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The role of plant processing for the cancer preventive potential of Ethiopian kale (Brassica carinata)

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ABSTRACT

Ethiopian kale (Brassica carinata) is a major crop for horticulture in many regions of East and Southern Africa, where it is grown for its green vegetables. Secondary plant metabolites have been found in the leaves, according to reports. However, there is a paucity of scientific evidence to support their health advantages.

The study's goal was to see whether B. carinata may prevent cancer in a human liver in vitro model by examining the effects of processing on secondary plant metabolite patterns and immunogenicity.

As a result of this study's design, B. carinata was grown under controlled circumstances and then processed either raw or cooked after harvesting. Ethanolic extracts of raw or processed B. carinata leaves were tested for their anti-genotoxic, anti-oxidant, and cytostatic properties on human liver cancer cells (HepG2). A variety of glucosinolates, including their breakdown products, phenolic compounds, carotenoids, and chlorophyll content, were analysed for their chemical properties.

B. carinata extracts concentrations were increased after pre-treatment. As shown by electron paramagnetic resonance spectroscopy, aflatoxin-induced DNA damage was decreased, reactive oxygen species were reduced, and Nrf2-mediated gene expression was in turn induced. Cytostasis was also aided by increasing extract concentrations. Secondary plant metabolite concentrations were significantly altered as a result of processing. Some endpoints tested showed an increase in protection against a variety of processing meth odologies, whilst others saw a reduction.

We conclude that B. carinata has cancer-preventive properties as shown by its ability to protect human liver cells against aflatoxin in vitro. B. carinata should be supported as part of chemopreventive strategies to reduce the occurrence of aflatoxin-induced illnesses in general.

Introduction

There are the most chronically malnourished individuals in Sub-Saharan Africa [1] than anywhere else in the world. A variety of factors contribute to Africa's severe food insecurity [2], but agronomic limits and limitations imposed by regionally suitable processing and cooking methods are particularly important [3,4]. African leafy vegetables (ALVs) have long been prized for their nutritious content and

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the presence of health-promoting secondary plant compounds that may help protect against noncommunicable diseases. It's for this reason that traditional ALV use is now on the rise throughout Africa. However, there is an unacceptable lack of scientific understanding about the effectiveness of these prospective health advantages. For example, Brassica carinata (also known as Ethiopian kale or Ethiopian mustard in English) is one of the most harmful plants in this context. In Ethiopia's highlands, it is grown for its oil seed and for its leaves.

While it's more common in Eastern and Southern Africa, it's less common in Western Africa and Central Africa. Glucosinolates, particularly 2-propenyl glucosinolate (sinigrin) and phenolic compounds, are found in abundance in the leaves and seeds of the plant [5, 8]. [9] These secondary plant metabolites have likely contributed to the Western idea of cancer prevention and management [9] and might account for a health-promoting impact of B. carinata. The fragile leaves and stems of this plant may be eaten fresh in salad, although they are most usually cooked or preserved. Biochemical characteristics and hence bioavailability of these secondary plant metabolisms may be dramatically altered by processing [10,11]. Long cooking times have been shown to rapidly degrade their nutritional value [12]. Extracts from B. carinata leaves were screened and evaluated for their cancer-prevention potential in this research. If food processing (fermentation or heating) alters the plants' ability to defend against aflatoxin B1 (AFB1), which is the most powerful naturally occurring chemical liver carcinogen, this study sought to answer that question. Despite the fact that the specific mechanism of AFB1-mediated carcinogenesis remains unknown, the fact that its conversion into the active, genotoxic AFB1-8,9-epoxide by phase I liver xenobiotic metabolism enzymes (primarily CYP3A4) is of critical significance [15]. AFB1-induced hepatocarcinogenesis has been shown to activate oxidative stress markers [16]. AFB1's genotoxicity in metabolically competent liver cells was investigated, as was the capacity to I act against oxidative stress and to (,) stimulate the cytostasis removal of malignant altered cells. The secondary plant metabolites found in the various extracts were also identified and quantified for their potential health benefits.

