Emerging Potential of Momordica’s Bioactive Phytochemicals in Cancer Prevention and Therapy

Subhayan Sur¹* and Ratna B. Ray²

¹Cancer and Translational Research Centre, Dr. D Y Patil Biotechnology and Bioinformatics Institute, Dr. D Y Patil Vidyapeeth (DPU), Pimpri, Pune, India.  
²Department of Pathology and Cancer Center, Saint Louis University, MO, USA.  
*Corresponding Author E-mail: subhayan.sur@dpu.edu.in

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Worldwide, cancer incidence and mortality are rising quickly. Cancer remains the biggest cause of death despite advances in therapy. Plants produce bioactive phytochemicals, and as a result, the bioactive elements have long been the focus of cancer research, both for medication discovery and for the discovery of alternative chemo-preventive methods. The medicinal plant Momordica charantia or bitter melon contains a wide variety of phytochemicals, such as triterpenoids, triterpene glycosides, phenolic acids, flavonoids, lectins, sterols, and proteins. In many pre-clinical systems, the Momordica charantia extract exhibits an anti-cancer action against various malignancies. The bioactive components of the extract play a significant role in its anti-cancer properties. With an emphasis on underlying molecular pathways, we address the roles of Momordica’s known bioactive components in several cancer models in this review. Through the inhibition of cancer cell proliferation and induction of cell death, several of active ingredients exhibit cancer prevention and therapeutic effects, at least in in-vitro models. Mechanistically examining the active components in pre-clinical systems may reveal a novel approach to cancer treatment.

Keywords: Bitter Melon; Cancer; Cancer Prevention; Cancer Therapy; Momordica charantia; Phytochemical.
death in the majority of countries throughout the
world, and it is the second highest cause of death
in the United States. The best ways to manage
diseases are early detection and prevention, which
can be accomplished by lowering the risk from
environmental and lifestyle variables. By removing
these external risk factors, over 80% of human
malignancies could be prevented. For example,
the results of the Pap test and HPV vaccines against
cervical cancer are exceptional achievements that
highlight the value of early detection and prevention
in treating the illness across a large population. 6
The major challenges in cancer therapy are tumor
heterogeneity, acquired resistance, and metastasis.
7 Significant advancements in targeted therapies
have been made in the past few years; however,
those methods show limited success with several
side effects and are sometimes expensive. To
effectively manage the disease, it is crucial to
comprehend the disease process and find better
therapeutic and preventive approaches. This will
allow for the provision of high-quality cancer care
that is both accessible and inexpensive.

For a very long period, dietary
phytochemicals have been a focus of cancer
research, including Leucovorin in the year 1950,
Carzinophilin in 1954, Vincristine in 1963, and
Actinomycin D in 1964. Plants can produce a
large number of bioactive phytochemicals and thus,
for many decades, the natural sources provided the
basis for drug discovery. Half of all cancer drugs and
antibiotics were discovered from natural sources.
For example, the medications podophyllotoxin
and its derivatives (topotecan, irinotecan), vinca
alkaloids (vinblastine, vincristine, vindesine,
vinorelbine), taxanes (paclitaxel, docetaxel),
and anthracyclines (doxorubicin, daunorubicin,
epirubicin, and idarubicin) are all produced from
either plant or microbial sources. A recent report
showed that out of a total of 1881 approved
drugs in the last four decades (year 1981- 2019),
929 drugs (anticancer, antibacterial, antifungal,
antiviral and antiparasitic) originated from natural
sources; whereas others are synthetic drugs with
natural pharmacophores or synthetic analogs of
natural product. Thus, research with bioactive
phytochemicals is an emerging field in drug
discovery and disease management. Many pre-
clinical and clinical investigations with newly
identified bioactive components or their analogs
are now underway. All of these studies show the
importance of phytochemicals as a single agent or
in combination with conventional therapy in the
treatment of various cancer types.

In the present review, we have focused
on the bioactive components of Momordica
charantia which is commonly known as bitter
melon, in cancer prevention and therapy. The
Momordica charantia is a tropical and sub-tropical
vine and widely cultivated in Asia, Africa, and
South America. The plant is popular due to its
medicinal value since ancient ages for the treatment
of diabetes, diarrhea, and toothache. In many
pre-clinical models of cancer, such as those of
the blood, breast, colon, head and neck (or oral),
kidney, liver, lung, ovary, pancreatic, prostate, skin,
stomach, and uterine cervix, crude extracts of the
fruit, leaves, or seeds of Momordica charantia
have shown potential anti-cancer actions. The
plant includes more than 30 therapeutic
compounds and has the most significant nutritional
content of all cucurbits. In the present review,
we have focused on the active phytochemicals of
Momordica in cancer prevention and therapy with
recent evidence. Thus, this review may be helpful
for basic researchers and pharmacologists for
attracting and/or designing future mechanistic pre-
clinical and clinical studies, and drug development
using pure components against different types of
cancer.

METHODS

In this review, we have discussed the
role of active phytochemicals of Momordica
charantia or bitter melon in cancer prevention
and therapy. Based on available studies from
“PubMed,” “PubMed Central,” “Google Scholar,”
“Science Direct,” and “Semantic Scholar,” we
have summarized the effects of every compound
separately, identified from the year 1980 to the
present, their isolation methods, source in the plant,
chemical composition, metabolism, bioavailability,
toxicity, chemical modifications, specific mode
of action, and future research direction in various
cancer models. ‘Bitter melon’, ‘Momordica
charantia’, ‘cancer’, ‘cancer prevention’, and
‘anticancer’ are the search terms utilized. The
data included in this study are from research
using in-vitro or in-vivo cancer models; while the
information on opinions, conference proceedings, news stories, or material specific to a certain profession are excluded.

