was collected, and stored at -80°C which was used for interleukin (IL) 6 and 10 asssays.

Biochemical assays: Relative gene expression of COX-2 and TNF-α was carried out according to the method described by Elekofehinti *et al*. [15]. Total RNA was extracted from colon samples using the Quick-RNA MiniPrep™ Kit (Zymo Research). DNA contamination was eliminated through DNAse I treatment (NEB, Cat: M0303S). RNA concentration was determined at 260 nm, and purity was assessed at 260 nm and 280 nm using an A&E Spectrophotometer (A&E Lab. UK). For cDNA conversion. One microgramme (1 µg) of DNA-free RNA underwent 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 (15). Polymerase chain reaction (PCR) for gene amplification utilized OneTaqR2X Master Mix (NEB) with specific primers (Inqaba Biotec, Hatfield, South Africa). The 25 µl reaction mixture contained cDNA, forward and reverse primers, and Ready Mix Taq PCR master mix. The conditions were as follows: Initial denaturation at 95 °C for 5 min, 30 cycles of amplification (95 °C for 30 s, annealing for 30 s, and extension at 72 °C for 60 s), and a final extension at 72 °C for 10 min. Amplicons were resolved on a 1.0% agarose gel. Normalization and quantification of gene expression were performed using the GAPDH gene and "ImageJ" software. The serum level of interleukin 6 and 10 were assayed using ELISA.

Total protein was determined using lowry's method [16] and catalase (CAT) was assayed according to the method of Cohen et al., [17]. Superoxide dismutase (SOD) was determined according to the method of Misra and Fridovich [18]. Glutathione Peroxidase (GPx) was determined according to the method of Paglia and Valentine, [19]. Isolation of the bioactive compounds was done using high-performance liquid chromatography (HPLC).

Statistical Analysis

Data obtained from the study were analyzed using one-way ANOVA and Graphpad prism 8.0.1 was used to compare the means and plot the graph. Data were presented as mean ± SEM. Values were considered statistically significant at $p < 0.05$.

Results

Identification of some bioactive compounds present in *F. exasperata*

The results obtained from the high-performance liquid chromatography (HPLC); isolation of the bioactive compounds present in *Ficus exasperata* are shown in **Figure 1**. The bioactive compounds present in high quantity include; alpha-caryophyllene, isoquinoline, quercetin, kaempferol and rutin. Garcin, caffeic acid, luteolin and linalool were moderately present but some bioactive compounds were present in minute quantity; catechin, epigallocatechin, stigmasterol, sitosterol, orientin, naringerin, hesperidin, isovitexin and isorhamnetin.

Effect of methanol extract of *Ficus exasperata* leaves (MEFE) on enzymatic antioxidants and total protein level

Colon tissues' total protein was significantly decreased in group 3 compared to other groups. Group 3 CAT activity was significantly reduced, the pretreated group also had a reduced level of CAT activities but not as low as group 3. This enzyme activity was significantly high in rats that were treated with a higher dose of the extract. SOD activity was significantly low in the pretreated group but higher in post-treated rats. GPx activity was significantly lower in groups 3 and 7 compared to groups 1 and 2. Groups 4, 5, 6 and 8 showed a significant increase in this enzyme activity as shown in **Figures 2–5**.

Effect of methanol extract of *Ficus exasperata* leaves (MEFE) on inflammatory markers

The relative gene expression of the inflammatory markers; IL-6, IL-10, COX-2 and TNF-α are shown in **Figures 6–9**. All the inflammatory markers were significantly high in group 3 compared to other groups.