Methods and materials

Chemicals

In addition to foetal calf serum (FCS), DMEM, trypsin, trypsin-EDTA, and phosphate buffered saline (PBS, Ca and Mg-free) were acquired from GibcoTM, Life Technologies GmbH. L-glutamine, penicillin, and streptomycin (P/S) solution were also purchased from Gibco (Darmstadt, Germany). For the Krebs HEPES buffer, deferoxamine methanesulfonate (DFO), diethyldithiocarbamic acid sodium (DETC), and 1-hydroxy-3-methoxycarbonyl 2,2,5,5-tetramethylpyrrolidine (CMH), Noxygen Science Tranfer & Diagnostics GmbH bought the products (Elzach, Germany). From Carl Roth GmbH & Co. KG (Karlsruhe, Germany), Triton-X 100 was bought, and dimethyl sulfoxide (purity > 99 percent) was acquired from Applichem GmbH. (Darmstadt, Germany). Sigma-Aldrich Chemie GmbH provided the following chemicals: ethanol (EtOH), hydrochloric acid (37 percent), trypan blue, aflatoxin B1 (98 percent purity), menadione, and ethidium bromide, as well as DEAE-Sephadex A-25 (Taufkirchen, Germany). Agar Serva GmbH was contacted for the acquisition of low melting point agarose (LMPA) and regular melting point agarose (Heidelberg, Germany). Roche Diagnostics GmbH bought Helix pomatia's Arylsulfatase (Mannheim, Germany). Methanol, ammonium acetate, tetrahydrofuran, methyl tert-butylether, dichloromethane, isopropanol, and zeaxanthin were purchased from Carl Roth GmbH & Co. KG (Karlsruhe). The other chemicals used for chemical analyses were purchased from VWR International GmbH, Chemsolute Th. Geyer GmbH, Chemsolute Th. Geyer GmbH, and Chemsolute Th. Geyer GmbH and Co. KG (Taufkirchen, Germany). It was procured from Carl Roth GmbH & Co. KG that chlorogenic acid, quercetin 3-O-glucoside, and iso rhamnetin-3–O-glucoside were obtained (Karlsruhe, Germany).

Plants and their components

Brassica carinata A. Braun (Figure 1) was studied with fully grown leaves (Figure 1). The World Vegetable Center gave seeds of B. carinata (AVRDC). At the Max Rubner Institute, Federal Research Institute of Nutrition and Food (location A; Karlsruhe, Germany), and at the Leibniz Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt e.V. (location B; Großbeeren, Germany; at 52° latitude North, 13° longitude East and altitude 43 m above sea level), the plants were cultivated. There, at the site A, B. carinata was grown using Gramoflor's

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standard potting substrate, which had the following specifications: pH of 5.8; N 140; P 2O5 160; and K 2 O 180. For four weeks, the plants were grown in a climatic room at 25°C day and night and 80% relative humidity.

B. carinata inflorescences (A) and leaves (B) are seen in this figure. Three weeks after the first four weeks, the plants were moved to the greenhouse and stayed there at an ambient temperature and humidity level (up to 40°C in the summer). A 5 L plastic pot containing potting substrate (Einheitserdewerke Werkverband, Sinntal Altengronau, Germany) with the following specifications: pH 5.8; KCl (g/L) 2; KCl (g/L) 2; P2O5 (Cal) 390; K2O (CaCl2 – mg/L) 420. B. carinata seeds were put in the pots at position B. After germination, the plants were kept in a greenhouse with adequate watering for seven weeks. It was 21/18.6 degrees Fahrenheit during the day and 54 percent humidity at night during this time period. At harvest, 200 g of fresh leaf material pooled from around 10 plants was taken in three replicates, each of which had 200 g of completely formed leaves. Freeze-drying or additional processing of the leaves was then carried out.

