Research Article

Anticancer Activity of Black Turtle Bean against Breast and Colorectal Adenocarcinoma: A Pre-clinical Study

R Durga Priyadarshini, D Annette Beatrice*

Department of Home Science, Women's Christian College (Affiliated to the University of Madras), Chennai, Tamil Nadu, INDIA.

Submission Date: 03-03-2022; Revision Date: 27-03-2022; Accepted Date: 07-04-2022.

ABSTRACT

Black turtle bean (BTB) is a nutrient rich common bean that remain least explored and underutilized in India. Throwing light to the known and discovering unknown phytochemicals present can further help narrow focus on treating chronic diseases such as cancer and reduce disease severity. Hence, this study aimed to analyse the phytochemical profile, dietary fiber content and anti-cancer activity of BTB against cancer cell lines. Raw and cooked BTB extracts were prepared using ethanol. GC-MS analysis was carried out to identify the phytochemicals present. Dietary fiber (DF) was estimated using the AOAC official method. The anticancer activity of BTB extracts against breast adenocarcinoma (MCF-7) and colorectal adenocarcinoma (HT-29) was evaluated by MTT anti-proliferative assay. Results showed the presence of various phytochemicals predominantly comprised of flavonoids, terpenoids, steroid, fatty acids, hydrocarbon, phenol and other compounds both in raw and cooked extracts of BTB. Compounds namely quercetin, myricetin, palmitic acid, vanillyl alcohol and gallic acid were commonly present in both extracts, however the levels were found to be reduced in the cooked extract. The total dietary fiber content of raw BTB extract was 9.03±0.36 g/100g (Insouble DF-7.97±0.15 g/100g; Soluble DF- 1.06±0.09 g/100g) and of cooked BTB extract was 6.56±0.16 g/100g (Insouble DF- 5.31±0.02 g/100g; Soluble DF-1.25±0.02 g/100g). A well pronounced anti-proliferative activity was expressed by raw BTB extract against MCF-7 cells with an IC_{50} of 10.25 µg/mL when compared to cooked BTB extract whose IC_{50} was 100 µg/mL. With respect to HT-29 cells, excellent dose-dependent inhibition was exhibited by both raw BTB extract (IC_{50}- 4.81 µg/mL) and cooked BTB extract (IC_{50}- 25 µg/mL). Results of this present study emphasizes the need for further investigation to determine the phytochemicals responsible, identify potential mechanism of dietary fiber and explore pharmacological relevance.

Keywords: Black turtle bean, Breast cancer, Colorectal cancer, Dietary fiber, Phytochemicals.

Correspondence:
Dr. D Annette Beatrice,
Associate Professor, Department of Home Science, Women's Christian College, (Affiliated to the University of Madras), College Road, Chennai-600006, Tamil Nadu, INDIA.

Email: annettebeatrice@yahoo.com

INTRODUCTION

Common beans (*Phaseolus Vulgaris L.*) are dry edible seeds that are cultivated in temperate and subtropical regions consumed globally. They are regarded as nutritional powerhouses for their ecologically sustainable protein source.[1] Common beans are available in varied shapes, sizes and colours with similar nutrient composition. They are energy dense foods with lower glycemic index and contain low fat, high fibre, rich in micronutrients and polyphenols.[2]

Evidences from epidemiological studies indicate that consumption of common beans are known to reduce the risk of cancer.[3] Pre-clinical studies have confirmed the positive effect of common beans on various cancer cell lines.[4-8] This protective role might be attributed to the rich polyphenol profile of common beans which are known to counteract the oxidative process by neutralizing free radicals and chelating metals, cause inhibition of mutagenic agents, reduce proliferation of cancer cells and modulation of inflammatory-related cell

SCAN QR CODE TO VIEW ONLINE

www.ajbls.com

DOI: 10.5530/ajbls.2022.11.27

signalling pathway.[9-11] Generally, phenolic compounds in common beans vary based on seed coat colour and type of cultivar where dark colours are due to higher anthocyanin content and light colours are attributed to higher condensed tannins.[12] Further, polyphenols are bound to dietary fibers which are also found to play a crucial role in cancer risk reduction. The potential mechanism of action might be linked to the short chain fatty acids (SCFA) produced by fermentation of dietary fibers which enhance gut microbiota composition. These SCFAs are found to possess numerous anticancer properties such as promotion of cancer cell cycle arrest, apoptosis, and inhibition of chronic inflammatory process within certain physiological range. In addition, fecal bulking caused by dietary fiber can potentially reduce the time for proteolytic fermentation and can shorten the contact between potential carcinogens and mucosal cells.[13] Though the beneficial prospect is wider, processing of beans for consumption leads to physical, chemical and structural changes thereby, causing variability in nutrient composition and its bioavailability. Hence, assessment of common beans before and after processing might prove crucial in confirmation of the stated effects.