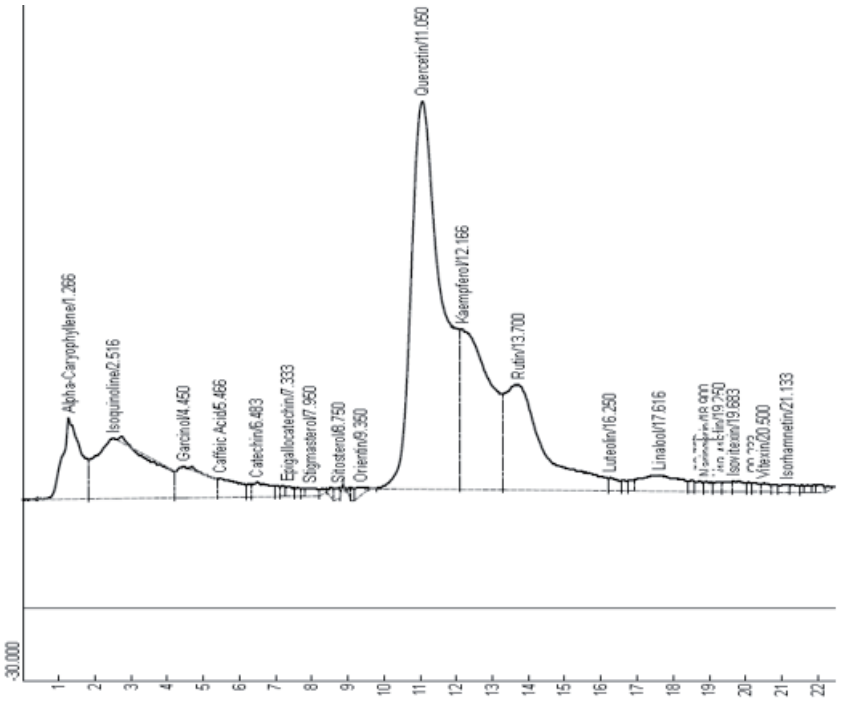

Figure 1. Phytochemical profile of the leaf extract using HPLC. Quercetin was the most numerous active compound present in this plant extract.

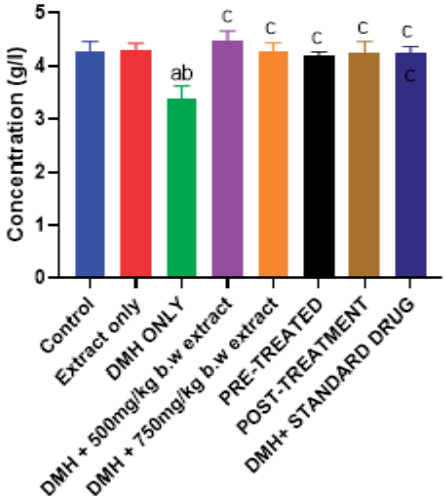

Figure 2. Colon total protein level. Values are expressed as mean ± SEM, n=6/group. Lowercase letters represent a significant difference at P < 0.05. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. The total protein of the groups that received methanol extract of Ficus exasperata leaves (MEFE) was statistically different from untreated group.

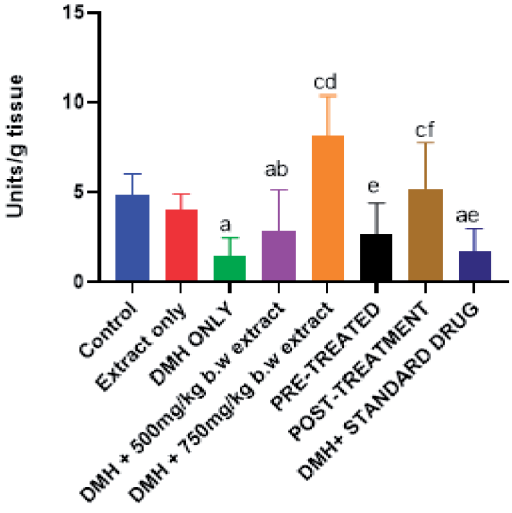

Figure 3. Colon catalase (CAT) activity. Values are expressed as mean ± SEM, n=6/ group. Lowercase letters represent a significant difference at P < 0.05. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. The lowercase letter "d" stands for a significant departure from co-treatment (DMH+ 500mg/kg MEFE). The lowercase letter "e" stands for a significant departure from co-treatment (DMH+ 750mg/kg MEFE). A statistically significant divergence from pre-treated is denoted by the lowercase letter "f". Post treated group expressed a catalase activity close to the control. All the MEFE-treated groups showed an increase in this enzyme activity, it was statistically significant in group 5 and 7 compared to group 3.

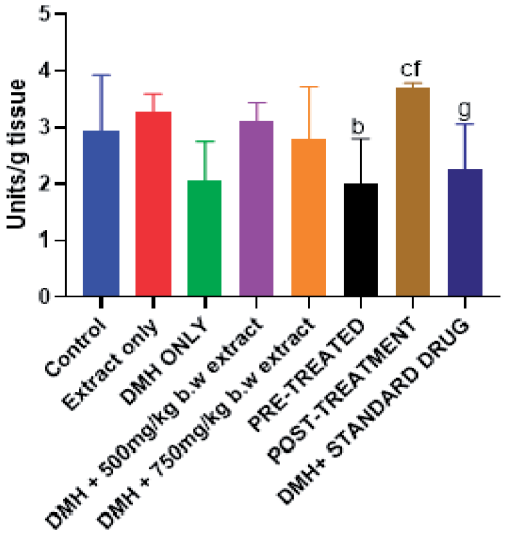

Figure 4. Colon superoxide dismutase (SOD) activity. Values are expressed as mean ± SEM, n=6/ group. Lowercase letters represent a significant difference at P < 0.05. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. A statistically significant divergence from pre-treated and post-treated is denoted by the lowercase letters "f" and "g" respectively. SOD activity in group 7 was statistically significant from group 6 and group 8 differs significantly from group 7. The enzyme activity didn't differ among the remaining groups.

