# MT VAJRA

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WHITE PAPER

**MT VAJRA**

# *Introduction:*

The healthy Avocado has nutrients such fatty acids, fibers, and vitamins which have positive effects on cholesterol, blood pressure, liver, as well as prevention of infection. Mettitech group together with Global Pharm Distribution were able to isolate 300 different types of fatty acids from avocado and study their effects (**Patent No.: US 10,888,105 B2**). Pathogen's capsid stability is based on an oligomerization process of "scaffolding proteins". Our study shows that **MT Vajra, as "food supplement",** will destabilize capsid of pathogen. Communicable diseases are commonly spread via respiratory droplets which can remain suspended in the air, travel long distances, and survive for long period on the surface. As per CDC protocol, our products will "prevent droplet of an infected person from infecting another person". The combination of our daily intake of MT VARJA's isolated fatty acids together with Vitamins (A, B, C, D, E and K) and minerals (Zn, Mg, Ca, and P) equate to 15 % or higher when compared to the Daily Nutritional Value or Daily Value (DV). Finally, natural food supplemental products that would play major role in antimicrobial therapies.

# *Abstract:*

Doctors and healthcare professionals view avocadoes as one of the world's healthiest foods. Avocados are rich in vitamins and minerals. They are a source of healthy fats such as Omega 3 and 6 fatty acids. Avocados also contain nutrients such as riboflavin, niacin, folate, magnesium, and potassium. They also contain vitamins C, E, and K.

As for the chemical composition, **MT Vajra** shows a high degree of fatty content:

- 71% monosaturated fatty acids
- 13% polyunsaturated fatty acids
- 16% saturated fatty acids
- A significant presence of sitosterol which is one chemical compound that promotes healthy cholesterol (HDL)