Material handling in the processing of plants

According to a prior report [17], the newly picked leaves (from site A) were fermented in 10 L crock pots characteristic of German Sauerkraut fermentation. A 2.5 percent brine solution was used to wash the leaves and ferment 700 g of leaves. The starter bacteria Lactobacillus plantarum BFE 5092 and Lactobacillus fer mentum BFE 6620 were injected into the fermentation and allowed to ferment for six days at 25°C. Following this fermentation, the brine was removed, the plant material was weighed, freeze dried, and ground into a fine powder. Leaves from site B were cut into 1 cm pieces using a kitchen knife and placed in boiling water for thermal treatment. After simmering for 20 minutes, they were drained in a strainer. The drained samples were frozen as soon as they reached room temperature using ice cubes. In order to get a fine powder out of them, they were freeze dried and pulverised.

Processing plant materials to get an ethanolic extract

With 70% EtOH at 1:10 and 2 minutes of vortexes, the freeze-dried plant material was extracted in a water bath heated to 50°C for 30 minutes. The extract was filtered and sterilised using a 0.22 m MillexR syringe-driven filter device after being strained through gauze (fast flow and low binding Millipore). One hundred milligrammes per millilitre were found in the final stock solution. Serial dilutions were made and eventually diluted in culture media by a factor of one hundred in order to expose samples (max. concentration of solvent: 0.7 percent). Stock extracts were evaporated under a nitrogen stream and resuspended in EtOH at a concentration of 7 percent for the Nrf2 reporter gene experiment. The ultimate dilution was 1:10 in culture media after a series of dilutions (max. concentration of solvent: 0.7 percent). Extracts from the various plant powders were made fresh and utilised within two hours for each separate cell culture experiment. Freshly produced extracts of each plant powder were used in the chemical analyses.

Cell lines and the circumstances under which they are grown

The German Collection of Microorganisms and Cell Cultures provided the ACC-180 HepG2 cell line (DSMZ; Braunschweig, Germany). At 37°C and 5 percent carbon dioxide, the cells were incubated for 95 percent of the time in a humidified incubator with 15% FCS and 1% penicillin/streptomycin solution. In order to study the Nrf2 antioxidant response pathway, we obtained a recombinant ARE reporter–HepG2 cell

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line from BPS Bioscience, Inc. (San Diego, USA) and grew it as described for HepG2 cells in the presence of 600 g/mL geneticin.

The comet test (single cell gel electrophoresis)

We followed Lamy et al. [18] for the alkaline Comet test, but made a few adjustments to better identify B. carinata's anti-genotoxic action. Two rounds of PBS washing followed by exposure to 10 M AFB1 or 0.1 percent DMSO incubated HepG2 cells prepared with the ethanolic plant extracts for 24 hours. A cell collection and preparation for Comet analysis was then completed. There were 100 cells on each slide that had to be examined, and they were analysed using an image analysis system (Comet 5.5) that was attached to the Leica fluorescence microscope (Leica DMLS; excitation filter; BP 546/ 10nm; barrier filter: 590 nm). Percent tail DNA was used as a marker for DNA damage.

Spectroscopy of electron paramagnetic resonance

The formation of reactive oxygen species (ROS) in HepG2 cells was detected using EPR spectroscopy and the Bio III (Noxygen) temperature and gas controller. The Lamy et al. [19] procedure was utilised with just a few alterations in this study. Prior to exposure to 200 M menadione (solvent) for 30 minutes, HepG2 cells were pre-treated with ethanolic extracts of B. carinata for 24 hours, washed twice with PBS, and then exposed to the solvent for 30 minutes. There followed 30 minutes of incubation at 37°C with the spin probe 100 M CMH in KHB supplemented with 25 nm DFO and 5 nm DETC in KHB. The cells were then rinsed with pre-warmed Krebs-HEPES buffer (KHB). Transferred supernatants were stored on ice in fresh reaction tubes. Glass capillaries of 50 L were used to measure EPR spectra. A total of ten scans were performed on each sample.

An experiment using the reporter gene for the antioxidant pathway Nrf2

In accordance with the manufacturer's instructions, the ONE-GloTM Luciferase Assay System was used to measure reporter gene activity (Promega GmbH, Mannheim, Germany). Experiments were conducted using HepG2-ARE cells, which were seeded into 96 wells and immediately subjected to B. carinata extracts, as described in the paper. A Tecan Group Ltd. Infinite M200 microplate reader (Männedorf, Switzerland) was used to detect luminescence 15 minutes after substrate addition after 18 hours of incubation.