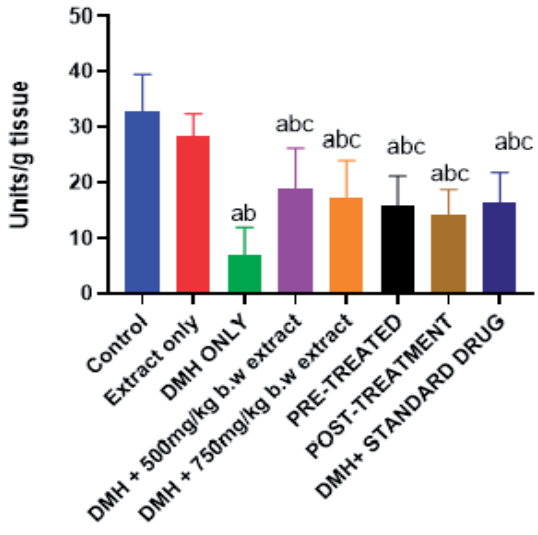

Figure 5. Colon glutathione peroxidase (GPx) activity. Values are expressed as mean ± SEM, n=6/ group. Lowercase letters represent a significant difference at P < 0.05. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. Cotreated and pretreated groups' GPx activities didn't differ from the group treated with standard drugs (p> 0.05) but were higher compared to group 3.

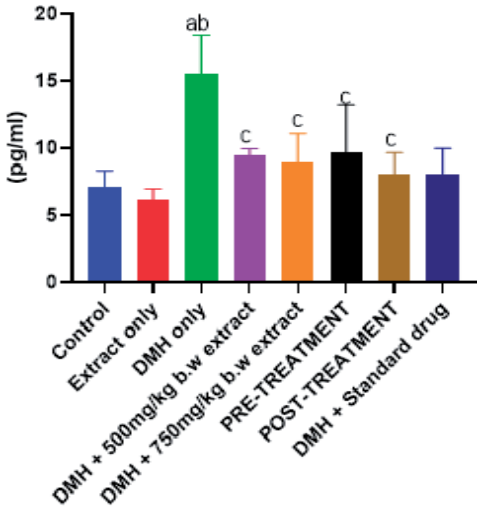

Figure 6. serum interleukin-6 (IL-6) level of rats in different groups. Values are expressed as mean ± SEM, n=6/ group. Lowercase letters represent a significant difference at P < 0.05. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. Interleukin 6 is a pro-inflammatory marker. MEFE-treated groups expressed a reduced level of IL-6 compared to group 3. The post-treated group's mean value was the closest to the standard drug, control and extract-only groups.

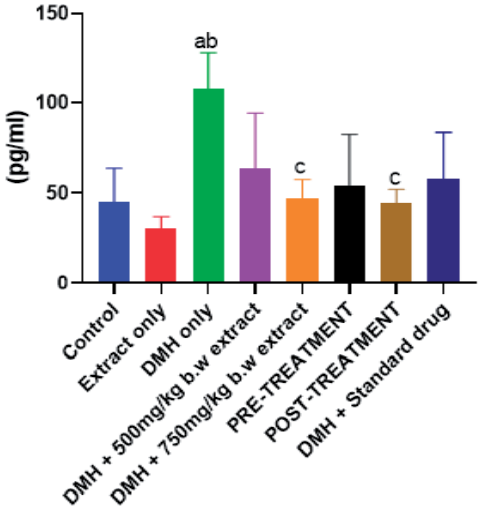

Figure 7. Interleukin-10 (IL-10) level of rats in different groups. Values are expressed as mean ± SEM, n=6/ group. Lowercase letters represent a significant difference at P < 0.05. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. Interleukin 10 is an anti-inflammatory marker. The post-treated group's IL-10 level was lowered compared to other groups that also received MEFE and carcinogen.

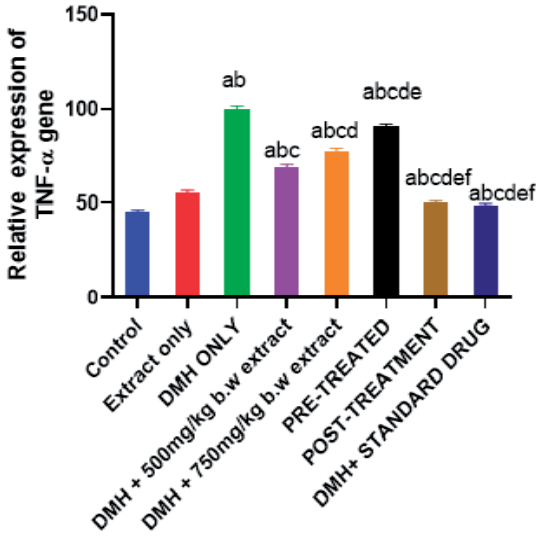

Figure 9. Relative expression of tumor necrosis factor-alpha (TNF-α) gene of rats in different groups. Values are expressed as mean ± SEM, n=6/ group. Lowercase letters represent a significant difference at $P < 0.05$. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. The lowercase letter "d" stands for a significant departure from co-treatment (DMH+ 500mg/kg MEFE). The lowercase letter "e" stands for a significant departure from co-treatment (DMH+ 750mg/kg MEFE). A statistically significant divergence from pre-treated is denoted by the lowercase letters "f". The pre-treated and co-treated groups could not reduce inflammation, unlike the post-treated groups. Tumor necrosis factor-alpha levels in the post-treated group did not differ from the groups treated with standard drugs.