Momordica charantia and its phytochemicals

The herbaceous plant *Momordica charantia* has a bitter flavor and slender stems, tendrils, bright yellow blooms, and light green fruits (Figure 1A). The plant contains a large number of phytochemicals and many of those showed potential biological roles. Depending on the varieties of extraction methods, researchers reported varying amounts of chemical constituents in the plant extract. The plant contains 94% water, 3.7% carbohydrate, 1% proteins, 0.17% fat, fibers, vitamins (A, B-complex, and C); minerals including Ca, Zn, Mg, K, Fe, and P; free amino acids like aspartic acid, alanine, butyric acid, glutamic acid, serine, and threonine; fatty acids including linoleic acid, linolenic acid, palmitic acid, oleic acid, stearic acid, and arachidonic acid; essential oils (α and β pinene, octanal, 1, 8-cineole, β- phellandrene, c-dihydrocarveol, carvone, safrole, methyl- eugenol, germacrene D, β-selinene, α-selinene, and myristicin) (Figure 1B) 14,18. The leaf and fruit contain higher amounts of carbohydrates than the seeds. Whereas, the seeds contain a high amount of fat and fibers; the protein content is almost comparable in different plant parts 20. Some of the identified and well-characterized proteins are α- and β-momocharin or momordica antiviral protein 30kDa (MAP30), 14 kDa Ribonucleases (RNase MC2), polypeptide-k, and marmorin13,17,18. Other constituents are phenolic acids and flavonoids which include gallic acid, tannic acid, (+)-catechin, caffeic acid, p-coumaric, gentisic acid, chlorogenic acid, and epicatechin 13.

The cucurbitane-type triterpenoids and cucurbitane-type triterpene glycosides are major phytochemicals in the plant and responsible for the bitterness of the plant 13,17,18. A total of 28 secondary metabolites were found in the water extract of fruits (without seeds) after being subjected to liquid chromatography high-resolution electrospray ionization mass spectrometry analysis (LC-HRESIMS). Of these, 4 metabolites belonged to the cucurbitane-type triterpenoids and 20 to the cucurbitane triterpene glycosides21. The cucurbitane-type triterpenoids were momordicine I (M-I); 7, 23-dihydroxy-3-O-malonylcucurbita-5,24-dien-19-al; (23E)-cucurbita-5,23,25-triene-3,7-dione (Figure 1C). A new malonylcucurbita-trien-19-al derivative of cucurbitane-type triterpenoid was also identified as having a molecular formula of C31H46O7 21. Other cucurbitane-type triterpenoids

![Fig. 1. Momordica charantia and active components. A: Momordica charantia plant [image source: www.omafra.gov.on.ca]. B: Nutritive in Momordica charantia per 100 g. [Source: U.S. DEPARTMENT OF AGRICULTURE; FDC ID: 168393; NDB Number: 11024; FDC: Published:4/1/2019]. C: Chemical structure of some cucurbitane-type triterpenoids and cucurbitane-type triterpene glycosides components (drawn in ChemDraw).](image-url)
reported in other studies are charantin, momordicine II and III, karavilagenin A, B, C, D and E, and kuguacins A-S from other studies are charantin, momordicine II and III, karavilagenin A, B, C, D and E, and kuguacins A-S (Figure 1C).

The cucurbitane-type triterpene glycosides in the water extract of fruit identified by LC-HRESIMS were momordicoside-B, C, K, L, N, O, V; momorcharaside-A and B, Karaviloside-II, III, X, XI; Goyaglycoside A, C, F; and charantosides-I, IV, V, VII (Figure 1C). Additionally, some other studies reported charantosides I-VIII, and kuguaglycoside13,17,18. Moreover, three monoterpenoid glycosides (vomifoliol β-D-glucopyranoside, sacranoside A and myrtenyl O-β-D-glucopyranoside) and one oleanane-type triterpene saponin (goyasaponin III) were identified in the extracts by the LC-HRESIMS21. Interestingly many of the compounds are not well characterized and have unknown biological activity.

**Momordica charantia extract and cancer**

The *Momordica charantia* or bitter melon crude extract (BME) isolated from fruit, leaf, or seed was studied in different cancer models. Many studies show that crude extract or a mixture of compounds acts better than individual pure compounds in an in-vivo system. The crude extract can be made using a variety of solvents, including water, acetone, methanol, ethanol, and n-butanol. The BME demonstrated potential anti-cancer properties against a variety of cancer types, including malignancies of the blood, breast, colon, head and neck, kidney, liver, pancreas, ovary, skin, stomach, and uterine cervix (Figure 2).

*Momordica charantia*’s putative anti-tumor properties were initially noted in a mouse lymphoma xenograft model, where the extract’s ammonium acetate precipitates inhibited tumor growth and stimulated immune response22. The cells were prior exposed to the extract in-vitro before animal injection. The study reported no toxic side effects of the dose to normal mice22. Similar to this, the water extract of fruit inhibited breast cancer cell proliferation in both in vitro and in vivo models, with superior results against a triple-negative breast cancer model23-25. The BME dose was non-toxic to normal mice or cell lines.