Of the varieties of common beans, the dark coloured variety namely black turtle beans (BTB) remain least explored and underutilized especially in India. Throwing light to the phytochemicals and dietary fiber present in BTB can further help narrow down the focus in treating chronic diseases such as cancer and also reduce disease severity. Hence, this study aimed to analyse the phytochemical profile and dietary fiber content of raw and cooked BTB and assess their anti-cancer activity on human cancer cell lines.

MATERIALS AND METHODS

Preparation of Extracts

Black turtle beans (*Phaseolus Vulgaris* L.) were purchased from a local market in Yercaud, Tamil Nadu, India. Yercaud is a quaint hill town in Salem district where different varieties of common beans including black turtle beans are cultivated. For the preparation of raw BTB extract, the seeds of BTB were rinsed in distilled water, air dried and pulverized into a course powder. The ground seeds were macerated in ethanol for 72 hrs. After 72 hrs, the supernatant was filtered through Whatman's filter paper no.1. The filtrate was evaporated in a petri dish to obtain condensed gummy extract. The condensed gummy extract was used for MTT assay. Stock solution was prepared and used for Gas Chromatography-Mass Spectrophotometry analysis. For the preparation of cooked BTB extract, the seeds were soaked for 12 hrs overnight in distilled water and pressure cooked for 12 min. The cooked beans were cooled and crushed coarsely. Same procedure was repeated to prepare the cooked BTB extract using ethanol.

Gas Chromatography - Mass Spectrophotometry (GC-MS)

GC-MS analysis was used to identify bioactive compounds present in the raw and cooked extracts of BTB. The samples were injected into an HP-5 column of 30 m × 0.25 mm i.d with 0.25 µm film thickness (Agilent technologies 6890 N JEOL GC Mate II GC-MS model). Helium was used as carrier gas with flow rate of 1mL/min where the injector was operated at 200°C and column oven temperature was set as 50-250°C at a rate of 10°C/min injection mode. For MS, an ionization voltage of 70 eV was set. Both ion source temperature and interface temperature was maintained at 250°C with a mass range of 50-600 mass units.

Identification of compounds

The bioactive compounds detected in the GC-MS were identified using the database of National Institute Standard and Technology (NIST). The spectrum of unknown components detected in the analysis was compared to the known spectrum values stored in the NIST library.

Estimation of Dietary Fibers

Dietary fiber was estimated using the AOAC official method 991.43 with slight modifications. In brief, this method is an enzymatic gravimetric method where three enzymes namely heat-stable a-amylase, protease and amyloglucosidase under different incubation conditions were used to remove starch and protein components. The enzyme digestate was filtered, washed with warm water to remove the residue and dried before weighing to determine the insoluble dietary fiber. For the estimation of soluble dietary fiber, the washed filtrate was precipitated with alcohol, re-filtered and dried before weighment. The sum of insoluble and soluble dietary fiber was used to obtain total dietary fiber.

Cell Lines

MCF-7 cells (Human breast adenocarcinoma) and HT-29 cells (human colorectal adenocarcinoma) were obtained from National Centre for Cell Science, Pune and cultured in Rose-well Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum, penicillin/streptomycin(250U/mL), gentamicin (100µg/mL) and amphotericin B (1mg/mL). All the chemicals used for cell culture was purchased

from Sigma Chemicals, MO, USA. The cultured cells were maintained at 37°C for 24 hrs in a humidified atmosphere of 5% CO_2.

MTT Antiproliferative Assay

MTT (2-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) was purchased from Invitrogen USA. Acridine orange, other fine chemicals and reagents were obtained from Sigma, Aldrich, USA. The conventional MTT assay was used to measure the proliferation rate and viability of cancer cells. MCF 7 cells and HT-29 cells were seeded in 96-well plates at density of $5x10^3$ cells/well in 200µL of RPMI with 10% FBS for 24 hrs. After the removal of culture supernatant, the RPMI containing raw BTB extract was added at various concentrations (1.625-250µg/mL) and kept for 48 hrs incubation. The cells were first incubated with MTT (10µL, 5mg/mL) for 4 hr at 37°C and later with DMSO for 1 hr at room temperature. Using a scanning multi-well spectrophotometer, the plates were read at 595nm. Doxorubicin was used as the reference drug. The cell viability was calculated using the following equation.