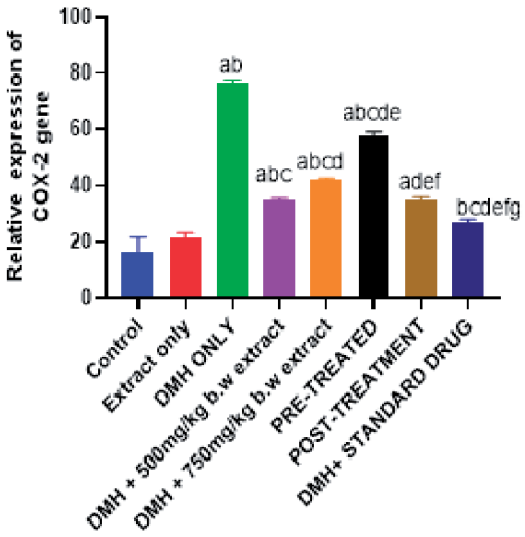

Figure 8. Cyclooxygenase-2 (Cox-2) gene expression of rats in different groups. Values are expressed as mean ± SEM, n=6/ group. Lowercase letters represent a significant difference at $P < 0.05$. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. The lowercase letter "d" stands for a significant departure from co-treatment (DMH+ 500mg/kg MEFE). The lowercase letter "e" stands for a significant departure from co-treatment (DMH+ 750mg/kg MEFE). A statistically significant divergence from pre-treated and post-treated is denoted by the lowercase letters "f" and "g" respectively. Cyclooxygenase-2 level was greatly increased in the DMH-only and pre-treated groups compared to other groups.

Discussion

The high-performance liquid chromatography phytochemical profile of *Ficus exasperata* leaves revealed the abundance of bioactive compounds contributing to its potency against inflammation and cancer. Quercetin, a potent antioxidant, was found in high amounts, capable of scavenging free radicals and inhibiting lipid peroxidation [20]. Studies suggest that quercetin down-regulates mutant p53 protein expression in cancer cell lines and inhibits the JAK-STAT signaling pathway in inflammatory disorders. It also plays a crucial role in cancer prevention and tumor suppression through activation of caspase-3 and cas-9, and increased translocation of proapoptotic Bax to the mitochondria membrane [21–24]. Kaempferol, also abundant in *F. exasperata*, exhibits its antioxidant potency by reducing free radicals and reactive oxygen species production [25]. Kaempferol, found abundantly in *Ficus exasperata*, exhibits cytotoxic effects on various human colorectal cancer cell lines, including HCT116, HT-29, HCT-15, LS174-R colon, and SW480 cells. It induces apoptosis, causes cell cycle arrest at G2/M, and reduces cell migration and invasion [26–29]. These properties likely contribute to the pharmacological actions of MEFE. Rutin, another compound present in appreciable amounts, is known for its anticancer, chemopreventive, and chemosensitizing properties against various cancers [30–32]. Studies have demonstrated rutin's anticancer effects on human neuroblastoma LAN-5 cells and its cytotoxicity against human colon adenocarcinoma SW480 cells, with additional antitumor and anti-angiogenic effects in vivo [33–35]. The presence of rutin in MEFE adds to its beneficial effects. Isoquinoline, identified in *Ficus exasperata* extract (MEFE), possesses antiproliferative and anticancer properties, inducing cell death in various cancer cell lines through mechanisms like cell cycle arrest,

apoptosis, and autophagy [36–42]. Additionally, though present in small amounts in MEFE, caffeic acid exhibits diverse pharmacological properties such as immunomodulation, neuroprotection, anti-inflammatory, antioxidant, and anticancer activities [43]. Caffeic acid is known for reducing oxidative stress, inhibiting DNA damage by free radicals, and demonstrating potential antitumor effects in cell cultures and animal models, suggesting a protective role against colorectal cancer [44–46]. The presence of caffeic acid in MEFE confirms its anticarcinogenic capability.