**Nutrition Value Property of MT VARJA**

**Figure 1.** Fatty alcohols in **MT Vajra**

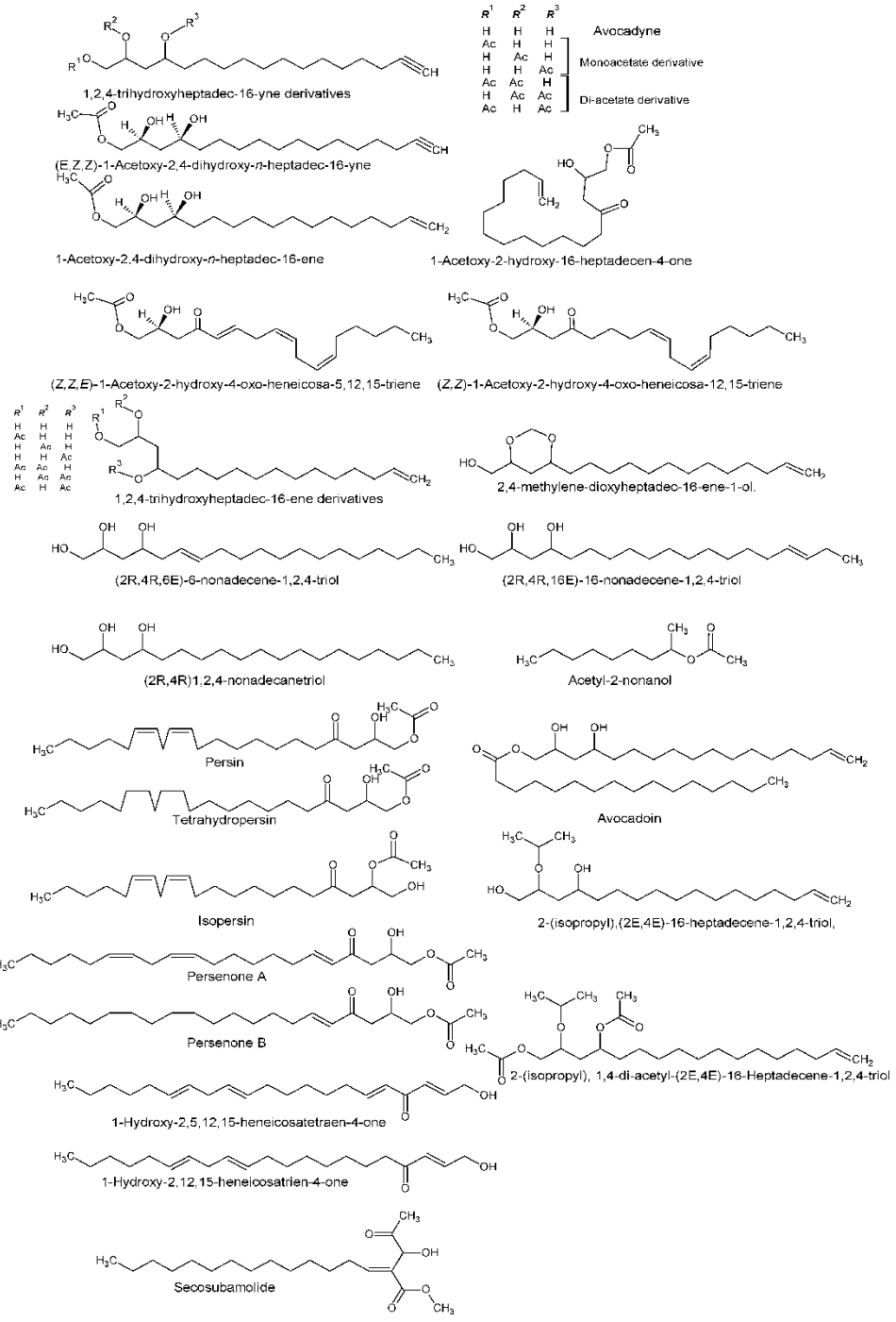


**Figure 2.** Phenolic compounds isolated in **MT Vajra**.

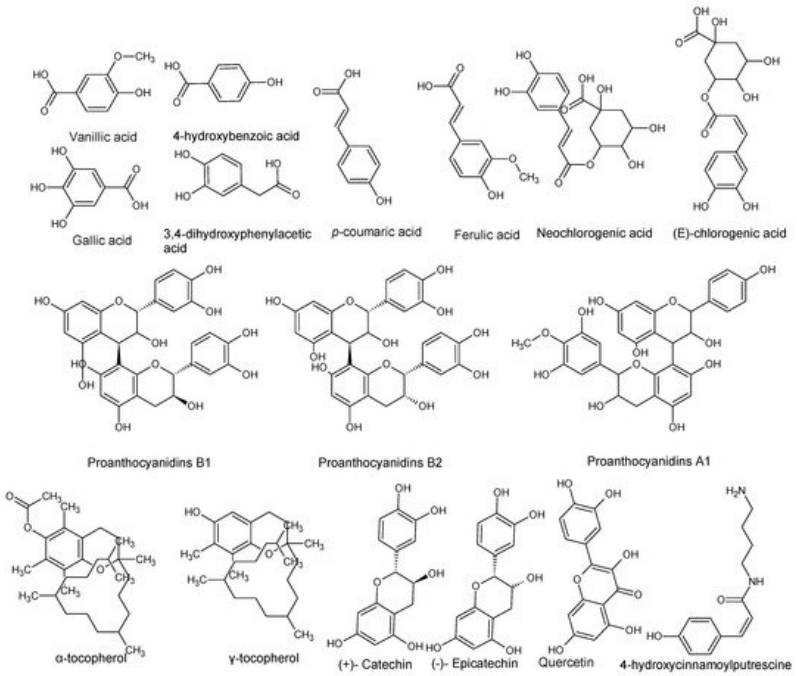



**Figure 3.** Carotenoids isolated in **MT Vajra**.

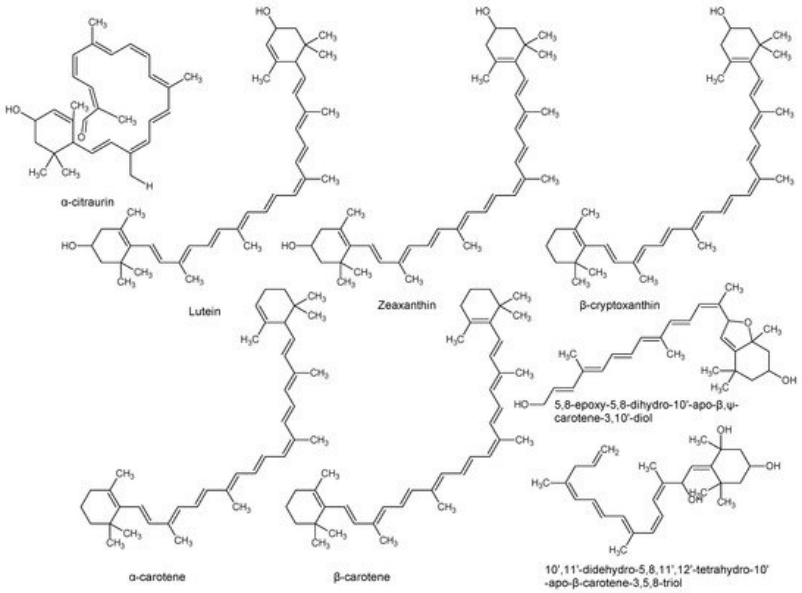


**Figure 4.** Sugars and sugar alcohol isolated in **MT Vajra**

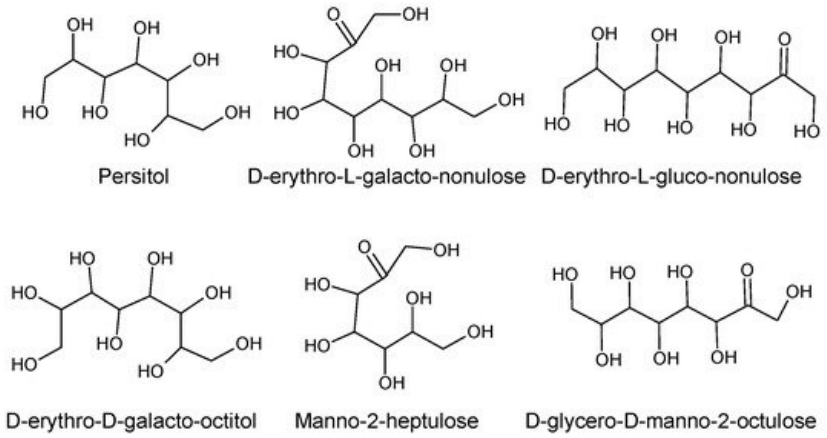



**Figure 5.** Furan and furanone derivatives isolated in **MT Vajra**.

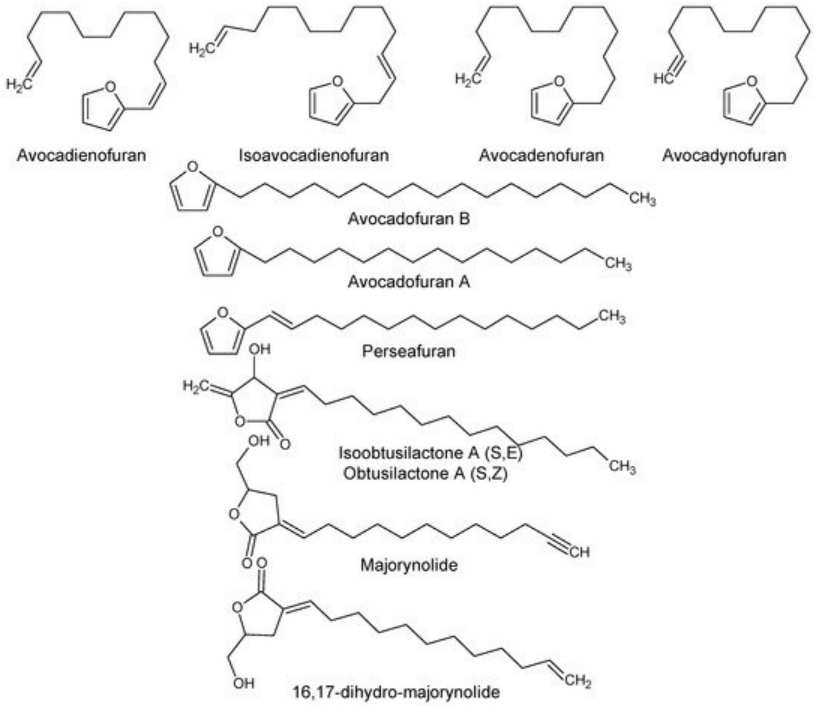


**Figure 5.** Diterpenoids isolated in **MT Vajra**.

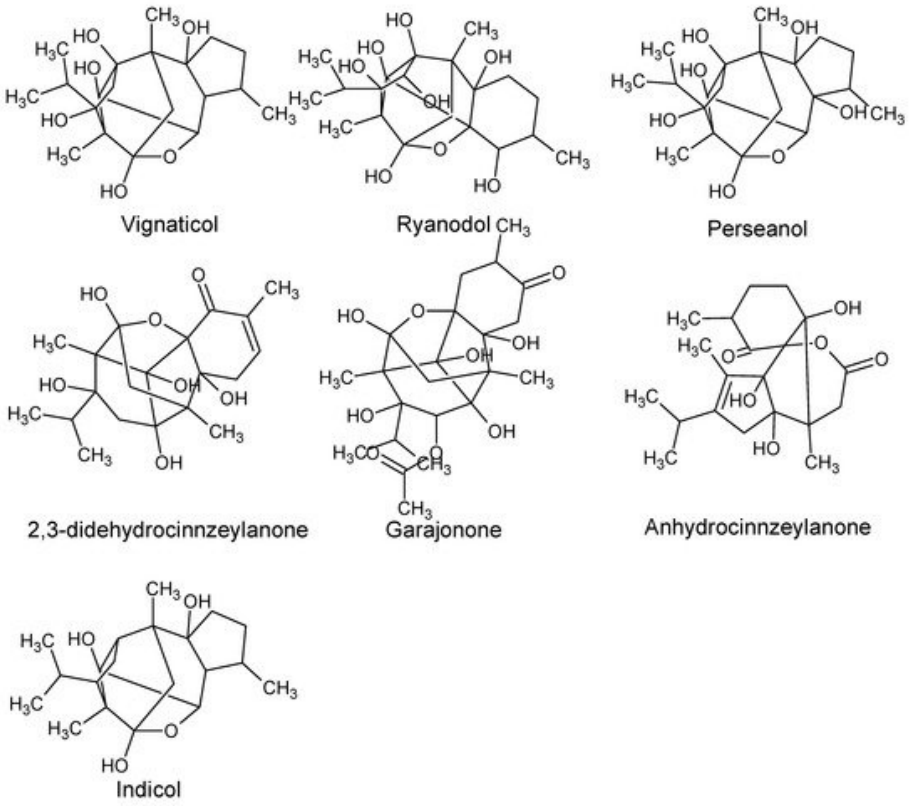



**Figure 6.** Norlignans, neolignans, and lignans in **MT Vajra**.

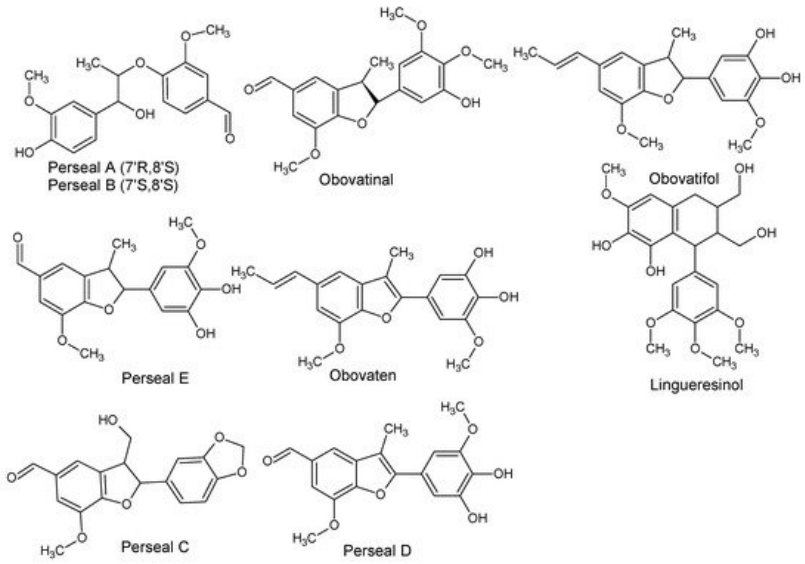
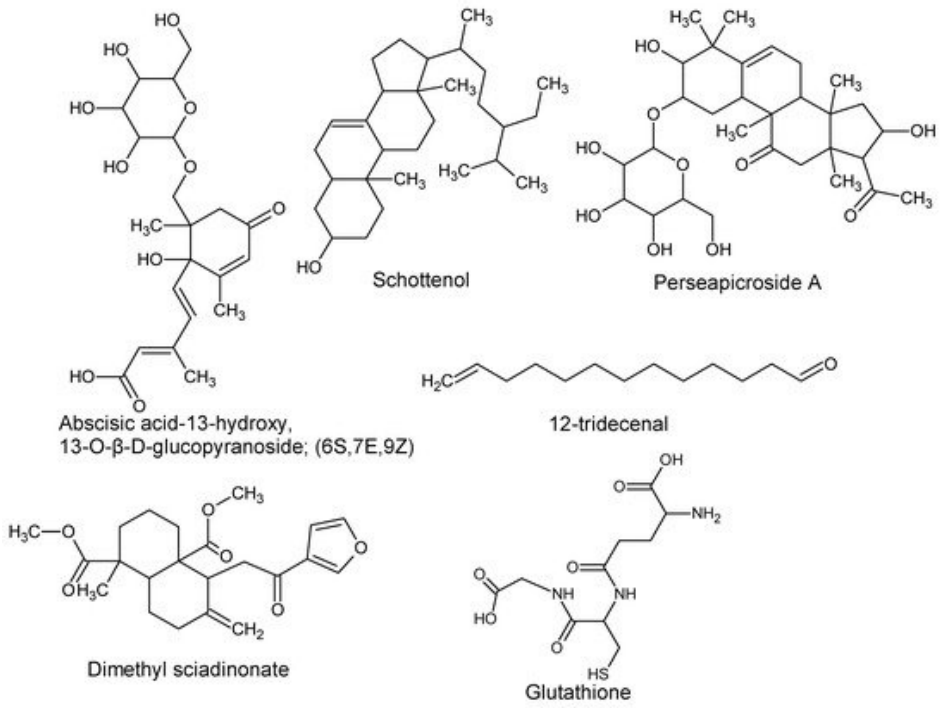


**Figure 7.** Miscellaneous compounds isolated in **MT Vajra**




**Pharmacokinetic of Compounds from Avocado's extract.**
Avocado is containing high levels of water and fat-soluble vitamins, plant sterols, MUFA, and phytochemicals. Avocado has been shown to improve the absorption of nutrients when used in combination with other foods and supplements; however, research on the pharmacokinetics of the avocado components alone is limited.

Vitamin A is fat-soluble in nature and present in many foods as retinol and in its provitamin A form (carotenes). Liver, fish, and cheese are rich sources of vitamin A. Carotenes (provitamin A) are converted to vitamin A in the body. However, plant-based foods typically present a challenging matrix for the utilization of vitamin A, hindering the absorption and conversion of provitamin A to vitamin A. Many commonly consumed plant-based foods contain higher levels of provitamin A such as sweet potato (709 µg/ 100 g), carrots (835 µg/100 g), and spinach (469 µg/100 g), especially compared to avocado (7 µg/100 g). Nevertheless, the levels of vitamins in the food are trivial if not absorbed and converted to their active chemical forms for the body to utilize. The absorption of provitamin A from plant sources is typically poor. In an in vitro digestion model, the accessibility of β-carotene in raw carrots was 1–3% and lycopene were <1%.

The consumption of lipid-rich food has been shown to improve the absorption of fat-soluble vitamins, including vitamin A. The presence of soluble fats during digestion facilitates the formations of mixed micelles, which facilitate absorption.