$$\text{Cell Viability (\%)} = \left\{\frac{\text{Mean OD}}{\text{Control OD}}\right\} \times 100$$

RESULTS

Phytochemical Profile of Raw and Cooked BTB

The phytochemical profile of raw and cooked BTB were obtained using GC-MS analysis. A total of 13 compounds were detected in the raw extract of BTB and 16 compounds were detected in the cooked extract of BTB. Both the extracts predominantly comprised of flavonoids, terpenoids, steroid, fatty acids, hydrocarbon, phenolic and other compounds. Table 1 presents the compound name, retention time and peak percentage of raw and cooked BTB extracts. The principal compound present in the raw extract was quercetin (15.41%) followed by phytol (13.37%) palmitic acid (10.82%), 1-pentadecene, 2 methyl hydrocarbon (10.44%) and myricetin (10.03%). In the cooked BTB extract, 3-phenyl-6,7-dicarboxyindan-1-one (10.1%) was the principal compound followed by quercetin (9.76%), myricetin (9.75%), palmitic acid (9.19%), 7-methyl-2,2,4,8 tetramethyl tricycle [5.3.1.0 (4,11)], undecane (8.10%) and flavone (8.05%). When compared, few compounds namely quercetin, myricetin, palmitic acid, vanillyl alcohol and gallic acid were commonly present in both extracts however the levels were found to be reduced in the cooked extract. The chromatogram of identified compounds present in the extracts are given in Figure 1. (a) and 1. (b).

Table 1: Phytochemical compounds identified in the ethanol extract of raw and cooked BTB (*Phaseolus Vulgaris L.*) measured by peak area.

Sl. No	Compound Name	RT		Peak Area (%)	
		Raw	Cooked	Raw	Cooked
1.	Myricetin	19.63	19.4	10.03	9.75
2.	Isoterpinolene	10.02	n.d	3.54	n.a
3.	Phenol, 2,4, 6-tris (1-methylethyl)-	16.12	n.d	5.11	n.a
4.	Pregna-5,8, 16 - triene - 3ά- ol - 20 - one acetate	23.12	n.d	4.84	n.a
5.	Phytol	18.8	n.d	13.37	n.a
6.	Vanillic acid, methyl ester	14.17	n.d	4.90	n.a
7.	Quercetin	19.03	19.03	15.41	9.76
8.	Palmitic Acid	17.78	17.73	10.82	9.19
9.	Vanillyl alcohol	12.1	12.1	3.84	3.34
10.	Phenol-2,6-bis(1,1-dimethylethyl)-4[(4-hydroxy-3,5-dimethylphenyl)methyl]-	21.62	n.d	5.71	n.a
11.	3-benzylidene-2,3-dihydro-5-phenyl-1H-1,4-benzodiazepin-2 -one	20.83	n.d	7.02	n.a
12.	1-Pentadecene, 2-methyl-	17.07	n.d	10.44	n.a
13.	Gallic Acid	15.95	16.12	4.92	6.48
14.	7-methoxy-2,2,4,8 - tetramethyl tricyclo[5.3.1.0 (4,11], undecane	n.d	17.07	n.a	8.10
15.	Dopacetic Acid	n.d	14.17	n.a	4.16
16.	Pregn-4-ene-3, 20* dione, 17 ethyl	n.d	22.42	n.a	4.25
17.	ά - pinene	n.d	9.67	n.a	2.86
18.	Isopropyl stearate	n.d	20.83	n.a	6.56
19.	3-phenyl-6, 7-dicarboxyindan-1-one	n.d	18.8	n.a	10.1
20.	Flavone	n.d	16.57	n.a	8.05
21.	Cholesta-7, 14-diene, [5ά]-	n.d	25.23	n.a	4.00
22.	Carveol [fr.2]	n.d	11.47	n.a	3.26
23.	Cyclobutanecarboxylic acid, heptadecyl ester	n.d	21.62	n.a	5.32
24.	Estra-1,3,5 (10), 6-tetraene-3,17-diol, diacetate, (17ά)-	n.d	23.15	n.a	4.72

Note: RT- Retention Time; n.d - not detected; n.a - not applicable.

Dietary Fiber Content of Raw and Cooked BTB

The raw BTB extract was found to contain 9.03±0.36 g/100g of total dietary fiber comprising majorly of insoluble dietary fiber (7.97±0.15 g/100g) and low amounts of soluble dietary fiber (1.06±0.09 g/100g).