DMH is a colon-specific carcinogen used to induce CRC in rodents. The methyldiazonium ion promotes oxidative stress through methylation of biomolecules in the epithelial cells of the colon. Most colon cancer is initiated by exposure to carcinogens, the cells may then progress through a series of precancerous lesions, premalignant, and malignant stages [14]. Superoxide dismutase (SOD), glutathione (GPx), and catalase (CAT) are more sensitive to oxidative damage induced by carcinogen treatment. These antioxidants play a crucial role in breaking down free radicals into less reactive molecules. During carcinogenesis, there is increased production of free radicals leading to more utilization of cells using antioxidants to break down this reactive species hence a reduction in antioxidant level. The reduced activities of these antioxidant enzymes contribute to an increase in the production of free radicals, surpassing the scavenging capacity of the antioxidant system in cancer. This imbalance can lead to a state of oxidative stress, which is known to play a pivotal role in the initiation and progression of cancer. The findings underscore the importance of maintaining a balanced antioxidant system to counteract the detrimental effects of oxidative stress associated with carcinogenesis. Thus, decreased activities of SOD and CAT observed in the DMH-treated rats in this study, may suggest their increased utilization to scavenge the dangerous increase in reactive oxygen species in the cancer tissues [47–48]. An increase in the activities of this enzyme in the administration of MEFE justifies that the plant is very potent in ameliorating colorectal cancer.

There was a decrease in total protein levels in colon tissues from animals in group 3 compared to animals that were treated with the methanol extract of *Ficus exasperata*. This might be a result of cancer cachexia [49]. The cachexia-inducing factors (CIFs) include tumor necrosis factor α (TNF-α), Interleukin 1 and 6 (IL-1, IL-6), Interferon γ [50]. These inflammatory markers were significantly high in group DMH-only group.

A high systemic level of pro-inflammatory cytokines (IL-6 and TNF-α) and an anti-inflammatory cytokine (IL-10) have been reported in colorectal cancer patients [51–52]. Data has suggested a potential role of the cytokine IL-6 in colon cancer. For instance, it has been shown that levels of IL-6 are increased in the serum of patients suffering from colon carcinoma, and IL-6 levels correlate with tumor size in colorectal cancer [53–55]. Gunasekaran *et al*. [56] also reported an increase in these inflammatory markers including COX-2 in Wistar rats treated with carcinogen. COX-2 is mainly expressed in cancerous conditions via the activation of inflammatory cytokines. It directs cancer cell proliferation by inhibiting apoptosis and enhancing cancer-induced angiogenesis. A similar trend was observed in this study, IL-6, IL-10, COX-2, and TNF-α were significantly increased in group 3 compared to groups 1 and 2. However, the levels of these markers were restored to normal on administration of the plant extract. The post-treatment appeared more effective in combating inflammation.

Conclusion

This study ascertained that *Ficus exasperata is* rich in anticancer compounds which contributed immensely to the potency of the plant in suppressing inflammation and oxidative stress induced by DMH colorectal cancer in rats.

Acknowledgements

Author contribution
Ngozi Paulinus OKOLIE and Olayemi Mujidat OLUDE prepared the materials used in this study. Olude OM. wrote the initial draft of the manuscript, while Okolie NP. designed the study and edited the written manuscript. The final paper was reviewed and approved by both authors.

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
There are no sources of funding to declare.