It has both cytotoxic and cytostatic properties.

At 37°C/95% humidity, the etha nolic plant extracts were exposed to the HepG2 cells in 12 well plates (105 cells/well) for 48 hours before the cells were harvested. Tests for cytotoxicity and cytostasis were performed 48 hours following treatment with the trypan blue dye. The equation was used to calculate cytotoxicity.

$$\%\text{viability} = \frac{no.\ of\ viablecells}{total\ no.\ of\ cells} * 100,$$

if viable cells are not stained by the dye and the total number of cells is the sum of viable cells and non-viable cells In order to measure cytostatic activity, the total number of extract-treated cells was compared to that of a corresponding solvent control.

Analytical chemistry

Glucosinolates and the byproducts of their metabolism

Glucosinolates were evaluated using Hanschen et al. [20]'s significantly modified methodology. Desulfo-GLSs were extracted from ethanolic plant extracts using aryl sulfatase and eluted in deionized water from DEAE-Sephadex A-25 ion-exchanger columns. As previously described [20], the Agilent 1290 Infinity UHPLC System was used to isolate and quantify desulfo-GLSs. Glucosinolate breakdown products were analysed by gas chromatography-mass spectrometry (GC-MS) after ethanolic fractions were extracted with 2 mL of water, following the methodology of Witzel and co-workers [21].

Compounds that are phenolic in nature

Minor adjustments were made to Schmidt et al. [22] in their analysis of phenolic chemicals.

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This plant extract was subjected to a series 1100 HPLC analysis (Agilent Technologies, Waldbronn, Germany) that was equipped with a degasser, binary pump, autosampler, column oven and a photodiode array detector in order to determine the composition and concentrations of the various compounds present. In order to separate the pounds, a Supelco Ascentis® Express F5 column was employed at a temperature of 25°C. A and B contained 0.5 and 100 percent acetonitrile, respectively, in their respective eluents. Only 5–12 percent of eluent B had the gradient of zero to three minutes, 12–25 percent of eluent B had the gradient of three minutes, and 25–90 percent had the gradient of 46 to 48 minutes. These wavelengths were used in the detection at the rate of 0.85 mL min1 and the flow rate of 0.85 M L1. Glycosides and hydroxycinnamic acid derivatives were identified as deprotonated molecular ions and characteristic mass fragment ions of fla vonoids using HPLC-DAD-ESI MSn on an Amazon SL Ion trap Mass Spectrometer from Bruker in negative ionisation mode, in accordance with previously described methods [22,23]. A capillary voltage of around 3500 volts was used to dry the sample with nitrogen (10 L min1 at 325°C) and nebulize it with nitrogen (40 psi). In the ion trap, the collision gas was helium. Helium is a noble gas. The mass spectrometer's ion optics were optimised for quercetin at m/z 301 or, more arbitrarily, at m/z 1000 for the purpose of mass optimization. From m/z 200 to 2000, the MSn tests were carried out in auto mode up to MS3. Chlorogenic acid, quercetin 3-O-glucose, kaempferol 3-O-glucoside, and isorhamnetin-3-O-glucoside were utilised as standards for external calibration curves in a semiquantitative approach to the study.

Chlorophylls and carotenoids

Using an Agilent Technologies 1290 Infinity UHPLC paired with an Agilent 6230 QTOF LC/MS as reported by Mageney et al. [24], carotenoids and chlorophylls were identified. Separation was carried out using a C30 column (YMC Co. Ltd Japan, YMC C30, 100 2.1 mm, 3 m) and different volume ratios of solvent A and B (81/15/4 and 6/90/4, both 20 mM ammonium acetate) as mobile phases at a flow rate of 0.2 mL min1 and mixtures of methanol, methyl tert-butyl-ether, and water as stationary phases Co-chromatography with reference substances allowed for identification. Quantification via dose–response curves was carried out using external standard calibration curves.