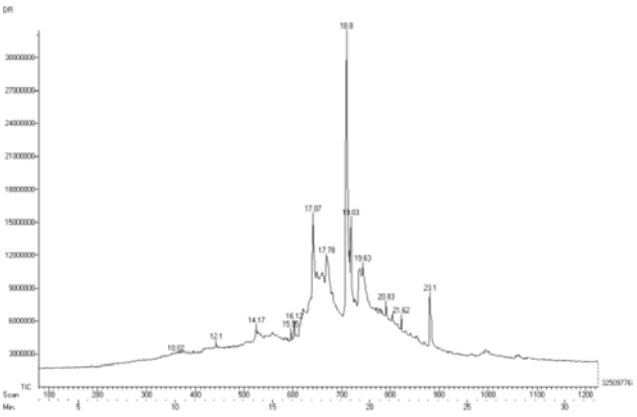

Figure 1: (a) Major bioactive compounds detected in raw BTB extract using GC-MS analysis.

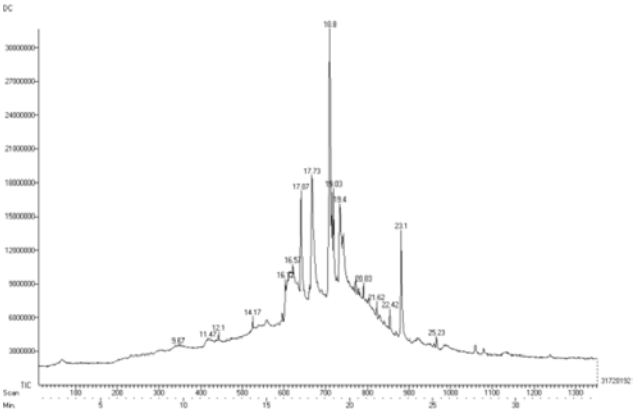

Figure 1: (b) Major bioactive compounds detected in cooked BTB extract using GC-MS analysis

Similar trend was observed with the dietary fibers present in the cooked BTB extract, however quantity was lesser in the cooked BTB extract (total dietary fiber 6.56±0.16 g/100g, insoluble dietary fiber 5.31±0.02 g/100g and soluble dietary fiber 1.25±0.02 g/100g) when compared to raw BTB extract.

Anticancer Activity of Raw and Cooked BTB

The cytotoxic activity of raw and cooked BTB extract against two cancer cells namely human breast cancer cells (MCF-7) and human colorectal cancer cells (HT-29) was determined determined using the MTT anti-proliferative assay. Results showed decreased viability of MCF-7 and HT-29 cells with increasing concentration of BTB extracts (Figure 2).

The IC_{50} value indicates the quantity of extract required to cause 50% reduction in the growth of cells. A well pronounced anti-proliferative activity was expressed by raw BTB extract against MCF-7 cells with an IC_{50} of 10.25 µg/mL when compared to cooked BTB extract whose IC_{50} was 100 µg/mL. With respect to HT-29

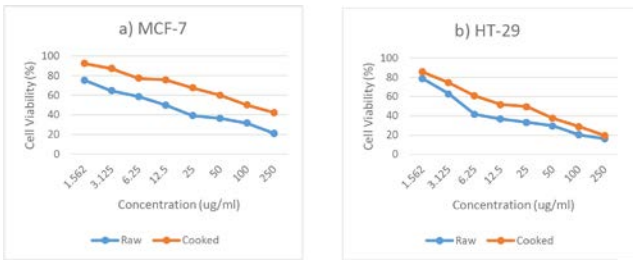

Figure 2: Dose-response curve showing % of cell viability of a) MCF -7 b) HT-29 by raw and cooked BTB extract.

cells, the raw BTB extract was found to have higher potency with an IC_{50} of 4.81 µg/mL. Whereas, the cooked BTB extract was found to perform 5 times lesser (than the raw BTB extract) with an IC_{50} of 25 µg/mL. Our results indicated superior anti-proliferative activity of raw BTB extract when compared to the reference drug doxorubicin (IC_{50} - 12.45 µg/mL). Overall, both raw and cooked BTB extract exhibited excellent cytotoxicity against both MCF-7 and HT-29 cells in a dose dependent manner.