References

1. Rajendran MS, Jayaraman S, Khan JS, Jasmine S, Prabhakaran R, Raju MV, Chandrasekaran MK, Ahalliya RM, Kannappan P, Palanisamy CP, Kanniappan GV. Investigating the colon toxicity and carcinogenic role of monosodium glutamate compared with Dimethylhydrazine in male Wistar rats: Exploring the link to childhood colon cancer risk. J. King Saud Uni. Sci. 2024; 36:103507. https://doi.org/10.1016/j.jksus.2024.103507
2. Burgos-Molina AM, Tellez-Santana T, Redondo M, Bravo-Romero MJ. The crucial role of inflammation and the immune system in colorectal cancer carcinogenesis: A comprehensive perspective. Int. J. Mol. Sci. 2024; 25:6188. https://doi.org/10.3390/ijms2511618
3. Mak WY, Zhao M, Ng SC, Burisch J. The epidemiology of inflammatory bowel disease: East meets west. JGH, 2020; 35:380–389 doi: 10.1111/jgh.14872
4. Brown JC, Ma C, Shi Q, Couture F, Kuebler P, Kumar P, Tan B, Krishnamurthi S, Chang V, Goldberg RM,. O'Reilly EM, Shields AF, Meyerhardt JA. Inflammation, physical activity, and disease-free survival in stage III colon cancer: Cancer and Leukemia Group B–Southwest Oncology Group 80702 (Alliance). JNCI, 2024; 116(12);2032-2039 doi: 10.1093/jnci/djae203.
5. Shahgoli VK, Noorolyai S, Youshanlui MA, Saeidi H, Nasiri H, Mansoori B, Holmskov U, Baradaran B. Inflammatory bowel disease, colitis, and cancer: unmasking the chronic inflammation link. Int. J. Colorectal Dis, 2024; 39:173 https://doi.org/10.1007/s00384-024-04748
6. Johnson DE, O'Keefe RA, Grandis JR. Targeting the IL-6/JAK/ STAT3 signalling axis in cancer. Nat. Rev. Clin. Oncol, 2018; 15(4):234-248 doi: 10.1038/nrclinonc.2018.8.
7. Janion K, Strzelczyk JK, Walkiewicz KW, Biernacki K, Copija A, Szczepanska E, Nowakowska-Zajdel E. Evaluation of Malondialdehyde Level, Total Oxidant/Antioxidant Status and Oxidative Stress Index in Colorectal Cancer Patients. Metabolites, 2022; 12:1118. https://doi.org/10.3390/ metabo12111118
8. Olude OM, Asoya VE, Uchenna HI. Phytochemical and nutritional composition of crude powder and ethanol extract of Annona muricata leaves. TJNPR, 2020; 4(7):315-318 doi.org/10.26538/tjnpr/v4i7.11
9. Rodríguez-Negrete EV, Morales-Gonzalez A, MadrigalSantillán EO, Sánchez-Reyes K, Álvarez-González I, MadrigalBujaidar E, Valadez-Vega C, Chamorro-Cevallos G, Garcia-Melo LF, Morales-González JA. Phytochemicals and Their Usefulness in the Maintenance of Health. Plants, 2024; 13:523. https://doi.org/ 10.3390/plants13040523
10. Zaman W. Morphology, Palynology and Phytochemicals of Medicinal Plants. Horticulturae, 2024; 10:202. https://doi.org/10.3390/ horticulturae10030202
11. Alemu M, Lulekal E, Asfaw Z, Warkineh B, Debella A, Abebe A, Degu S, Debebe E. Antibacterial activity and phytochemical screening of traditional medicinal plants most preferred for treating infectious diseases in Habru District, North Wollo Zone, Amhara Region, Ethiopia. PLOS ONE, 2024; 19(3):e0300060. https://doi.org/10.1371/journal. pone.0300060
12. Raudone L, Savickiene N. Phytochemical Profiles of Plant Materials: From Extracts to Added Value Ingredients. Plants, 2024; 13:964. https://doi.org/10.3390/plants13070964
13. Olude OM, Omoregie, FO. Antioxidant potential of ethanol extract of annona muricata leaves and its inhibitory effect on lipid peroxidation in 1,2-dimethylhydrazine induced colon carcinogenesis. J. Biol. Res. 2023b; 21(3):2079-2090 https://dx.doi.org/10.4314/br.v21i3.2
14. Olude OM, Chukwu OJ. Protective effect of ethanol extract of annona muricata leaves on hematological profile, histology and oxidative stress in 1,2 dimethylhydrazine-induced colorectal carcinogenesis in rats. JASEM, 2023a; 27(2):257-265 DOI: https://dx.doi.org/10.4314/jasem.v27i2.11
15. Elekofehinti OO, Lawal AO, Ejelonu OC, Molehin OR, Famusiwa CD. Involvement of fat mass and obesity gene (FTO) in the anti-obesity action of Annona muricata Annonaceae: in silico and in vivo studies. IJDMD, 2020; https://doi.org/10.1007/s40200-020-00491-7.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J. Biol. Chem. 1951; 193:265–275 PMID: 14907713.
17. Cohen G, Denbliec D, Marcus S. Measurement of catalase activity in tissue extracts. Anal. Biochem, 1970; 34:30-38 doi: 10.1016/0003-2697(70)90083-7
18. Misra HP, Fridovich I. The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem, 1972; 247:3170-3175 PMID: 4623845.
19. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med, 1967; 70:158-169 PMID: 6066618.
20. Cizmarova B, Hubkova B, Birkova A. Quercetin as an effective antioxidant against superoxide radical. J. Funct. Foods, 2023; 3(3):5-25 . DOI: https://www.doi.org/10.31989/ffs.v3i3.1076
21. Khan F, Niaz K, Maqbool F, Hassan FI, Abdollahi M, Venkata KCN, Nabavi SM, Bishayee A. Molecular targets underlying the anticancer effects of quercetin: an update. Nutrients, 2016; 8:529; doi:10.3390/nu8090529
22. Massi A, Bortolini ID, Ragno D, Bernardi T, Sacchetti G, Tacchini M, De-Risi C, Research progress in the modification of quercetin leading to anticancer agents. Molecules, 2017; 22:1270; doi: :10.3390/molecules22081270
23. Hashemzaei M, Far AD, Yari A, Heravi RE, Tabrizian K, Taghdisi SM, Sadegh SE, Tsarouha KN, Kouretas D, Tzanakakis G, Nikitovic D, Anisimov NY, Spandidos DA, Tsatsakis AM, Rezaee R. Anticancer and apoptosis-inducing effects of quercetin in vitro and in vivo. Oncol. Rep, 2017; 38:819-828 doi: 10.3892/or.2017.5766
24. Adorisio S, Argentieri MP, Avato P, Caderni G, Chioccio S, Cirmi S, Delfi DV, Greco G, Hrelia P, Iriti M, Lenzi, M, Lombardo GE, Luceri C, Maugeri A, Montopoli