Analyze the data

In order to evaluate all of the data, we used GraphPad Prism 6. (GraphPad Software Inc., San Diego, USA). Findings from

At the very least, three separate experiments are used to illustrate the results of the cell culture trials. It is deemed significant when the difference between two groups is less than 0.05 (*), 0.01 (**), 0.01 (***), and 0.01 or less (****). One-way analysis of variance (ANOVA) and the Dunnett multiple comparisons test were used to determine the study's statistical significance.

Results

Afb1-induced DNA damage is reduced by B. carinata.

The Comet test was used to investigate the anti-genotoxic impact of B. carinata on human liver cancer (HepG2) cells. Percent tail DNA (percent tail DNA) was employed as a metric to measure DNA damage in this experiment. All data were computed in terms of AFB1-treated cells, which were set to 100% in order to make comparisons easier. 9.23 percent of tail DNA was damaged by AFB1 at concentrations of 10 M, which is about 600 times more than the solvent control (1.54 percent tail DNA). Plant extracts were shown to diminish AFB1-mediated genotoxicity (Figure 2) regardless of the method of processing, in a concentration-dependent manner. For some reason, the raw extract from site A (Figure 2(A)) reduced AFB1-induced genotoxicity by 48.4% at the highest dose tested (111.1g/mL), but the fermen ted extract reduced it by 61.8percent at 37.0g/mL. An AFB1-induced genotoxicity of 49.1% at the maximum concentration of the raw extract from site B (Figure 2(B)) compared to the cooked extract. Fermentation and boiling had no effect on the anti-genotoxic properties of B. carinata leaves when taken simultaneously.

By using ethanolic B. carinata plant extracts, we found antioxidative activity and an increase in ARE/Nrf2 gene expression.

A concentration-dependent induction of reactive oxygen species (ROS) was observed by EPR spectroscopy in the range of 50 to 200 M menadione, which was exposed for 30 minutes in this experiment (data not shown). When compared to the control,

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ROS generation increased by 500% at 200 M, hence this concentration was chosen in following experiments for challenge tests. The raw B. car inata extract reduced ROS generation in HepG2 cells by 11.5 percent (location A) and 19.7 percent (location B) at the highest concentration tested. Keeping the same level of Focus

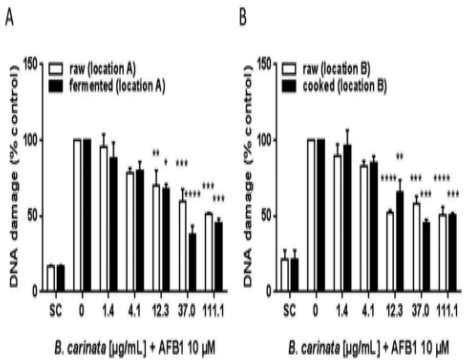
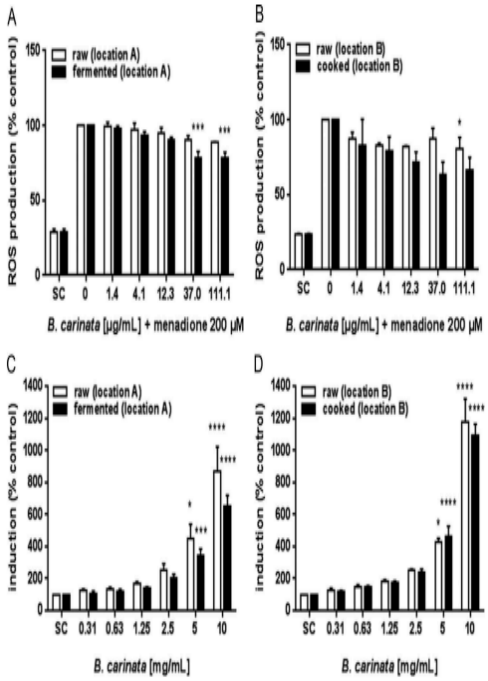