DISCUSSION

Phytochemicals play a crucial role in the prevention and treatment of non-communicable diseases such as cardiovascular diseases, diabetes and cancer.[9] These phytochemicals are known to exert beneficial effect on varying magnitude depending on the type and percentage of phytochemicals present. Hence screening of phytochemicals is essential to further understand its significance. GC-MS analysis of raw and cooked BTB showed presence of various phytochemicals predominantly comprising of flavonoids, terpenoids, steroid, fatty acids, hydrocarbon, phenol and other compounds in both extracts of BTB. Similar results were reported in a study by[5] where analysis of phenolic compounds in black beans grown in Egypt showed the abundant presence of vanillic acid, followed by catechin, cholorogenic acid, benzoic acid, epicatechin, ferulic acid, p-coumaric acid and galic acid in raw extract. However, the quantity of phenolic compounds were reduced after soaking and cooking of the beans thereby implying the effect of processing treatments similar to our results. Likewise,[14] assessed the flavonoids present in hulls of black beans (turtle eclipse cultivar) grown in Mexico and found the presence of myricetin 3-O-glucoside, quercetin 3-O galactoside and kaempferol 3-O glucoside. In contrast, analysis of black beans grown in New Zealand was found to contain high levels of polyunsaturated fatty acids namely linoleic and α-linolenic acid; moderate levels of palmitic and oleic

acid; low levels of stearic acid. Further, 68 volatile compounds were detected with abundant presence of alcohols and other class of compounds namely terpenes and aldehydes.[15] Similarly, GC-MS analysis of black beans grown in China detected the presence of 11 phytosterols namely cholesterol, cholestanol, brassicasterol, ergosterol, spinasterol, campesterol, stigmasterol, sitostanol, cycloartanol, cycloartenol and 2, 4, methylenecycloartanol which were not detected in the beans used in present study.[16] These variations in the phytochemicals detected in black beans from above quoted studies might be attributed to the difference in solvents used for extraction, growth environment of beans, processing methods and cultivars.

Dietary fiber being an essential nutrient are found to modulate human health through complicated modes of action alongside other phytochemicals and nutrients present in the food.[13] Our results showed that both raw and cooked BTB had higher proportion of insoluble dietary fibers. This finding is in line with the composition of dietary fiber present in other varieties of common bean which are also known to contain higher amounts of insoluble dietary fiber than the soluble dietary fiber.[17,18] Further, numerous studies have explored the role of dietary fiber in cancer prevention where a systematic review and meta-analysis has indicated that for every 10g/day increment in the dietary intake of fiber, a 4% reduction in breast cancer risk was observed.[19] Similar yet profound results were revealed in a study by[20] were the relative risk of developing colorectal cancer was 62% lower for every 10g of fiber from legume sources. This effect can jointly be attributed to the nature and quantity of dietary fiber and phytochemicals present in the beans since polyphenols may work synergistically with dietary fiber.[17] Further research on animal models and human intervention would help to evaluate and understand the complete role of dietary fiber present in common beans variety against cancer.

The prime focus of the present study was to examine the *in vitro* anticancer potential of raw and cooked BTB extracts. Results indicated that both raw and cooked BTB extracts exhibited dose dependant cytotoxicity against MCF-7 and HT-29 cells. Review of literature retrieved few studies exploring the anticancer potential of black beans. A study by[4] explored the anti-proliferative effect of BTB against human breast cancer cells (MCF-7 and MDA-MB231 cell line). This study used crude extract of BTB and results revealed that crude BTB extract inhibits the viability of MCF-7 and MDA-MB231 cells in a dose-dependent manner both with an IC_{50} of 50µg/mL. The anti-proliferative effect of crude BTB reported in the above cited study is found to be 5 times less effective than the raw BTB extract (IC_{50} - 10.25µg/mL) used in the present study. Similarly,[21] assessed the anti-proliferative activity of black beans (turtle eclipse cultivar) against human breast cancer cells (MCF-7), colorectal cancer cells (Caco-2 and SW480) and 7 other cancer cells (Tongue squamous carcinoma cell line- CAL27, gastric adenocarcinoma cell line - AGS, hepatocellular carcinoma cell line - HepG2, prostate carcinoma cell line - DU145, ovary adenocarcinoma cell line - SK-OV-3, leukemia cell line - HL-60). Hydrophilic extract of black bean had been used and results showed dose-dependent inhibition against all nine types of cancer cells where the effect was strongest against HL-60 and AGS cells with an IC_{50} of 700 µg/mL and 740 µg/mL respectively. With respect to MCF-7, Caco-2 and SW480 cells, a weak inhibitory effect (IC_{50} of 1910µg/mL, 1140µg/mL and 930µg/mL, respectively).[21] Moreover, the anti-proliferative potential of black beans against all 9 cancer cells cited in the above quoted study is apparently found to be much weaker than the results of the present study thereby highlighting the potency of raw and cooked BTB extracts. Moreover, our results also revealed a well pronounced anti-proliferative activity expressed by raw BTB extract and cooked BTB extract especially against HT-29 cells. This effect can be attributed to the rich phytochemical profile and dietary fiber present in BTB as research evidences have also highlighted the protective role of dietary fiber and phytochemicals in colorectal and breast cancer incidence.[19,20,22,23]