M, Muscari I, Nani, MF, Navarra M, Gasperini S, Turrini E, Fimognari C. The molecular basis of the anticancer properties of quercetin. Pharmadvances, 2021; 3(3):496-520 Doi: 10.36118/pharmadvances.2021.10
25. Imran M, Salehi B, Sharifi-Rad J, Gondal TA, Saeed F, Imran A, Shahbaz M, Fokou PVT, Arshad MU, Khan H, Guerreiro SG, Martins N, Estevinho LM. Kaempferol: A key emphasis to its anticancer potential. Molecules, 2019; doi:10.3390/molecules24122277
26. Riahi-Chebbi I, Souid S, Othman H, Haoues M, Karoui H, Morel A, Srairi-Abid N, Essafi M, Essafi-Benkhadir K. The Phenolic compound kaempferol overcomes 5-fluorouracil resistance in human resistant LS174 colon cancer cells. Sci. Rep, 2019; 9:195 |DOI:10.1038/s41598-018-36808-z.
27. Choi JB, Kim JH, Lee H, Pak JN, Shim BS, Kim SH, Reactive oxygen species and p53 mediated activation of p38 and caspases is critically involved in kaempferol induced apoptosis in colorectal cancer cells. J. Agri. Food Chem, 2018; 66:9960-9967 doi: 10.1021/acs.jafc.8b02656
28. Lee HS, Cho HJ, Yu R, Lee KW, Chun HS, Park JH. Mechanisms underlying apoptosis-inducing effects of kaempferol in HT-29 human colon cancer cells. Int. J. Mol. Sci, 2014; 15:2722-2737 doi: 10.3390/ijms15022722.
29. Shahbaz M, Imran M, Alsagaby SA, Naeem H, Al-Abdulmonem W, Hussain M, Abdelgawad MA, El-Ghorab AH, Ghoneim MM, El-Sherbiny M, Atoki AV, Awuchi CG. Anticancer, antioxidant, ameliorative and therapeutic properties of kaempferol. Int. J. Food Prop, 2023; 26(1):1140-1166 DOI: 10.1080/10942912.2023.2205040
30. Lin JP, Yang JS, Lin JJ, Lai KC, Lu HF, Ma CY, Wu RSC, Wu KC, Chueh FS, Wood WG, Chung JG. Rutin inhibits human leukemia tumor growth in a murine xenograft model in vivo. Environ. Toxicol, 2012; 27(8):480-484 doi: 10.1002/tox.20662
31. Jayameen P, Sivakumari K, Ashok K, Rajesh S. Rutin: a potential anticancer drug against human colon cancer (hct116) cells. IJHPAS, 2018; 7(9):1731-1745
32. Nouri Z, Fakhri S, Nouri K, Wallace CE, Farzaei MH, Bishayee A. Targeting multiple signaling pathways in cancer: the rutin therapeutic approach. Cancers, 2020; 12:2276; doi:10.3390/cancers12082276
33. Chen H, Miao Q, Geng M, Liu J, Hu Y, Tian L, Pan J, Yang Y. Anti-tumor effect of rutin on human neuroblastoma cell lines through inducing G2/M cell cycle arrest and promoting apoptosis. Sci. World J, 2013; http://dx.doi.org/10.1155/2013/269165
34. Alonso-Castro AJ, Dominguez F, Garcia-Carranca A. Rutin exerts antitumor effects on nude mice bearing SW480 tumor. Arch. Med. Res, 2013; 44:346-351. doi: 10.1016/j.arcmed.2013.06.002.
35. Prasad R, Prasad B. A review on the chemistry and biological properties of rutin, a promising nutraceutical agent. Asian j. pharm. pharmacol., 2019; 5:1-20 DOI:10.31024/AJPP.2019.5.S1.1
36. Iranshahy M, Quinn RJ, Iranshah M. Biologically active isoquinoline alkaloids with drug-like properties from the genus Corydalis. RSC Adv, 2014; 4:15900–15913. DOI: 10.1039/c3ra47944
37. Danao KR, Malghade PM, Mahapatra DK, Motiwala MN, Mahajan UN. Progressive insights into the pharmacological importance of isoquinoline derivatives in modern therapeutics. Int. J. Curr. Res. Rev, 2021; 13(4):83-90. DOI: 10.31782/IJCRR.2021.13421
38. Lizuka N. Inhibitory effect of Coptidis Rhizoma and berberine on the proliferation of human esophageal cancer cell lines. Cancer Letters, 2000; 148:19-25. https://doi.org/10.1016/S0304-3835(99)00264-5
39. Choi JB, Kim JH, Lee H, Pak JN, Shim BS, Kim SH. Reactive oxygen species and p53 mediated activation of p38 and caspases is critically involved in kaempferol induced apoptosis in colorectal cancer cells. J. Agri. Food Chem, 2018; 66:9960-9967. doi: 10.1021/acs.jafc.8b02656
40. Havelek R, Seifrtova M, Kralovec K, Krocova E, Tejkalova V, Novotny I, Cahlíkova L, Safratova M, Opleta L, Bilkova Z, Vavrova I, Rezacova M. Comparative cytotoxicity of chelidonine and homochelidonine, the dimethoxy analogues isolated from Chelidonium majus L. (Papaveraceae), against human leukemic and lung carcinoma cells. J. Phymed 2016; 23:253–266. DOI: 10.1016/j.phymed.2016.01.001
41. Al-ghazzawi, A.M.; Anti-cancer activity of new benzyl isoquinoline alkaloid from Saudi plant Annona squamosa. BMC Chemistry, 2019; 13:1-6. https://doi.org/10.1186/s1306501905364
42. Yun D, Yoon SY, Park SJ, Park YJ. The anticancer effect of natural plant alkaloid isoquinolines. Int. J. Mol. Sci, 2021; 22:1653. https://doi.org/10.3390/ijms22041653
43. Alam M, Ahmed S, Elasbali AM, Adnan M, Alam S, Hassan MI, Pasupuleti VR. Therapeutic implications of caffeic acid in cancer and neurological diseases. Front. Oncol, 2022; 12:860508. doi: 10.3389/fonc.2022.860508
44. Korkina, L.; Phenylpropanoids as naturally occurring antioxidants: from plant defense to human health. Cell. Mol. Bio, 2007; 53(1):15–25. PMID: 17519109.
45. Zdunska K, Dana A, Kolodziejczak A, Rotsztejn H. Antioxidant properties of ferulic acid and its possible application. Skin Pharmacol. Physiol, 2018; 31(6):332–6. doi: 10.1159/000491755.
46. Dik VK, Bueno-de-Mesquita HB, Van-Oijen MG, Siersema PD, Uiterwaal CS, Van-Gils CH. Coffee and tea consumption, genotype-based CYP1A2 and NAT2 activity and colorectal cancer risk—results from the epic cohort study. Int. J. Cancer, 2014; 135(2):401–12. doi: 10.1002/ijc.28655
47. Manju V, Nalini N. Effect of ginger on lipid peroxidation and antioxidant status in 1,2- dimethyl hydrazine induced experimental colon carcinogenesis. J. Biochem. Tech, 2010; 2(2):161-167
48. Khan NG, Kumar N, Ballal R, Datta D, Belle VS. Unveiling antioxidant and anti-cancer potentials of characterized Annona reticulata leaf extract in 1,2-dimethylhydrazine-induced colorectal cancer in Wistar rats. J Ayurveda Integr Med, 2021; 12:579-589. oi: 10.1016/j.jaim.2021.05.010.
49. Kasprzak A. The role of tumor microenvironment cells in colorectal cancer (CRC) cachexia. Int. J.