The absorptions of provitamin A including β-carotene, α-carotene, β-cryptoxanthin, lutein, and zeaxanthin were enhanced when co-consumed with avocado. This can perhaps be attributed to the high MUFA content of avocado. In salsa, the absorption of lycopene and β-carotene was increased by 4.4 and 2.6 times respectively when avocado was added. In salad (150 g), the addition of avocado (24 g) increased the absorption of α and β-carotene and lutein by 7.2, 15.3, and 5.1 times, respectively. In addition to the improved absorption, avocados were shown to enhance the utilization of provitamin A by increasing the conversion rate to vitamin A in participants with low conversion efficacy. The enhanced absorption of provitamin A has been attributed to the improved formation of mixed micelles in the lumen, increasing solubility and facilitating uptake by enterocytes. Improved vitamin A uptake has been observed with other high lipid foods such as eggs and oil. Likewise, the consumption of salad rich in carotenes, with canola oil, resulted in significantly higher carotene concentrations in chylomicrons. As avocado is a rich source of fat and high in monosaturated fatty acids, it presents an alternative from sources high in unsaturated fats.

Avocado is the most concentrated source of β-sitosterol. Plant sterols share similar chemical structures with cholesterol; however, they are poorly absorbed compared to cholesterol, (with about 10% systematically absorbed compared to 50–60% for cholesterol). Like other lipophilic compounds, phytosterols are incorporated into mixed micelles before being taken up by enterocytes. Plant steroids may assist in lowering cholesterol absorption by acting as a competitive inhibitor. Interestingly, plant sterols have also been observed to lower dietary carotene plasma levels by 10–20%. As established for vitamin A and carotene, it is likely that the absorption of other lipophilic compounds may similarly be enhanced by consumption with avocado. Within avocado, this may apply to vitamin E, vitamin K, chlorophylls, and phytochemicals such as acerogenins. Further pharmacokinetic research is necessary to determine if the absorption of other lipophilic compounds is enhanced in combination with avocado. The current literature does not provide any information regarding the effect of avocado matrix on the absorption of water-soluble vitamins and phytochemicals. Moreover, further pharmacokinetic research should be directed to understand the bioavailability of pharmaceutically promising phytochemicals such as acerogenins from avocado.

The known positive effects of avocado consumption on cardiovascular health have caught the attention of MettiTech and Global Pharm. Their research and development (R&D) departments have focused on the purported benefits of avocados' fatty acids in

combating the capsid content in bacteria and viruses. The combination of isolated fatty acids together with Vitamins (A, B, C, D, E and K) and minerals (Zn, Mg, Ca, and Phosphate) equate to 15% of Daily Nutrition Value or Daily Value (DV). Table 1

**Table 1.** Composition of MT VAJRA as nutrition supplement

| Nutritional Composition | Unit | Value Per 100 g | 1 Fruit 136 g | 1 Serving 30 g |
|---|---|---|---|---|
| **1. Proximate** | | | | |
| Water | g | 72.3 | 98.4 | 21.7 |
| Energy | kcal | 167 | 227 | 50 |
| Energy (insoluble fiber adjusted) | kcal | 148 | 201 | 44 |
| Protein | g | 1.96 | 2.67 | 0.59 |
| Total lipid (fat) | g | 15.41 | 21 | 4.62 |
| Ash | g | 1.66 | 2.26 | 0.5 |
| Carbohydrate | g | 8.64 | 11.8 | 2.59 |
| Fiber | g | 6.8 | 9.2 | 2 |
| Sugars | g | 0.3 | 0.41 | 0.09 |
| Starch | g | 0.11 | 0.15 | 0.03 |
| **2. Minerals** | | | | |
| Calcium | mg | 13 | 18 | 4 |
| Iron | mg | 0.61 | 0.83 | 0.18 |
| Magnesium | mg | 29 | 39 | 9 |
| Phosphorus | mg | 54 | 73 | 16 |
| Potassium | mg | 507 | 690 | 152 |
| Sodium | mg | 8 | 11 | 2 |

| Nutritional Composition | Unit | Value Per 100 g | 1 Fruit 136 g | 1 Serving 30 g |
|---|---|---|---|---|
| Zinc | mg | 0.68 | 0.92 | 0.2 |
| Copper | mg | 0.17 | 0.23 | 0.05 |
| Manganese | mg | 0.15 | 0.2 | 0.05 |
| Selenium | ug | 0.4 | 0.5 | 0.1 |
| **3. Vitamins and Phytochemicals** | | | | |
| Vitamin C | mg | 8.8 | 12 | 2.6 |
| Thiamine | mg | 0.08 | 0.1 | 0.02 |
| Riboflavin | mg | 0.14 | 0.19 | 0.04 |
| Niacin | mg | 1.91 | 2.6 | 0.57 |
| Pantothenic acid | mg | 1.46 | 2 | 0.44 |
| Vitamin B-6 | mg | 0.29 | 0.39 | 0.09 |
| Folate, dietary folate equivalents | µg | 89 | 121 | 27 |
| Choline total | mg | 14.2 | 19.3 | 4.3 |
| Betaine | mg | 0.7 | 1 | 0.2 |
| Vitamin B-12 | µg | 0 | 0 | 0 |
| Vitamin A | µg | 7 | 10 | 2 |
| β-Carotene | µg | 63 | 86 | 19 |
| α-Carotene | µg | 24 | 33 | 7 |
| β-Cryptoxanthin | µg | 27 | 37 | 8 |
| Lutein + zeaxanthin | µg | 271 | 369 | 81 |
| Vitamin E (α-tocopherol) | mg | 1.97 | 2.68 | 0.59 |

| Nutritional Composition | Unit | Value Per 100 g | 1 Fruit 136 g | 1 Serving 30 g |
|---|---|---|---|---|
| Tocopherol β | mg | 0.04 | 0.05 | 0.01 |
| Tocopherol γ | mg | 0.32 | 0.44 | 0.1 |
| Tocopherol δ | mg | 0.02 | 0.03 | 0.01 |
| Vitamin K1 (phylloquinone) | µg | 21 | 28.6 | 6.3 |
| **4. Lipids** | | | | |
| Fatty acids, total monounsaturated | g | 9.799 | 13.3 | 2.94 |
| 16:1 | g | 0.698 | | |
| 17:1 | g | 0.01 | | |
| 18:1 | g | 9.066 | | |
| 20:1 | g | 0.025 | | |
| Fatty acids, total saturated | g | 2.126 | 2.9 | 0.64 |
| 8:0 | g | 0.001 | | |
| 16:0 | g | 2.075 | | |
| 18:0 | g | 0.049 | | |
| Fatty acids, total polyunsaturated | g | 1.816 | 2.47 | 0.55 |
| 18:2 | g | 1.674 | | |
| 18:3 | g | 0.125 | | |
| 18:3 n-3 c,c,c (ALA) | g | 0.111 | | |
| 18:3 n-6 c,c,c | g | 0.015 | | |
| 20:3 | g | 0.016 | | |
| Cholesterol | mg | 0 | 0 | 0 |

| Nutritional Composition | Unit | Value Per 100 g | 1 Fruit 136 g | 1 Serving 30 g |
|---|---|---|---|---|
| Stigmasterol | mg | 2 | 3 | 1 |
| Campesterol | mg | 5 | 7 | 2 |
| β-sitosterol | mg | 76 | 103 | 23 |