It is crucial to understand the mechanism of action and identify compounds responsible to further facilitate pharmacological relevance. In our study, the phytochemicals predominantly present in both extracts were quercetin, myricetin, palmitic acid, vanillyl alcohol and gallic acid. A review of potential mechanism of phytochemicals against cancer cells reported in preclinical studies on breast cancer cells has revealed a dose and time dependent effect of quercetin which induced apoptosis, caused upregulation of Bax, down regulation of Bcl-2 and was found to modulate β-catenin and its target genes.[24,25] Similarly, myricetin also exhibited apoptopic effects by inducing the BRCA1- GADD45 pathway, suppressing the protein expression of PAK1, MEK and ERK1/2 signalling, activation of GSK3β and Bax protein expression, inhibition of β-catenin , cyclin D1, PCN antigen and promotion of caspase-3 activity in MCF-7 cells.[26-27] In a study by,[28] palmitic acid was found to alter the nuclear morphology of breast cancer cells and caused significant DNA damage. Further, expression analysis showed expression of p53, Bax, caspase 3, caspase 9 and inhibition of Bcl12, an anti-apoptopic protein.[28]

Exposure of breast cancer cells to gallic acid revealed that both extrinsic (Fas/FasL pathway) and intrinsic pathway (mitochondrial pathway) were triggered to induce apoptosis.[29] Experimental evidences against colorectal/colon cancer cells has shown that quercetin reduced AMPK activity leading to apoptosis.[30-31] Similarly, myricetin was found to modulate the Bax/Bcl-2 dependent pathway thereby inducing apoptopic cell death in human colon cancer cells.[32] In a study by,[33] gallic acid upregulated caspase-3/pro-caspase-3 ratio, cleaved caspase-9/procaspase-9 ratio and decreased the level of (p)-SRC, p-EGFR, p-AKT and p-STAT3, thereby causing Apoptosis of colon cancer cells.[33] These above stated mechanisms support the present results with relevance to the phytochemical profile and antiproliferative potency of BTB extracts.

CONCLUSION

In summary, the present study revealed the presence of various phytochemicals predominantly comprised of flavonoids, terpenoids, steroid, fatty acids, hydrocarbon, phenol and other compounds both in raw and cooked extracts of BTB. The anti-proliferative activity of BTB extracts observed against breast and colorectal adenocarcinoma deserves further investigations in order to determine the phytochemicals responsible, their potential mechanism and development of anti-cancer drugs. Not all fibers possess same properties, hence it is vital to characterize and quantify the types of dietary fibers present to further understand the role of dietary fibers in cancer prevention. These results also encourage the consumption of BTB owing to the rich phytochemicals profile, dietary fiber content and anti-cancer potential.