![Fig. 2. Effect of Momordica charantia or bitter melon extract (BME) on different cancers. Sharp arrows indicate activation or induction and blunt arrows indicate inhibition by BME. (Created with BioRender.com).](image-url)
The extract caused G2/M phase cell cycle arrest, enhanced p53, p21, pChk1/2, inhibited cyclin B1 and cyclin D1 expression and induced apoptosis by increasing PARP cleavage and caspase activation in MCF-7 and MDA-MB-231 cells. In breast cancer syngeneic and xenograft mice models, the extract could increase both apoptosis and autophagy through modulation of AMPK/mTOR pathways. Further in the TNBC model, the extract inhibited the accumulation of esterified cholesterol by inhibiting acyl-CoA: cholesterol acyltransferase 1 (ACAT-1), sterol regulatory element-binding proteins-(SREBP-1 and -2), and FASN expression of lipid metabolism.

A diet containing 0.01% - 1% *Momordica charantia* seed oil prevented azoxymethane-induced rat colon cancer development by enhancing peroxisome proliferator-activated receptor- gamma (PPARγ) protein expression. Furthermore, a rise in the lipid component of CLA (c9, t11-18:2) was seen in the colonic mucosa and liver. In the human colon cancer cell line (HCT1116), the water extract of seeds showed potential anticancer effects by activation of PARP cleavage. The methanolic extract of whole fruit showed a better anticancer effect as compared to only skin extract on colon cancer cell lines. The whole fruit extract effectively inhibited proliferation, colony formation, sphere formation, induced S-phase arrest and autophagic cell death in colon cancer cells.

A diet containing *Momordica*’s fruit extract prevented benzo-(a)-pyrene-induced forestomach papillomagenesis in Swiss albino mice. In addition, the methanol extract of the leaf inhibited proliferation and induced apoptosis in different human cancer cell lines, including nasopharyngeal carcinoma cells (Hone-1), gastric adenocarcinoma cells (AGS), colorectal carcinoma cells (HCT-116), and lung adenocarcinoma cells (CL1-0) in a dose-dependent way. In head and neck cancer models, the aqueous extract of fruit suppressed tumor growth in vitro and in vivo by reducing the expression of c-Met and its downstream signaling components phospho-Stat3, c-Myc, and Mcl-1. The BME also induced T-regulatory cell activation and stimulated NK cell-mediated cytotoxicity of head and neck cancer cells. Furthermore, regular oral drinking protected carcinogen-induced tongue squamous cell cancer development in a mouse model while having no harmful effect in normal animals. RNA sequence analysis followed by subsequent validation showed that the BME inhibits pro-inflammatory genes s100a9, IL23a, IL1β and immunological checkpoint gene PDCD1/PD1 during cancer prevention. In addition, the extract could modulate glucose metabolism by significantly reducing pyruvate and lactate levels by inhibiting key regulatory genes of the glycolysis pathway (GLUT-1, PFKP, LDHA, PKM, and PDK3). Also, in lipid metabolism, the BME significantly reduced membrane phospholipids-phosphatidylcholine, phosphatidylethanolamine and plasmenylethanolamine, and inhibited calcium-independent Phospholipase A2 (iPLA2) as well as fatty acid biogenesis genes (ACLY, ACC1, and FASN), which resulted in the endoplasmic reticulum (ER) stress and reactive oxygen species (ROS) associated cell death.

Pre- and post-treatment of methanolic extract of bitter melon prevented diethyl-nitrosamine (DENA) and carbon tetrachloride (CCl4) induced rat hepatocellular carcinoma (HCC) development by decreasing Cox-2, VEGF,
HDAC and MMP-2,-9 and increasing expression of Caspase-3, and 831.

The water and methanol extract of the leaf inhibited human non-small cell lung cells A549 and lung adenocarcinoma cells CL1 in a dose-dependent manner respectively32,33. The anticancer mechanism was linked to increased apoptosis, ROS production, and activation of Src and FAK, which reduced the expression of downstream Akt, \(\beta\)-catenin, and MMPs.

The water extract of fruit inhibited proliferation, induced apoptosis and activated AMPK in human pancreatic carcinoma cells (BxPC-3, MiaPaCa-2, AsPC-1 and Capan-2) and xenograft model in nude mice38. The extract demonstrated potential efficacy against gemcitabine-resistant pancreatic cancer cells, as well as decreased Akt and ERK1/2 phosphorylation19. Furthermore, in pancreatic cancer models, bitter melon extract suppressed sphere formation and decreased cancer stem cell-related genes SOX2, OCT4, NANOG, and CD4440. In the pancreatic carcinoma model, bitter melon extract also modulated glucose metabolism and lactate export by blocking the GLUT1 and MCT4 transporters41. Interestingly, the combinatorial treatment of BME with gemcitabine significantly reduced pancreatic cancer patient-derived xenograft tumor growth in mice34.

Bitter melon extract inhibited prostate cancer cell PC3 and LNCaP proliferation in vitro, and induced S-phase cell cycle arrest by modifying expression of Cyclin D1, Cyclin E, and p21, and increased apoptosis by increasing Bax and PARP cleavage43. In TRAMP (transgenic adenocarcinoma of mouse prostate) mice, oral gavage of bitter melon extract as a dietary component prevented the progression of high-grade prostatic intraepithelial neoplasia43. Bitter melon leaf extract lowered MMP-2 and MMP-9 levels in a rat prostate cancer cell line and enhanced rat survival in a prostate cancer metastasis model44. A diet containing bitter melon leaf extract (1% and 5%) inhibited PC3 xenograft development by 63% and 57%, respectively, with no deleterious effect on mice’s body weight45. The aqueous extract suppressed rat prostatic adenocarcinoma by inhibiting the G2/M phase of the cell cycle and cyclic GMP46.