Figure 2. Anti-genotoxic properties of ethanolic extracts of raw and processed B. carination. DNA damage in AFB1-treated cells is indicated as percent of control. Data are means ± SEM of three separate trials. Asterisks indicate statistically significant differences between the particular treatment and the positive control (= without B. carination extract). SC = solvent control (0.1 percent DMSO) (0.1 percent DMSO).

the fermented extract demonstrated a higher decrease of 21.5 percent , while the cooked extract prevented menadione driven ROS generation by 33.3 percent (Figure 3(A,B)). Further, all B. carination extracts were able to stimulate ARE/Nrf2-mediated gene expression in a concentra tion-dependent manner (Figure 3(C,D)). At the lowest concentration, raw material increased ARE/Nrf2- mediated gene expression by 23 percent (location A) to 29 percent (location B) (location B). This induction was demonstrated with fermented extracts at 630 µg/mL. At the highest dose evaluated in this experiment, the raw extracts from site A and B revealed an increase of more than 800 percent and 1180 percent in ARE/Nrf2-mediated gene expression, respec tively. Compared to that, the fermented extract.



Ethanolic extracts of raw and processed B. carination have anti-oxidative properties. In reaction to 200 nM menadione, cells pre-treated with B. carination extracts produced ROS as detected by the EPR technique. ARE/Nrf-2-mediated gene expression in cells treated with B. carination extracts (C) and (D). The results are presented as a percentage of three separate trials' means SEM. It is important to note that asterisks denote statistically significant differences between the relevant treatment and the positive control (i.e. the extract of B. carination) or the SC (C, D). SC stands for solvent-control (0.7 percent EtOH).

Figure 3(C) shows an increase in gene expression of 650 percent, whereas Figure 3(D) shows a rise of 1090 percent. Fermentation and cooking combined improved the plant extract's ability to combat ROS generation. However, the potential of the plant extract to trigger ARE/NRF2-mediated gene expression was reduced by fermentation, but not by heating.

Ethanolic plant extracts of B. carination induce cytotoxicity and cytostasis.

At the highest concentration examined, cells treated with fresh and cooked ethanolic extracts of B. carination exhibited only a slight loss in viability

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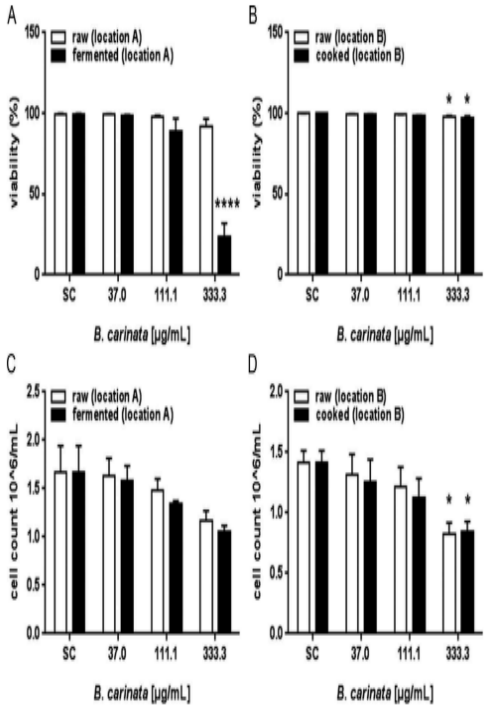
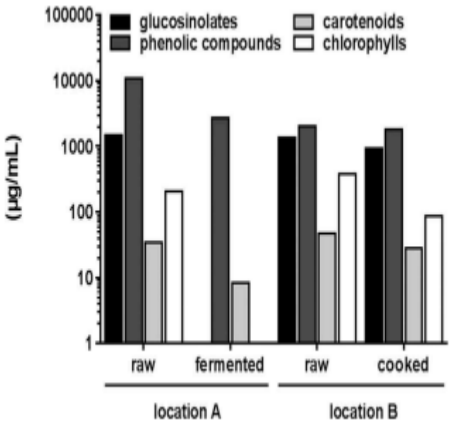
(Figure 4(A, B)). At the maximum dosage, the fermented extract drastically decreased viability by 76% compared to control cells (Figure 4(A)). B. carinata's cytostatic activity increased with increasing concentration, but was unaffected by the processing of the plant (Figure 4). From site A (Figure 4(A)) to location B (Figure 4(B)), the extracts lowered cell numbers by 30 to 40 percent at a 333.3 g/mL concentration.