ACKNOWLEDGEMENT

The authors would like to thank the Junior Research Fellowship (JRF) scheme of the University Grants Commission (UGC) for their financial support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Nchanji EB, Ageyo OC. Do common beans (*Phaseolus vulgaris L.*) promote Good Health in humans? A systematic review and meta-analysis of clinical and randomized controlled trials. Nutrients. 2021;13(11):3701. doi: 10.3390/nu13113701, PMID 34835959.
2. Mudryj AN, Yu N, Aukema HM. Nutritional and health benefits of pulses. Appl Physiol Nutr Metab. 2014;39(11):1197-204. doi: 10.1139/apnm-2013-0557, PMID 25061763.
3. Campos-Vega R, Oomah BD, Loarca-Piña G, Vergara-Castañeda HA. Common beans and their non-digestible fraction: Cancer inhibitory activity-an overview. Foods. 2013;2(3):374-92. doi: 10.3390/foods2030374, PMID 28239123.
4. Kumar S, Sharma VK, Yadav S, Dey S. Antiproliferative and apoptotic effects of black turtle bean extracts on human breast cancer cell line through extrinsic and intrinsic pathway. Chem Cent J. 2017;11(1):56. doi: 10.1186/s13065-017-0281-5, PMID 29086840.
5. Eshraq KB, Mona AM, Sayed FA, Abdel-Rahim EA. Bioactive components in black beans for inhibition of cancer cell growth. Res J Pharm Biol Chem Sci. 2016;7(6):1068-80.
6. Cruz-Bravo RK, Guevara-González RG, Ramos-Gómez M, Oomah BD, Wiersma P, Campos-Vega R, *et al*. The fermented non-digestible fraction of common bean (*Phaseolus vulgaris L.*) triggers cell cycle arrest and apoptosis in human colon adenocarcinoma cells. Genes Nutr. 2014;9(1):359. doi: 10.1007/s12263-013-0359-1, PMID 24293398.
7. Bobe G, Barrett KG, Mentor-Marcel RA, Saffiotti U, Young MR, Colburn NH, *et al*. Dietary cooked navy beans and their fractions attenuate colon carcinogenesis in azoxymethane-induced ob/ob mice. Nutr Cancer. 2008;60(3):373-81.
8. Hangen L, Bennink MR. Consumption of black beans and navy beans (*Phaseolus vulgaris*) reduced azoxymethane-induced colon cancer in rats. Nutr Cancer. 2002;44(1):60-5. doi: 10.1207/S15327914NC441_8, PMID 12672642.
9. Huber K. Phenolic Acid, Flavonoids and Antioxidant Activity of Common Brown Beans (*Phaseolus vulgaris* L.) Before and After Cooking. J Nutr Food Sci;06(5). doi: 10.4172/2155-9600.1000551.
10. Frassinetti S, Gabriele M, Caltavuturo L, Longo V, Pucci L. Antimutagenic and antioxidant activity of a selected lectin-free common bean (*Phaseolus vulgaris L.*) in two cell-based models. Plant Foods Hum Nutr. 2015;70(1):35-41. doi: 10.1007/s11130-014-0453-6, PMID 25631277.
11. Zhang C, Monk JM, Lu JT, Zarepoor L, Wu W, Liu R, *et al*. Cooked navy and black bean diets improve biomarkers of colon health and reduce inflammation during colitis. Br J Nutr. 2014;111(9):1549-63. doi: 10.1017/S0007114513004352, PMID 24521520.
12. Juárez-López BA, Aparicio-Fernández X. Polyphenolics concentration and antiradical capacity of common bean varieties (*Phaseolus vulgaris L.*) after thermal treatment. In: Nevárez-Moorillón GV, Ortega-Rivas E, editors. Food science and food biotechnology essentials: A contemporary perspective. Mexico: Asociación Mexicana de Ciência de los Alimentos, A.C [Mexican Association of Food Science]; 2012. p. 25-33.
13. Zeng H, Lazarova DL, Bordonaro M. Mechanisms linking dietary fiber, gut microbiota and colon cancer prevention. World J Gastrointest Oncol. 2014;6(2):41-51. doi: 10.4251/wjgo.v6.i2.41, PMID 24567795.
14. Aregueta-Robles U, Fajardo-Ramírez OR, Villela L, Gutiérrez-Uribe JA, Hernández-Hernández J, López-Sánchez RDC, *et al*. Cytotoxic activity of a black bean (*Phaseolus vulgaris L.*) extract and its flavonoid fraction in both *in vitro* and *in vivo* models of lymphoma. Rev Invest Clin. 2018;70(1):32-9. doi: 10.24875/RIC.17002395, PMID 29513299.
15. Khrisanapant P, Kebede B, Leong SY, Oey I. A comprehensive characterisation of volatile and fatty acid profiles of legume seeds. Foods. 2019;8(12):E651. doi: 10.3390/foods8120651, PMID 31817745.
16. Wang D, Zhang Y, Fan B, Zang L, Shen C, Wang F. GC-MS analysis on form and content of phytosterol in the beans from different producing areas. Asian Agric Res. 2017;9:77-83.
17. Luna-Vital DA, Ramirez-Jimenez AK, Gaytan-Martinez M, Mojica L, Loarca-Pine G. Biological effect of antioxidant fiber from common beans (*Phaseolus vulgaris L.*). In: Hosseinian F, Oomah BD, Campos-Vega R, editors. Dietary fiber functionality in food and nutraceuticals. New York: John Wiley and Sons; 2016. p. 98.
18. De Almeida Costa GEde A, Da Silva Queiroz-Monici K, Pissini Machado Reis SM, De Oliveira AC. Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. Food Chem. 2006;94(3):327-30. doi: 10.1016/j.foodchem.2004.11.020.
19. Chen S, Chen Y, Ma S, Zheng R, Zhao P, Zhang L, *et al*. Dietary fibre intake and risk of breast cancer: A systematic review and meta-analysis of epidemiological studies. Oncotarget. 2016;7(49):80980-9. doi: 10.18632/oncotarget.13140, PMID 27829237.