**Fig. 4.** Mode of action of Momordica antiviral protein 30 kd (MAP30) in cancer prevention/therapy (created with BioRender.com). Sharp arrows indicate activation or induction and blunt arrows indicate inhibition. (For ease of illustration, the graphic shows MAP30’s structure or intracellular location).
In a DMBA-induced skin tumorigenesis model, mice given *Momordica* fruit and leaf extracts at doses of 500 and 1000 mg/kg body weight for 30 days had a longer life span and tumor volume was significantly reduced when compared to control values. Similarly, oral administration of the fruit extract protected against the formation of skin tumors and enhanced life expectancy by reducing lipid peroxidation in the liver and DNA damage in lymphocytes. Moreover, the fruit extract was observed to strongly stimulate the glutathione-S-transferase, glutathione peroxidase, and catalase during cancer prevention. Similarly, the BME showed a potential cytotoxic effect against adrenocortical cancer cells and ovarian cancer cells and cervical cancer cells.

Most of the studies reported the non-toxic effect of the extract either in normal cells or normal animal models. Different *Momordica charantia* cultivars are geographically available. A comparative study reported the similar anticancer effects of different *Momordica charantia* varieties on pancreatic cancer pre-clinical models. As shown in Figure 2, the mechanism of cancer therapy and prevention against many cancer types is more or less comparable. Pre- or post-initiation stages of carcinogenesis determine the therapeutic or preventative effects of the extracts.

**Momordica charantia bioactive phytochemicals in cancers**

The *Momordica charantia* plant includes a variety of phytochemicals, and the crude extract’s biological activity is influenced by the interaction of these bioactive substances. The next section and Table 1 provide an overview of some bioactive phytochemicals’ anti-cancer properties.

**Momordicine-I (M-I)**

The Momordicine-I or M-I (C_{30}H_{48}O_{4}) is a cucurbitane-type triterpene. The secondary metabolite was initially discovered in plants’ leaves and vines. The fruit extracts in ethanol, n-butanol, and water have all been found to contain the same substance. The M-I was discovered to be effective in promoting insulin secretion from pancreatic beta cell lines and reducing diabetes-related cardiac fibrosis in rats.

The anticancer role of M-I was studied in oral cancer (head and neck) models. The M-I inhibited human oral cancer cells in a dose-dependent way (IC_{50} dose at 48 hr = 7 µg/mL in Cal27, 17 µg/mL in JHU022, and 6.5 µg/mL in JHU029 cells). Mechanistically the M-I inhibited the c-Met expression resulting reduction of downstream signaling molecules c-Myc, STAT3, survivin, and cyclin D1 (Figure 3). In the oral cancer xenograft model, daily IP injection of M-I could regress tumor growth in nude mice by inhibiting c-Met signaling. Oral cancer is frequently characterized by increased expression of c-Met signaling which is linked to metastasis and a poor prognosis. The *Momordica* crude extract also has a similar function to M-I in the inhibition of c-Met signaling in the prevention of oral cancer. Thus, it appears that one of the main factors influencing *Momordica*’s anti-cancer effectiveness may be the M-I.

A pharmacokinetic study showed that the M-I is quite stable in mouse blood and maximum peak concentration is achieved at 1 hr post-IP and oral treatment (PO) with mean clearances at 30.8 mL/min/kg (IP) and 534.8 mL/min/kg (PO). A drug permeation study using human intestinal epithelial cell monolayers (Caco-2) showed permeation across the basolateral side with an apparent permeability coefficient at 8.12 × 10^{-6}. In addition, a small amount of the M-I was found to be absorbed inside the Caco-2 cells. The substance is not toxic to mice as evidenced by the lack of alterations in body weight and blood parameters associated with liver and kidney functions after routine IP administration. Normal oral keratinocytes (NOK) are the least affected by the substance. Thus, M-I may be an important therapeutic agent in the treatment of oral cancer. However, more pre-clinical studies are needed in the presence of intact immune systems in this regard. Also, the role of M-I in other cancers is not known and needs to be evaluated.

**Momordica antiviral protein 30 kd (MAP30) or β momorcharin**

The MAP30 or β momorcharin is one of the well-studied phytochemicals of *Momordica charantia* which is highly present in mature fruit and seeds. It is a 30 kDa molecular weight type I single-chain ribosome-inactivating protein. It has an immune-modulatory function and has antiviral properties against the herpes simplex virus (HSV) and the human immunodeficiency virus (HIV). Additionally, MAP30 has anti-cancer properties against several malignancies.
Table 1. The anticancer effect of *Momordica*'s bioactive phytochemicals