Raw and processed B. carination have different secondary plant metabolites composition.

The glucosinolate, phenolic component (particularly flavonoids), carotenoid, and chlorophyll content of ethanol extracts from raw, fermented, and cooked B. carination leaves was examined (Figure 5 and supplemental Tables S1–S3).. As has been shown in previous research, such as [25], the variations in secondary plant metabolite concentrations in raw plant material between the two sites are most likely related to the unique environmental conditions present during plant culture at each location. In ethanolic extracts of raw B. carination, sinigrin was the most abundant glucosinolate. Concentration decreased 37% in the cooked extract, but glucosinolate levels were completely eliminated from fermented samples (Table S1). B. carination extracts included only a very small quantity of allyl isothiocyanate, which was not present in extracts of cooked or fermented material; 1.0 to 0.22 g/mL of allyl isothiocyanate was discovered in the raw material, and 3.3 g/mL was found in the extract of fermented material.



(A,B) and (C,D) Potential cytotoxic and anti-cytostatic effects of B. carination ethanolic extracts. Three separate trials yielded the mean SEM of the data. There was a statistically significant difference between the treatment and the control (SC). SC stands for solvent-control (0.7 percent EtOH).



In etha nolic extracts of raw, fermented, and cooked B. carinata the total amount of secondary plant metabolites is shown in Figure 5. The results are given in logarithmic units of g/mL. A and B are the locations where the first raw and fermented extracts

come from, and the second raw and cooked extracts come from.

During fermentation, phenolic compounds were decreased by 75%. There was, nevertheless, a structural decline. Caffeoylquinic acid, one of the simpler hydroxycinnamic acid derivatives, was able to withstand fermentation better than others (e.g. caffeoylquinic acid). Glycosides of most flavonol glycosides fell by more than 50%. Quercetin-3-O-sophoroside 7O-D-glucoside (37 percent), kaempferol-3-O-feruloyl sophoraside-7-D-glucoside (17 percent), and isorhamne tin-3-O-feruloyl sophoroside-7-O glucose (7 percent) were the most stable compounds. Cooking, on the other hand, reduced flavonoid glycosides and hydroxycinnamic acid derivatives by just 11 percent. It's possible to find a structure-specific deterioration as well.

There was a decrease in the concentration of the more complex flavonoid glycosides (kaempferol tri- and tetragylcosides), whereas the concentration of the simpler ones (kaempferol diglycosides) increased. These included the 3-O-caffeoyl-sphoroside, 3-O-sinapoyl-sphoroside, and 3-O-feruloyl-sophoroside. Cooking of B. carination seems to result in the deglycosylation of complex flavonoid glycosides. Cooking had less of an impact on caffeoylquinic acid, 3-p-coumarolyquinic acid, and 5-p-coumaroylquinic acid. In terms of carotenoids and chlorophylls, etha nolic extract included lutein, zeaxanthin, and -carotene, along with Chl a and B. (Table S3). The fresh samples had the highest quantities of chlorophylls, zeaxanthins, and -carotene, whereas the cooked or fermented samples had the lowest concentrations. A 75 percent reduction in total carotenoid content occurs during fermentation, whereas a 40 percent reduction occurs after cooking. After fermentation, lutein lost 98% of its content, whereas boiling reduced it by 42% of its original content. During fermentation, the content of zeaxanthin went up. Cooking maintained 23% of the original chlorophyll, whereas fermentation degraded it completely.

Discussion

Global activities and scientific studies aimed at promoting the positive impacts of ALVs are rapidly being acknowledged as a valuable resource for consumers' health [3]. Around the globe, chronic aflatoxic exposure and the diseases it causes are a persistent issue. Practical and economic measures must be taken to deal with this obvious hazard to human health in poor nations [13,25]. Ethanolic extracts from raw B. carination plant material were shown to have a protective effect against AFB1. An anti-oxidant as well as a cytostatic effect was seen in a human-derived liver cell culture after taking this supplement.