## Anti-Cancer Properties of MT VARJA

Their research and development (R&D) departments have focused on the purported benefits of avocadoes' fatty acids in combating the capsid content in bacteria and viruses. Multiple studies have pointed out that capsid is an oligomerization process where its assembly requires "scaffolding proteins" resulting in a stable coat structure. Study shows how avocadoes' fatty acids destabilized cancer cells. (Table 2 and Table 3)

Scopoletin, a plant coumarin and phytoalexin found in avocado, reduced the carcinogens-induced toxicity and the size of skin papilloma in vivo. Further mechanistic study revealed the modulation of various key cell cycle, apoptotic and tumor invasion markers by scopoletin. Notably, the downregulation of AhR (aryl hydrocarbon receptor), CYP1A1 (cytochrome P450 1A1), PCNA (proliferating cell nuclear antigen), stat-3 (signal transducer and activator of transcription 3), surviving, MMP-2 (matrix metalloproteinase-2), cyclin D1 and c-myc (avian myelocytomatosis virus oncogene cellular homolog); and the upregulation of p53, caspase-3 and TIMP-2 (tissue inhibitor of metalloproteinases-2) by scopoletin were demonstrated. Of note, the expression of p53 and its target genes (~500) regulate a wide range of cellular processes, including apoptosis, cell cycle arrest, and DNA repair. Additionally, the upregulation of TIMP-2 inhibits MMP-2 expression, which consecutively leads to the reduction of cellular migration and invasion (metastasis). Therefore, MMP-2 upregulation has been correlated with poor prognosis and relapse in cancer patients. Another study by Roberts et al. also indicated synergistic interaction between the breast cancer standard drug—tamoxifen—and persin isolated from avocado leaves against MCF-7 (Michigan cancer foundation-7), T-47D, and SK-Br3 breast cancer cells in vitro. The authors reported a significant reduction of tamoxifen $IC_{50}$ values when it was combined with avocado persin. The synergistic interaction was Bim-dependent and mediated by the modulation of ceramide metabolism. Bim is a member of the Bcl-2 (B-cell lymphoma 2) family of proteins that play a key role in the intrinsic (mitochondrial) pathway of apoptosis. In particular, Bim is linked with microtubule-stabilizing properties, which mediate the formation of microtubule bundles with subsequent mitotic arrest and apoptosis .

Chemical synthesis of the most potent anticancer compounds found in avocado has also been carried out in a number of studies. Similar to avocado crude extracts, chemically synthesized avocado peptide PaDef defensin was recently found to induce apoptosis via caspase 7, 8, and 9 expressions in K562 chronic myeloid leukaemia and MCF-7 breast cancer cells in two studies by the same research group. Moreover, PaDef defensin was previously demonstrated to have antimicrobial properties. The induction of apoptosis and abrogation of the cell cycle were also observed earlier in the human breast, lung, ovarian, and colorectal cancer cells when treated with chemically synthesized avocado β-hydroxy-α,β-unsaturated ketones by Leon et al. Although many preclinical studies were performed to elucidate the cytotoxicity of extracts derived from different parts of the avocado plant and their components, very few of them have investigated their molecular mechanisms of action. Interestingly, contradicting information regarding avocado extract-induced genotoxicity is also available. For instance, Kulkarn et al. found out that avocado fruit and leaf extracts can induce chromosomal aberrations in human peripheral lymphocytes, with leaf extract being more genotoxic. The same research group later reported that avocado fruit extract can reduce cyclophosphamide-mediated chromosomal aberrations in human lymphocytes, which was perhaps due to the antagonistic effects of the extract on cyclophosphamide.

Traditionally, an avocado leaf decoction is used for the treatment of tumors and tumor-related diseases in Nigeria. Despite their health benefits highlighted in numerous reports, clinical studies examining the direct correlation between avocado consumption and the prevention and treatment of cancer are scarce. Only one case-control study involving 243 men with prostate cancer and 273 controls in Jamaica demonstrated that MUFA from avocado may reduce the risk of prostate cancer. However, it should be noted that bioactive compounds that are also commonly found in avocados such as α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin were found to have inverse associations with cancers of the mouth, larynx, pharynx, and breast in few clinical trials. According to the USDA, avocados contain a significantly higher amount of glutathione per average serving compared to other fruits. Glutathione is a potent tripeptide antioxidant that plays a major role in detoxification pathways and the reduction of oxidative stress and risk of cancer. Notably, it has been linked with the reduction of chemotherapy-associated toxicity and risks of oral cancer in a few clinical studies. Nonetheless, the molecular mechanism of how glutathione reduces the side effects of chemotherapeutic regimens remains largely speculative. To precisely understand the anticancer mechanisms of action of avocado extracts and their bioactive compounds, more in vitro and in vivo studies are warranted. As very few studies have identified the solitary bioactive compounds responsible for the growth inhibition of different cancer cells, more research should be undertaken to gain a comprehensive understanding of the chemical profiles of the active extracts. Notably, bioassay-guided fractionation and

the subsequent isolation and characterization of biologically active compounds from different parts of the avocado plant may lead to the identification of many novel anticancer compounds. Randomized controlled trials should be designed to evaluate the efficacy of bioactive compounds derived from avocado in the prevention and treatment of different cancer types. Furthermore, the chemoprotective properties of avocado and the possibility of using its bioactive compounds as an adjunct therapy for cancer should also be explored.