<table>
<thead>
<tr>
<th>Compound</th>
<th>Compound type</th>
<th>Isolated from</th>
<th>Cancer type</th>
<th>Cancer model</th>
<th>Mechanism</th>
<th>Phenotypic changes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Momordicin Cucurbitan C</td>
<td>Fruit</td>
<td>Head and neck cancer</td>
<td><em>In vitro</em> and <em>in vivo</em></td>
<td>Inhibits c-Met signaling</td>
<td>Inhibits tumor growth</td>
<td>(21)</td>
<td></td>
</tr>
<tr>
<td>Antiviral Protein 30 Kd (MAP-30)</td>
<td>Protein and seeds</td>
<td>Cancers in breast, glioma, liver, lung, ovary, prostate and leukemia</td>
<td><em>In vitro</em> and <em>in vivo</em></td>
<td>Type I ribosome-inactivating protein; inhibits HDAC1, Wnt signaling; reduces expression of LGR5, NF-kB, JNK, MMP2 and induces expression of PTEN, AMPK, Caspase 3, 8 and 9, and induces ROS generation</td>
<td>Inhibits cell proliferation, migration, invasion, induces S-phase arrest, apoptosis and chemotherapy sensitivity</td>
<td>(58-70)</td>
<td></td>
</tr>
<tr>
<td>Alpha-eleostearic acid (α-EESA)</td>
<td>Fatty acid</td>
<td>Cancers in breast, colon, cervix, and leukemia</td>
<td><em>In vitro</em></td>
<td>Inhibits Akt signaling, induces expression of PPARα, p21, Bax, and Caspase-3</td>
<td>Inhibits proliferation, colony formation, induces G2/M cell cycle arrest, apoptosis, and autophagy</td>
<td>(71-79)</td>
<td></td>
</tr>
<tr>
<td>Kuguacin J</td>
<td>Cucurbitan e-type triterpenoid</td>
<td>Leaf</td>
<td>Prostate, ovary and cervical cancer</td>
<td><em>In vitro</em></td>
<td>Inhibits P-glycoprotein, reduces expression Cyclin D1, Cyclin E1, Cdk2, Cdk, PCN, survivin, MMP-2, MMP-9 and uPA, and induces p21, p27, Caspase-3 and PARP cleavage, Bax/Bcl-2 and Bad/Bcl-xL ratio</td>
<td>Inhibits cell cycle arrest, apoptosis and chemotherapy sensitivity</td>
<td>(45, 81-83)</td>
</tr>
<tr>
<td><em>Momordica charantia</em> lectin (MCL)</td>
<td>Carbohydrate conjugated protein</td>
<td>Leukemia, liver, and nasopharyngeal carcinoma</td>
<td><em>In vitro</em> and <em>in vivo</em></td>
<td>Type II ribosome inactivating protein, reduces Cyclin D1 expression, modulates RB and p38 MAPK, JNK, and ERK phosphorylation, induces Bid, cytochrome c release, Caspases-3, 8, 9 and PARP cleavage</td>
<td>Inhibits cell cycle arrest, mitochondrial injury, autophagy, apoptosis, and chemotherapy sensitivity</td>
<td>(86-88)</td>
<td></td>
</tr>
<tr>
<td>α-momorcharin</td>
<td>Protein</td>
<td>Breast and lung cancer</td>
<td><em>In vitro</em> and <em>in vivo</em></td>
<td>Ribosome inactivating protein, increases Caspase-3</td>
<td>Inhibits proliferation, induces G1 or G2/M phase arrest and apoptosis</td>
<td>(63, 89)</td>
<td></td>
</tr>
<tr>
<td>BG-4</td>
<td>Protein</td>
<td>Colon cancer</td>
<td><em>In vitro</em></td>
<td>Protease inhibitor, reduces Bcl-2 and increases expression of p21, Bax and Caspase-3</td>
<td>Not known</td>
<td>(93)</td>
<td></td>
</tr>
<tr>
<td>Charantagen ins-D and Goyaglycoside de-d (modified)</td>
<td>Cucurbitan e-type triterpene glycosides</td>
<td>Fruit</td>
<td>Glioblastoma, lung and liver cancer</td>
<td><em>In vitro</em></td>
<td>Not known</td>
<td>Inhibits proliferation</td>
<td>(94)</td>
</tr>
<tr>
<td>Karaviloside III</td>
<td>Cucurbitan e-type triterpene glycoside</td>
<td>immaturity fruit</td>
<td>Liver cancer</td>
<td><em>In vitro</em></td>
<td>Not known</td>
<td>Inhibits proliferation</td>
<td>(95)</td>
</tr>
<tr>
<td>Kuguaglycoside C</td>
<td>Leaves</td>
<td>Neuroblastoma</td>
<td><em>In vitro</em></td>
<td>Caspase independent cell death</td>
<td>Not known</td>
<td>Inhibits proliferation and induced cell death</td>
<td>(96)</td>
</tr>
<tr>
<td>RNase MC2</td>
<td>Protein</td>
<td>Breast and</td>
<td><em>In vitro</em></td>
<td>Induces phosphorylation</td>
<td>Not known</td>
<td>Inhibits</td>
<td>(97, 98)</td>
</tr>
</tbody>
</table>
The MAP30 showed potential anticancer effects in different in vitro cancer models including acute myeloid leukemia, glioma, breast cancer, hepatocellular carcinoma, ovarian and prostate cancers in a time-dependent and dose-dependent way by inhibiting cellular proliferation, migration, invasion and inducing S-phase cell cycle arrest and apoptosis. In ovarian cancer cells, the MAP30 was found to induce cisplatin sensitivity upon combinatorial treatment. Importantly, the MAP30 dose was found to be non-toxic in normal cells.

In vivo anti-cancer effect of MAP30 was studied in various pre-clinical models. The MAP30 treatment effectively prevented xenograft tumor growth of breast, liver, and prostate cancer, ovarian cancer ascites in a mouse model, and increased mice survival. It is worth noting that the MAP30 showed no toxic effect on liver and kidney function-related serum parameters in the treated mice.