Many of the Brassicaceae family's bioactive secondary plant metabolites are well-known, and some of these metabolites have been implicated in cancer prevention studies [26,27]. Cabbage seed ethanolic preparations have previously been proven to protect rats from aflatoxicosis caused by aflatoxins [28]. Also in rats, cabbage inhibited AFB1 binding to hepatic DNA and enhanced the activity of liver microsomal and cytosolic enzymes [29]. There was also a reduction in the creation of AFB1-DNA adducts in liver cells and the development of AFB1-induced tumours in mice when Chinese cabbage was added to the diet. B. carination may be a promising option for dietary cancer chemoprevention because of our findings, which provide evidence of low-dose exposure in humans. ALVs, on the other hand, are prepared for consumption rather than ingested raw. Cooking [31,32] and fermenting [33] are common practises in Kenya. Cooking leafy vegetables for many hours in the traditional manner is common in Western Kenya, where it is believed to diminish their vitamin content and impair their bioactivity [31]. Glucosinolates, phenolic compounds, carotenoids, and chlorophyll concentration and composition were shown to be altered in processing of B. carination leaves. Although brief cooking or fermentation could not eliminate anti-genotoxicity or the anti-antigen effects, they were still present.

the extract's anti-oxidant properties Isothiocyanates, which are well-known cancer-fighting chemicals, have also been hypothesised to play a significant role

in our work. In fact, sinigrin, a glucosinolate found in raw plant material, was found in significant concentrations in extracts of raw plant material, but not in extracts of cooked or fermented plant material. Low quantities of allyl isothiocyanate (AITC) were found in the ethanolic extracts. The lack or presence of these chemicals may not have a significant impact on the reported preventative effects, as a result. The extracts from raw material from site A had a larger total phenolic content than extracts from location B, and this difference was greatly reduced throughout processing. Only the minor hydroxycinnamic acid derivatives (caf feoylquinic acid, 3-p-coumarolyquinic acid, 5-p coumaroylquinic acid) were identified in almost identical levels in extracts from both locations. According to research into anti-genotoxicity and antioxidant efficacy, this is consistent with comparable bioactivity. The processing of these three compounds had no effect on their phenolic concentration, in contrast to the overall results from the bioassays. There have been reports of caffeoylquinic acid's anti-oxidative and anti-carcinogenic actions, for example, previously. Antioxidant and liver-protective properties in human HepG2 cells have been shown [34, 35, 36], as has the ability to prevent methylazoxymethanolacet ate-induced carcinogenesis in the livers of hamsters [35, 36]. In order to account for potential synergistic effects of various bioactive chemicals and the activity of de-glycosylated substances and breakdown products (e. g. complex flavonoid glycosides), it is necessary to correlate analytical findings with observed bioactivity. Despite the decomposition of the original chemical combination, polyphenol radical scavenger activity was shown to be persistent in kale [37,38]. Another research found that the total of the individual phenolic compounds in red wine might only account for up to 24% of the anti-oxidant activity seen [39]. Further research is needed to determine the role played by individual carotenoids and chlorophylls in the reported effects.

Conclusion

B. carination, like other Brassicas, is a rich source of cancer-fighting chemicals. The leaf extracts of B. carination demonstrated protective action in vitro when extracted with andethanolic solvents. B. carination leaves may be significantly altered by post-harvest processing such as short-boiling or fermenting after harvest. For other endpoints studied, however, the bioactivity of leaves was not significantly reduced by the employed processing methods; instead, the protective impact was strengthened. It seems that brief time boiling does not negatively effect the plant's preventative potential, based on the current in vitro data, as an alternative to ingesting the raw leaves. Lactobacillus plantarum and Lactobacillus fermentum fermentation should also be considered since it avoids spoiling and development of harmful pathogenic bacteria while at the same time without diminishing the product's bioactivity [40,41]. If you want to keep your family safe from aflatoxin-related ailments, you might consider eating B. carination, which is an excellent source of this nutrient.

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