**Table 2.** Clinical studies demonstrating the anticancer activity of bioactive compounds

| Bioactive Compounds | Type of Cancer | Type of Study | Major Findings |
|---|---|---|---|
| Carotenoids- α-carotene, β-cryptoxanthin, lycopene, and lutein/ zeaxanthin | Breast cancer | A nested case-control study in women consisting of 604 breast cancer cases and 626 controls. | In women with high mammographic density, plasma levels of carotenoids reduced breast cancer risk significantly (40–50% reduction, $p < 0.05$). |
| | | An ancillary study involving 207 women ages 18 to 70 years who had been successfully treated for early-stage breast cancer. | An inverse association between total plasma carotenoid concentrations and the oxidative stress biomarkers (urinary 8-hydroxy-2′-deoxyguanosine and 8-isoprostaglandin-F2α) was observed. |
| | Larynx, pharynx and oral cancers | The study population involving 52 patients curatively treated for early-stage larynx, pharynx or oral cavity during 1997–2001. | An inverse association was observed between individual/ grouped xanthophylls and urinary F2-isoprostanes ($F_2$-IsoPs), a biomarker of oxidative stress. However, individual/grouped carotenes did not show such association with $F_2$-IsoPs. |
| Glutathione | Advanced colorectal carcinoma | A randomized, double blind, placebo-controlled trial in 52 patients. | Prevented of oxaliplatin-induced neuropathy without reducing the clinical efficacy of oxaliplatin. |
| | Ovarian cancer | A multicenter, randomized, double-blind, parallel group design with 51 women. | Reduced the cisplatin-associated toxicity and improved the quality of life. |

| Bioactive Compounds | Type of Cancer | Type of Study | Major Findings |
|---|---|---|---|
| | Oral cancer | A population-based case-control study involving 1,830 Caucasian participants (855 cases and 975 controls) in during 1984–1985 in the United States. | Reduced oral cancer risk was associated with glutathione when fruit and vegetable were commonly consumed raw. |

**Table 3.** Preclinical and clinical studies highlighting the anticancer properties.

| Preclinical Studies | | | | | | |
|---|---|---|---|---|---|---|
| **Variety** | **Parts** | **Type of Extracts** | **Bioactive Compounds** | **Type of Cell Lines** | **Major Findings and Molecular Mechanisms of** | **References** |
| Hass | Seeds | Methanol | - | MCF-7 breast, H1299 lung, HT29 colon, and LNCaP prostate cancer cells | Dose-dependent inhibition of all cells with $IC_{50}$ values 19–132 µg/mL after 48 h of treatment. In LNCaP prostate cancer cells, the induction of caspase 3-mediated apoptosis, PARP cleavage, downregulation of cyclin D1 and E2, cell cycle arrest at $G_0$/ | [140] |
| Hass | Seeds | High-speed countercurrent chromatographic fraction of methanol- | Proanthocyanidins B1, B2 and A-type trimer. Traces of abscisic acid glucosides. | HaCaT immortalized nontumorigenic human epidermal cells | Significant inhibition of cell proliferation, increased LDH activity. Molecular mechanisms of action were not investigated. | [23] |
| Hass | Pulp | Chloroform-soluble | Two aliphatic acetogenins-(2S,4S)-2,4-dihydroxyheptadec 16-enyl acetate] and 2 [(2S,4S)-2,4-dihydroxyheptadec-1 | 83–01-82CA human oral cancer cell line, MEK overexpressing cell line 83–01-82CA/MEKCA | The two aliphatic acetogenins targeted the EGFR/RAS/RAF/MEK/ERK1/2 cancer pathway by synergistically inhibiting c-RAF (Ser338) and | [165] |

| Preclinical Studies | | | | | | |
|---|---|---|---|---|---|---|
| **Variety** | **Parts** | **Type of Extracts** | **Bioactive Compounds** | **Type of Cell Lines** | **Major Findings and Molecular Mechanisms of** | **References** |
| Hass | Pulp | Chloroform | - | 83-01-82CA human oral cancer and TE1177 normal epithelial cell lines | In the oral cancer cells, the extract induced apoptosis by increasing the levels of reactive oxygen species by twofold to threefold. Apoptosis was not | [141,142] |
| Hass | Pulp | Acetone | Lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene, α-tocopherol and γ- | LNCaP androgen-dependent and PC-3 androgen-independent prostate cancer cell lines | Inhibited the growth of both the prostate cancer cell lines. Arrested PC-3 cells at the $G_2$/M phase and | [24] |
| Lulu | Unripe fruit pulp | 95% (*v/v*) ethanol extracts and its fractions | 1,2,4-Trihydroxynonadecane, 1,2,4-Trihydroxyheptadec-16-ene and 1,2,4-Trihydroxyheptadec-16-yne. | A-549 human lung, MCF-7 human breast, HT-29 human colon, A-498 human Kidney, MIA PaCa-2 human pancreatic carcinoma, PC-3 | All three compounds were active against six human tumor cell lines and exhibited selectivity against PC-3 cells. Molecular mechanisms were not | [21] |
| - | Seeds | Ethanol extract and its hexane and dichlorometh | - | Lung A549 and gastric BGC823 cancer cells | Growth inhibition at 200 µg/mL. The $IC_{50}$ values and molecular mechanisms of action were not investigated. | [166] |
| - | Pulp and seed extracts | Lipids | Fatty acids, hydrocarbon, and sterols. | HCT116 colon and HePG2 liver cancer cell lines | Seed extract showed greater activity against HCT116 ($IC_{50}$< 4 µg/mL) and HePG2 ($IC_{50}$ < 20 µg/mL) cell lines compared to the pulp extract. Molecular | [98] |
| - | Seeds | Chloroform extracts and its soluble methanol fraction (FML) and non-soluble methanol | - | MCF-7 breast cancer cell line | Chloroform extract, FML, and FTML inhibited cell growth in a dose-dependent manner and displayed $IC_{50}$ values of 94.87, 34.52, and 66.03 µg/mL, respectively. FML | [167] |

| Preclinical Studies | | | | | | |
|---|---|---|---|---|---|---|
| **Variety** | **Parts** | **Type of Extracts** | **Bioactive Compounds** | **Type of Cell Lines** | **Major Findings and Molecular Mechanisms of** | **References** |
| - | Leaves | Silver nanoparticles | | MCF-7 breast and HeLa cervical cancer cells | Dose-dependent cytotoxicity was observed at concentrations above 50 µM in MCF-7 but not in HeLa cells. Downregulation of p53 | [168] |
| - | Leaves | Aqueous-ethanol (5% v/v) | - | Larynx cancer tissue | Significant increase in adenosine deaminase activity in cancerous tissues derived from 13 patients who underwent surgery for larynx cancer (median age of 57 years) compared to | [169] |
| - | Seeds | Fraction of ethanol extract | Triterpenoid | MCF-7 breast and HepG2 liver cancer cells | Inhibited MCF-7 ($IC_{50}$ = 62 µg/mL) and HepG2 ($IC_{50}$ = 12 µg/mL) cells with no activity against normal cells. Molecular mechanisms of action | [170] |
| - | Pulp | Ethanol, chloroform, ethyl acetate, and | - | Esophageal squamous cell carcinoma and colon adenocarcinoma cell | Moderate activity. The $IC_{50}$ values and molecular mechanisms of action were not | [171] |
| - | Pulp | Aqueous | - | A549 lung, HepG-2 liver, HT-29 colon, and MCF-7 breast cancer cells. | Exhibited $LC_{50}$ values in the range of 13.3–54.5 µg/mL against the tested cell lines. Molecular mechanisms | [172] |
| - | Root bark | Methanol extract and its fractions. | 4-hydroxy-5-methylene-3-undecyclidenedihydrofuran-2 (3H)- | MCF-7 breast cancer cell line | Antiproliferative activity with an $IC_{50}$ value of 20.48 µg/mL with induction of apoptosis. | [36] |
| - | Endocarp, whole seed, seed and leaves | Ethanol | - | Jurkat lymphoblastic leukemia cells | Induced significant oxidative stress-dependent apoptosis via mitochondrial membrane depolarization. Activated transcription factor p53, protease | [138] |