The MAP30 protein was modified by mono-PEGylation and the modified MAP30 was seen as similar cytotoxic to the A549 cells. Liposomal MAP30 showed a better cytotoxic effect on bladder cancer cells T24. As the Momordica charantia plant contains traces of MAP30, researchers have cloned the gene, expressed it in bacterial culture, and generated recombinant MAP30. The recombinant MAP30 also inhibited cell proliferation and migration, and induced apoptosis in human bladder cancer cell T24, colorectal carcinoma LoVo, and uterine cervical cancer cell HeLa in a time-dependent and dose-dependent manner. In an animal model, the recombinant MAP30 inhibited bladder cancer xenograft tumor growth in mice. It significantly increased the level of reactive oxygen species (ROS), but reduced glutathione levels and activities of catalase and glutathione peroxidase in the prevention of bladder cancer. However, histological analysis showed mild changes in the liver and kidney following MAP30 treatment in the mice.

Mechanistically, the MAP30 is a ribosome-inactivating protein (Figure 4). It binds both ribosomal RNA and the HIV-1 long-
terminal repeat. Apart from that, it acts as a potent inhibitor of histone deacetylase-1 (HDAC-1) resulting in modulation in gene expression. The MAP30 inhibited the self-renewal Wnt pathway by reducing the expression of effector molecule β-catenin and its downstream genes c-Myc and Cyclin D1 in glioma and prostate cancer models. The MAP30 also inhibited the expression of G-protein-coupled receptor 5 (LGR5), NF-κB, JNK and MMP2 and induced expression of PTEN and AMPK signaling. The MAP30 has a positive role in the induction of the expression of apoptosis genes Caspase 3, 8, and 9. It was observed that the MAP30 induces intracellular Ca2+ ion concentration, ROS level, and ROS-mediated apoptosis and ferroptosis. Thus, MAP30 is one of the important contributors to the biological activity of Momordica and it may be a potential therapeutic agent against different cancers (Figure 4).

**Alpha-eleostearic acid (αESA)**

The αESA or (9Z,11E,13E)-octadec-9,11,13-trienoic acid (C_{18}H_{30}O_{2}), is an essential fatty acid present in *Momordica charantia* seed oil which contains 60% of αESA. The ESA was found to be converted to conjugated linoleic acid (CLA; 9,11-18:2) in the liver and plasma in the rat model.

The potential anti-cancer effect of αESA was seen in human leukemia cells HL60. The αESA inhibited proliferation, colony formation, induced G2/M cell cycle arrest, and apoptosis in breast cancer cells. Mechanistically, the αESA induced expression of PPARγ, p21, Bax, p53, and caspase-3 in the breast cancer cells. The αESA reduced mitochondrial membrane potential and induced translocation of apoptosis-inducing factor (AIF) and endonuclease G from the mitochondria to the nucleus resulting in apoptosis in breast cancer cells. In another study of a breast cancer model, the αESA was found to reduce the expression of HER2/HER3 and increase the expression of PTEN resulting in reduced expression of activated Akt and its downstream signaling molecules GSK-3β and BAD proteins.

In addition, the αESA could decrease proliferation and induce DNA fragmentation in the human colon cancer cells Caco-2 and HT-29. On the other hand, β-eleostearic acid showed a better effect in Caco-2 cells than the αESA. The αESA also inhibited proliferation and induced both apoptosis and autophagy in human cervical cancer cell HeLa. It inhibited the expression of phospho-AKT and phospho-P70S6K, increased phospho-ERK1/2 expression and conversion of LC3 I to LC3 II resulting in autophagy-mediated HeLa cell death. The αESA was also found to induce ferroptosis in diverse cancer cells. The αESA was found to incorporate into cellular lipids and promote lipid peroxidation and cell death mediated by acyl-CoA synthetase long-chain isofrom 1. The αESA increased the total antioxidant capacity in plasma and successfully maintained the RBC membrane integrity against stress indicating its protecting role. Thus, αESA may be a potential therapeutic agent in many cancers. However, one study reported the toxic effect of the compound on normal human fibroblast cell WI38. Also, the effect of αESA in *in-vivo* cancer models is scarce. In addition, its bio-availability, stability, and toxicity information are not known clearly.

**Kuguacin j (KuJ)**

The KuJ (C_{30}H_{46}O_{3}) is a cucurbitane-type triterpenoid present in *Momordica charantia* leaves and vines. The biological activity of KuJ was studied in *in-vitro* cancer cells. The compound was isolated from the methanol extract of leaves.

Similar to the crude extract from the leaf, the KuJ inhibited the proliferation of human prostate cancer cells LNCaP and PC3 in a time-dependent and dose-dependent manner. Both the leaf extract and KuJ showed minimum toxicity in the normal prostate cell line, PNT1A. It induced G1/S cell cycle arrest and apoptosis in the cells. KuJ effectively reduced the expression of cell cycle genes Cyclin D1, Cyclin E1, Cdk2, Cdk4, and PCNA, and induced the expression of p21 and p27. In apoptosis pathways, it induced Caspase-3 and PARP cleavage, Bax/ Bcl-2, and Bad/ Bcl-xl ratio and reduced survivin expression. In addition, it reduced migration and invasion-associated molecules MMP-2, MMP-9 and uPA in PC3 cells.

The KuJ inhibited human ovarian cancer cells (SKOV3 and A2780). Interestingly, co-treatment of KuJ with cisplatin or paclitaxel increased cytotoxicity in both cells. In addition, it induced apoptosis by increasing Caspase-3 and PARP cleavage and reducing the expression of survivin.
The KuJ also increased the sensitivity of vinblastine and paclitaxel in the human cervical carcinoma cell line (KB-V1)\textsuperscript{71}. Mechanistically, the KuJ directly interacts with the drug-substrate-binding site on P-glycoprotein (P-gp) and thus inhibits the function of P-gp in the KB-V1 cells. The P-gp is a type of ABC drug transporter that induces efflux of chemotherapeutic drugs from cancer cells. However, induction of cisplatin or paclitaxel sensitivity in ovarian cancer cells by the KuJ was P-gp independent\textsuperscript{70}. Thus, KuJ may have a potential therapeutic role. However, further mechanistic studies in different cancer pre-clinical models are needed.