20. Aune D, Chan DS, Lau R, Vieira R, Greenwood DC, Kampman E, *et al*. Dietary fibre, whole grains, and risk of colorectal cancer: Systematic review and dose–response meta-analysis of prospective studies. BMJ. 2011;343:d6617. doi: 10.1136/bmj.d6617, PMID 22074852.
21. Xu B, Chang SK. Comparative study on antiproliferation properties and cellular antioxidant activities of commonly consumed food legumes against nine human cancer cell lines. Food Chem. 2012;134(3):1287-96. doi: 10.1016/j.foodchem.2012.02.212, PMID 25005945.
22. Veettil SK, Wong TY, Loo YS, Playdon MC, Lai NM, Giovannucci EL, *et al*. Role of diet in colorectal cancer incidence: Umbrella review of meta-analyses of prospective observational studies. JAMA Netw Open. 2021;4(2):e2037341. doi: 10.1001/jamanetworkopen.2020.37341, PMID 33591366.
23. Vitelli-Storelli F, Zamora-Ros R, Molina AJ, Fernández-Villa T, Castelló A, Barrio JP, *et al*. Association between polyphenol intake and breast cancer risk by menopausal and hormone receptor status. Nutrients. 2020;12(4):E994. doi: 10.3390/nu12040994, PMID 32260135.
24. Srinivasan A, Thangavel C, Liu Y, Shoyele S, Den RB, Selvakumar P, *et al*. Quercetin regulates β-catenin signaling and reduces the migration of triple negative breast cancer. Mol Carcinog. 2016;55(5):743-56. doi: 10.1002/mc.22318, PMID 25968914.
25. Duo J, Ying GG, Wang GW, Zhang L. Quercetin inhibits human breast cancer cell proliferation and induces apoptosis via Bcl-2 and Bax regulation. Mol Med Rep. 2012;5(6):1453-6. doi: 10.3892/mmr.2012.845, PMID 22447039.
26. Sajedi N, Homayoun M, Mohammadi F, Soleimani M. Myricetin exerts its apoptotic effects on MCF-7 breast cancer cells through evoking the BRCA1-GADD45 pathway. Asian Pac J Cancer Prev. 2020;21(12):3461-8. doi: 10.31557/APJCP.2020.21.12.3461, PMID 33369440.
27. Jiao D, Zhang XD. Myricetin suppresses p21-activated kinase 1 in human breast cancer MCF-7 cells through downstream signaling of the β-catenin pathway. Oncol Rep. 2016;36(1):342-8. doi: 10.3892/or.2016.4777, PMID 27122002.
28. Zafaryab Md, Fakhril KU, Asad KMd, Hajela K, Rizvi MMA. *In vitro* assessment of cytotoxic and apoptotic potential of palmitic acid for breast cancer treatment. Int J Life Sci Res. 2019;7(1):166-74.
29. Wang K, Zhu X, Zhang K, Zhu L, Zhou F. Investigation of gallic acid induced anticancer effect in human breast carcinoma MCF-7 cells. J Biochem Mol Toxicol. 2014;28(9):387-93. doi: 10.1002/jbt.21575, PMID 24864015.
30. Velázquez KT, Enos RT, Narsale AA, Puppa MJ, Davis JM, Murphy EA, *et al*. Quercetin supplementation attenuates the progression of cancer cachexia in ApcMin/+ mice. J Nutr. 2014;144(6):868-75. doi: 10.3945/jn.113.188367, PMID 24759931.
31. Kim HS, Wannatung T, Lee S, Yang WK, Chung SH, Lim JS, *et al*. Quercetin enhances hypoxia-mediated apoptosis via direct inhibition of AMPK activity in HCT116 colon cancer. Apoptosis. 2012;17(9):938-49. doi: 10.1007/s10495-012-0719-0, PMID 22684842.
32. Kim ME, Ha TK, Yoon JH, Lee JS. Myricetin induces cell death of human colon cancer cells via BAX/BCL2-dependent pathway. Anticancer Res. 2014;34(2):701-6. PMID 24511002.
33. Lin X, Wang G, Liu P, Han L, Wang T, Chen K, *et al*. Gallic acid suppresses colon cancer proliferation by inhibiting SRC and EGFR phosphorylation. Exp Ther Med. 2021;21(6):638. doi: 10.3892/etm.2021.10070, PMID 33968169.

Cite this article: Priyadarshini RD, Beatrice DA. Anticancer Activity of Black Turtle Bean against Breast and Colorectal Adenocarcinoma: A Pre-clinical Study. Asian J Biol Life Sci. 2022;11(1):193-9.