| Preclinical Studies | | | | | | |
|---|---|---|---|---|---|---|
| **Variety** | **Parts** | **Type of Extracts** | **Bioactive Compounds** | **Type of Cell Lines** | **Major Findings and Molecular Mechanisms of** | **References** |
| - | Pulp | 50% (*v/v*) Methanol | - | Human lymphocyte cells | Chemoprotective against cyclophosphamide-induced chromosomal | [158] |
| - | Seeds and peel | Methanol | - | MDA-MB-231 breast cancer cells | Apoptosis due to activation of caspase-3 and its target protein, | [144] |
| - | Leaves | - | Persin | In vitro: MDA-MB-231, MCF-7, and T-47D breast cancer cells<br>In vivo: Quackenbush lactating mice | In vitro: Persin selectively arrested cells at the $G_2$/M phase and induced caspase-dependent apoptosis. Apoptosis was dependent on the expression of Bim protein, which also indicated the microtubule-stabilizing properties of persin. Overall, MCF-7 and T-47D cells were more sensitive to persin | [139] |
| | | | | MCF-7, T-47D, and SK-Br3 breast cancer and MCF-10A human mammary epithelial cells. | Synergistic interaction between tamoxifen and persin against the tested breast cancer cells was observed. Significant reduction of $IC_{50}$ values of tamoxifen when combined with 13.8 µmol/L of persin. The synergistic cytotoxicity was Bim-dependent and | [149] |
| - | Fruit | - | Persenone A | In vitro: RAW 264.7 mouse macrophage cells<br>In vivo: Female ICR mice (7 weeks old) | Downregulated the expression of iNOS/COX-2 (nitric oxide synthase/ cyclooxygenase-2) in macrophage cells. When applied topically, | [173] |

| Preclinical Studies | | | | | | |
|---|---|---|---|---|---|---|
| **Variety** | **Parts** | **Type of Extracts** | **Bioactive Compounds** | **Type of Cell Lines** | **Major Findings and Molecular Mechanisms of** | **References** |
| - | Fruit | - | (2R)-(12Z,15Z)-2-hydroxy-4-oxoheneicosa-12,15-dien-1-yl acetate (1), persenone A (2) and B (3) | HL-60 acute promyelocytic leukemia and RAW 264.7 mouse macrophage cells. | Suppressed the growth of HL-60 cells (compound 1, $IC_{50}$ = 33.7; compound 2, $IC_{50}$ = 1.4; compound 33, $IC_{50}$ = 1.8 μM). Inhibited nitric oxide generation induced by lipopolysaccharide in | [19] |
| - | - | - | Scopoletin | In vivo: Skin papilloma in mice induced by 7,12-dimethylbenz(a)anthracene and croton oil | Reduced carcinogen-induced toxicity and led to decrease in the size of skin papilloma. Downregulated AhR, CYP1A1, PCNA, stat-3, survivin, MMP-2, cyclin D1, and c-myc, and | [26] |
| **Chemical synthesis** | | | **Type of cell lines** | | **Major findings and molecular mechanisms of action** | **References** |
| Antimicrobial peptide-PaDef defensin | | | K562 chronic myeloid leukemia cells | | Cytotoxic with an $IC_{50}$ value of 97.3 μg/mL. Activated caspase-8 and induced | [153] |
| | | | MCF-7 breast cancer cell line | | Inhibited the growth in a concentration-dependent manner ($IC_{50}$ = 141.62 μg/mL). Induced cytochrome c, APAF-1, and the caspase 7 and 9 expressions, loss of mitochondrial Δψm and | [143] |
| Persin and tetrahydropersin | | | Breast cancer: MCF-7, T-47D, MDA-MB-468, MDA-MB-157, SkBr3, Hs578T, MDA-MB-231 cells, normal mammary epithelial MCF-10A cells, Ovarian cancer: OVCAR3 and IGROV-1 cells Prostate cancer: PC-3 and LNCaP cells | | Persin was more potent compared to tetrahydropersin against most of the tested cancer cell lines with $IC_{50}$ values in the range 15.1 ± 1.3 to more than 39 μM. | [154] |

| Preclinical Studies | | | | | | |
|---|---|---|---|---|---|---|
| Variety | Parts | Type of Extracts | Bioactive Compounds | Type of Cell Lines | Major Findings and Molecular Mechanisms of | References |
| β-Hydroxy-α,β-unsaturated ketones | | | | A2780 human ovarian, SW1573 lung, HBL-100 human breast, T-47D human breast and WiDr colorectal cancer cells. | $GI_{50}$ values in the range of 0.5–3.9 μM. Induced apoptosis and dose-dependent cell cycle arrest in the S and $G_2$/ | [145] |
| **Case-control studies** | | | | | | |
| **Type of cancer** | | | **Major findings** | | | **Referen** |
| Prostate cancer | | | A study involving 243 men with prostate cancer and 273 controls in Jamaica reported that monounsaturated fat from avocado was | | | [160] |

## Anti-Microbial Properties of MT VAJRA

Currently, there is a growing interest in finding alternatives to the synthetic antimicrobial agents that are commonly used in the food and pharmaceutical industries. This is due to the concerns of the consumers regarding the safety of products containing synthetic chemicals and their associated health risks. Seeds (endocarp) and peels (exocarp) being the by-products of the avocado industry are generally disposed of as wastes and have been investigated for their antimicrobial properties. Most of the studies conducted thus far have noted the antimicrobial activity of the extracts derived from different avocado varieties, while only a few have reported insignificant antimicrobial activity. The antimicrobial activity of avocado extracts might be influenced by (i) the variety of the avocado, (ii) the parts used for investigation (i.e., exocarp, endocarp, or mesocarp), (iii) the solvent type used for extraction, and iv) the bacterial species examined. Raymond and Dykes investigated the antimicrobial activity of ethanolic and aqueous extracts of seeds and peels of three different avocado varieties. The authors reported that ethanolic extracts had antibacterial activity against both Gram-positive and Gram-negative bacteria (except for Escherichia coli) ranging from 104.2 to 416.7 μg/mL, while aqueous extracts exhibited activity against Listeria monocytogenes and Staphylococcus epidermidis. Rodriguez-Carpena et al. investigated the antibacterial activity of the extracts derived from different avocado parts (peel, seed, and pulp) of a number of varieties against Bacillus cereus, S. aureus, L. monocytogenes, E. coli, Pseudomonas spp., and Yarrowia lipolytica. The highest inhibitory activity against the Gram-positive bacteria- B. cereus and L. monocytogenes was observed, while E. coli was the most sensitive among the tested Gram-negative bacterial species. The authors mentioned that all avocado parts had