**Momordica charantia lectin (MCL)**

The MCL is a carbohydrate-conjugated protein isolated from *Momordica charantia* seeds. It is a type II ribosome-inactivating protein and shows some degree of sequence homology to \( \beta \)-momorcharin from *Momordica charantia* and to lectins from *Cucurbita maxima*, *Cucurbita argyrosperma*, *Sambucus nigra* and *Ricinus communis*\textsuperscript{72}. MCL is an important contributor to the anti-viral effect and hypoglycemic effect of *Momordica charantia* extract\textsuperscript{73}.

The MCL inhibited protein and subsequently DNA synthesis in human peripheral blood lymphocytes of leukemia patients\textsuperscript{74}. The MCL inhibited the viability of HCC cells (HepG2 and PLC/PRF/5) in a dose-dependent and time-dependent way\textsuperscript{75}. It induced G2/M phase cell cycle arrest, DNA fragmentation, mitochondrial injury, autophagy and apoptosis in the HCC cells. The MCL induced p38/MAPK kinase pathway, Bid activation, PARP cleavage and expression of Caspase 8 and 9. In addition, MCL treatment prevented HCC xenograft growth in mice. The MCL in combination with sorafenib showed a better therapeutic effect against the pre-clinical HCC model without showing toxic side effects. The MCL treatment inhibited proliferation in nasopharyngeal carcinoma (NPC) cell lines CNE-1 and CNE-2 without affecting normal cells NP69\textsuperscript{76}. Mechanistically, the MCL induced G1-phase cell cycle arrest, DNA fragmentation, mitochondrial injury and apoptosis in the NPC cells by modulating Cyclin D1 expression, RB and p38/MAPK, JNK, and ERK phosphorylation. In addition, it induced cytochrome-c release, Caspases-3, 8, 9 and PARP cleavage. Intraperitoneal administration of MCL effectively prevented CNE-2 xenograft tumor growth in nude mice\textsuperscript{76}. A combination of MCL with sorafenib showed a better therapeutic effect than the individual effect against the in-vivo NPC growth. No toxic side effect was seen during the MCL treatment in the mice. Thus, MCL may be a potential therapeutic agent.

**Alpha momorcharin (\( \alpha \)-MMC)**

Alpha momorcharin (\( \alpha \)-MMC) is another ribosome inactivating protein isolated from *Momordica charantia* seeds. The effect of \( \alpha \)-MMC was studied on the human lung adenocarcinoma epithelial cell A549. The \( \alpha \)-MMC inhibited cell proliferation in a dose dependent and time dependent manner and induced S-phase cell cycle arrest and apoptosis on the cells\textsuperscript{50}. The \( \alpha \)-MMC also inhibited proliferation and induced apoptosis in human breast cancer cells MDA-MB-231 and MCF-7 and xenograft tumor growth in mice\textsuperscript{77}. However, \( \alpha \)-MMC has severe in-vivo hepatotoxicity and stimulates inflammatory responses in human monocytes\textsuperscript{78,79}. To reduce its immunogenicity and toxicity and to increase stability, \( \alpha \)-MMC was PEGylated. The PEGylated \( \alpha \)-MMC could preserve the anti-tumor effect against lung cancer cell A549\textsuperscript{53}, in-vivo murine breast cancer cell (EMT-6) and human breast cancer cell (MDA-MB-231) transplanted mouse tumor model\textsuperscript{80}. Interestingly, the PEGylation increased the plasma half-life in rats and reduced its non-specific toxicity\textsuperscript{80}.

**Other components**

BG-4 is a novel and low-molecular weight (4 kDa) protease inhibitor, isolated from *Momordica charantia* seeds. BG-4 showed a cytotoxic effect and induced apoptosis in human colon cancer cells HCT-116 and HT-29\textsuperscript{81}. The BG-4 reduced expression of Bcl-2 and increased expression of p21, Bax and Caspase-3 in the cells.

Two cucurbitane-type triterpene glycosides isolated from fruits: charantagenins-D and goyaglycoside-d with an –(OMe) substitution in side chain exhibited significant cytotoxic effects against lung cancer cell line A549, glioblastoma cell line U87, and hepatoma carcinoma cell line Hep3B\textsuperscript{82}.

Karaviloside-III is a cucurbitane-type triterpene glycoside isolated from immature fruit. It showed a potent cytotoxic activity against activated murine hepatic stellate cells (t-HSC/Cl-6) and
human HCC cells Hep3B and HepG2 suggesting its potential role against hepatic fibrosis and cancer.

Kuguaglycoside C is a triterpene glycoside isolated from the leaves of *Momordica charantia*. The Kuguaglycoside C showed cytotoxic effects against human neuroblastoma IMR-32 cells and induced caspase-independent cell death.

RNase MC2 is a 14-kDa unique ribonuclease isolated and purified from *Momordica charantia* seeds. It showed RNase activity on tRNA in baker’s yeast, calf liver, and rRNAs from breast cancer cell MCF-7. The RNase MC2 inhibited cell proliferation and induced apoptosis in the MCF-7 and HCC cell HepG2. It induced phosphorylation of Akt, p38, and ERK.