Mol. Sci, 2021; 22, 1565. https://doi.org/10.3390/ijms22041565
50. Fearon KC, Glass DJ, Guttridge DC. Cancer cachexia: Mediators, signaling, and metabolic pathways. Cell Metab. 2012; 15(2):153–166. doi: 10.1016/j.cmet.2012.06.011.
51. Stanilov N, Miteva L, Deliysky T, Jovchev J, Stanilova S. Advanced colorectal cancer is associated with enhanced IL-23 and IL-10 serum levels, Lab Med 2010; 41(3):159b–163, https://doi.org/10.1309/LM7T43AQZIUPIO
52. Szkaradkiewicz A, Marciniak R, Chudzicka–Strugała I, Wasilewska A, Drews M, Majewski P, Karpinski T, Zwozdziak B. Proinflammatory cytokines and IL-10 in inflammatory bowel disease and colorectal cancer patients. Arch. Immunol. Ther. Exp. 2009; 57:291–294 DOI: 10.1007/s00005-009-0031-z
53. Lin Y, He Z, Ye J, Liu Z, She X, Gao X, Liang R. Progress in understanding the il-6/stat3 pathway in colorectal cancer. Onco Targets Ther, 2020; 13:13023–13032 doi: 10.2147/OTT.S278013
54. Waldner MJ, Foersch S, Neurath MF. Interleukin-6 – A key regulator of colorectal cancer development. Int. J. Bio Sci, 2012; 8(9):1248-1253. DOI: 10.7150/ijbs.4614
55. Zeng J, Tang Z, Liu S, Guo S. Clinicopathological significance of overexpression of interleukin-6 in colorectal cancer. World J Gastroenterol. 2017; 14; 23(10):1780-1786 doi: 10.3748/wjg.v23.i10.1780
56. Gunasekaran S, Venkatachalam K, Namasivayam N. Anti-inflammatory and anticancer effects of p-methoxycinnamic acid, an active phenylpropanoid, against 1,2-dimethylhydrazine-induced rat colon carcinogenesis. Mol. Cell. Biochem, 2019; 451:117–129. doi: 10.1007/s11010-018-3398-5.