Plumericin, an iridoid lactone, isolated from the leaves of *Momordica charantia* vine, showed antibacterial and anti-proliferative activities. The compound potentially inhibited proliferation, and induced G2/M cell cycle arrest and apoptosis in leukemia cells. It also inhibited proliferation and induced G2/M arrest in HCC cells Hep3B and HepG2. It significantly decreased expressions of COX 2 and VEGF in the cells.

Atriterpenoid compound, 3β, 7β, 25-trihydroxy cucurbita-5, 23(E)-dien-19-al (TCD), isolated from the whole plant, inhibited proliferation and induced autophagy in breast cancer cells MCF-7 and MDA-MB-231. Mechanistically, the TCD down-regulated Akt-NF-κB signaling, induced p38/ MAPK, p53, inhibited expression of histone deacetylases, and increased ROS generation in the cells.

A similar compound, known as triterpenoid, 3β, 7β-dihydroxy-25-methoxy-cucurbita-5,23-dien-19-al, inhibited proliferation and induced apoptosis in breast cancer cells. Mechanistically, it modulated the expression of PPARγ and mTOR-p70S6K signaling molecules Cyclin D1, CDK6, Bel-2, XIAP, COX-2, NF-κB, ERα, and Akt, activated AMPK and induced endoplasmic reticulum (ER) stress.

A series of cucurbitane-type triterpene glycosides isolated from methanol extract of the fruits were studied for anti-viral and anti-cancer effects. Among those, two compounds: (19R, 23E)-5β, 19-epoxy-19-methoxy- cucurbita-6,23,25-trien-3β-ol and (19R, 23E)-5β, 19-epoxy-19,25- dimethoxy- cucurbita-6,23-dien-3β-ol showed inhibitory effects in dimethylbenz[a] anthracene (DMBA) and peroxynitrite induced mouse skin carcinogenesis. In another study, 15 cucurbitane-type triterpene glycosides were isolated from ethanol extract of fruits including kuguaolesides A-D, charantoside A, momordicosides I, gogayglycosides-b and -d, 7β, 25-dihydroxy cucurbita-5,23(E)-dien-19-al 3-O-β-d-allopyranoside, and 25-hydroxy-5β, 19-epoxycucurbita-6,23-dien-19-on-3β-ol 3-O-β-d-glucopyranoside. Many of those compounds showed anti-proliferative effects against MCF-7 (human breast adenocarcinoma), Doay (human medulloblastoma), HEP-2 (human laryngeal carcinoma), and WiDr (human colon adenocarcinoma) with IC50 values ranging from 10–20 µg/mL for 72 h treatment.

**Conclusion**

As discussed in this review, *Momordica charantia* is a medicinal plant and the crude extract of the whole plant or plant parts (fruit, leaves, or seeds) exhibits effective cancer-preventative and therapeutic activities. The extract contains many bioactive phytochemicals, including triterpenoids, triterpene glycoside, phenolic acids, flavonoids, lectins, sterols, proteins, and saponins. Studies suggested that the combined impact of these bioactive phytochemicals determines the biological activity of the extract. Among the bioactive phytochemicals, Momordicine-I (M-1), MomordicaAntiviral Protein 30 Kd (MAP30) or Momorcharin, alpha-Eleostearic acid (ESA), kuguacin J (Kuj), Momordica charantia lectin (MCL), RNase MC2, alpha-Momorcharin (α-MMC), BG-4, and karaviloside-III show potential anticancer effects. By modifying the gene expression of related pathways, the majority of the compounds prevent the growth of cancer cells, cause cell cycle arrest in either the S or G2/M phase, and promote either apoptotic or autophagic cell death or both of cancer cells. Most of the compounds show minimum side effects at least in *in-vitro* models. Each compound has specific mechanisms, sometimes targets multiple molecules at a time, and plays potential anti-cancer
effects against multiple types of cancer. However, to design prospective studies for interventional therapies, more analysis of active components and in-depth mechanistic study in pre-clinical systems are required.

**Future directions**

There are several nutrients and bioactive elements in *Momordica charantia*. *In vitro* and *in vivo* cancer models have been used to characterize and assess some of the components. However, to identify a specific target in cancers, detailed mechanistic studies are highly recommended. There is a need for extensive validation or follow-up research as well as effects when combined with traditional therapy. Additionally, for many of the components found, the data on bioavailability, stability, metabolism, and toxicity are not well evaluated. Many components still need to be assessed for their functional characterization.

Complex interactions between tumor cells and the immune microenvironment closely control multistep carcinogenesis. One crucial step in the development of cancer is immune suppression which is accomplished by inflammation in tumor-bearing hosts 93, modulation in natural killer (NK) cells 94,95 and myeloid-derived suppressor cells (MDSCs) 96, suppression and dysfunction of CD8+T-cells, and activation of regulatory T-cells (Tregs) in the microenvironment 97,98. Effective anti-tumor immunity must elicit both innate and adaptive immune responses. Naturally occurring anti-inflammatory or immunomodulatory plant extracts contribute to anticancer effects in modulating immune signaling pathways 99,100. Activation of NK cells treated with *Momordica charantia* extract enhances killing of cancer cells *in vitro* and inhibits CD4+FoxP3+T cell populations following the extract treatment in syngeneic head & neck tumor-bearing mice 100,101. Therefore, it will be critical to study the active components of *Momordica charantia* in regulating the tumor microenvironment.

**Conflict of interest**

The authors declare that they do not have any Conflicts of Interests.

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