antimicrobial properties, with pulp (mesocarp) showing the highest activity. In addition, authors reported that the Gram-positive bacteria were more sensitive in comparison to the Gram-negative bacteria. The Gram-negative bacteria have an extra protective outer membrane, which makes them more resistant to antibacterial agents compared to the Gram-positive bacteria. β-sitosterol in avocados was also shown to play a key role in strengthening the immune system and the suppression of human immunodeficiency virus and other infections. In particular, it has been found to enhance the proliferation of lymphocytes and natural killer cell activity for invading pathogens. Salinas-Salazar et al. investigated the antimicrobial activity of seed extracts of avocado enriched with acetogenin against L. monocytogenes and reported growth inhibition at 37 °C and 4 °C with MIC (minimum inhibitory concentration) values of 15.6 and 7.8 mg/L, respectively. Acetogenins of avocados are fatty acid derivatives with a long unsaturated aliphatic chain (C19–C23). Owing to the structural similarities between acetogenins and fatty acids, authors hypothesized that acetogenins may penetrate the cell membranes of bacteria and physically disrupt their functionality. Indeed, several compounds might be associated in the antimicrobial activity of avocado extracts. Polyphenols have been previously reported for their antimicrobial properties. However, the contribution of the phenolic compounds toward the antimicrobial activity of avocado extracts needs to be investigated. Rodriguez-Carpena et al. found that avocado pulp extract had a higher antimicrobial activity than peel and seed extracts, despite having lower polyphenol content. Future studies should be conducted to isolate individual phenolic compounds from different parts of avocado and investigate their antimicrobial properties.

**Table 4.** Summary of studies that have been conducted that investigated the antimicrobial activity MT VARJA.

| Variety/ies | Bacteria | Highlights |
|---|---|---|
| Hass Shepard Fuerte | *Listeria monocytogenes*<br>*Staphylococcus epidermidis*<br>*Staphylococcus aureus*<br>*Enterococcus faecalis*<br>*Escherichia coli*<br>*Salmonella Enteritidis*<br>*Citrobacter freundii*<br>*Pseudomonas aeruginosa*<br>*Salmonella Typhimurium*<br>*Enterobacter aerogenes* | The antimicrobial activity of peel and seed extracts was evaluated.<br>Ethanol extracts showed antimicrobial activity against both Gram-positive and Gram-negative bacteria (except *E. coli*).<br>Aqueous extracts had antimicrobial activity against *L. monocytogenes* and *S. epidermidis*. |

| Variety/ies | Bacteria | Highlights |
|---|---|---|
| Hass<br>Fuerte | *Bacillus cereus*<br>*S. aureus*<br>*L. monocytogenes*<br>*E. coli*<br>*Pseudomonas spp.*<br>*Yarrowia lipolytica* | All components have antimicrobial activities.<br>Pulp showed the highest antimicrobial activity.<br>Gram-positive bacteria were found to be more sensitive than Gram-negative bacteria. |
| Hass | *L. monocytogenes* | The antilisterial properties of an enriched acetogenin extract from seed were determined.<br>Seeds had higher acetogenin content than pulp.<br>The antimicrobial effect was probably caused by increased membrane permeability. |
| Lorena Hass | *S. aureus*<br>*E. coli* | Extracts did not have antimicrobial activity against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 |
| Hass | *Listeria innocua*<br>*E. coli*<br>*Lactobacillus sakei*<br>*Weissella viridescens*<br>*Leuconostoc mesenteroides* | Peel and seed extracts did not present antimicrobial activity against any bacteria analyzed. |

## Anti-Inflammatory Properties of MT VAJRA

Several studies have investigated the anti-inflammatory properties of avocado via modulation of inflammatory responses. The aqueous extract of avocado leaves showed an anti-inflammatory effect in vivo by inhibiting carrageenan-induced rat paw oedema. Persenone A, an active constituent of avocado, reduced inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in murine macrophages. Similarly, (2*R*)-(12*Z*,15*Z*)-2-hydroxy-4-oxoheneicosa-12,15-dien-1-yl acetate, persenone A and B isolated from the avocado fruit, decreased the generation of nitric oxide in mouse macrophages. Avocado oil contains a high amount of oleic acid and essential fatty acids. A study by highlighted the wound-healing properties of avocado fruit oil by increasing collagen synthesis and decreasing inflammation in Wistar rats. They also reported that oleic acid was the predominant unsaturated fatty acid (47.20%) present in the fruit oil.

Inflammation in joints causes damage to the joint cartilage due to degenerative changes leading to a loss of joint function and stability. Osteoarthritis (OA) is considered a non-inflammatory disease; recent studies have shown that inflammation is a leading cause for the initiation and continuation of the disease process. Non-pharmacological agents that modulate the expression of pro-inflammatory mediators are highly promising as safe and effective ways to treat OA. Avocado–soybean unsaponifiable (ASU) combination represents one of the most commonly used treatments for symptomatic OA . ASU is a combination of avocado oil and soybean oil, which has been accepted as a

medication/food supplement in many countries. Three ratios of avocado (A) and soybean (S) unsaponifiable combinations (A:S = 1:2, 2:1, and 1:1) were studied for their anti-inflammatory properties on chondrocyte cells. All the ratios showed significant inhibition compared to the individual extracts on collagenase, stromelysin, interleukin 6 (IL-6), interleukin 8 (IL-8), and prostaglandin $E_2$(PGE2) release. In particular, 1:2 was found to be the most effective combination that exhibited chondroprotective effects in vivo by stimulating glycosaminoglycan and hydroxyproline synthesis and inhibiting the production of hydroxyproline in the granulomatous tissue. In another study, the unsaponifiable of avocado alone indicated a significant chondroprotective effect. Several preclinical and clinical studies conducted in the last few decades have revealed the modulation of different pathways and molecular targets associated with OA pathogenesis by ASU. For instance, the anti-OA properties of ASU are mediated via the suppression of critical regulators of the inflammatory response such as iNOS/COX-2, and PGE-2, and the reduction of catabolic enzymes (matrix metalloproteinases-3 and -13) and Gabay et al. demonstrated the inactivation of the mitogen-activated protein kinases such as the extracellular signal-regulated kinase (ERK 1/2) and NF-$\kappa$B as the molecular mechanism of action for the anti-inflammatory effects of ASU. A recent study showed the potential bone repair properties of ASU by the modulation of molecular targets *Rankl* and *Il1β*, *RANKL*, and *TRAP* using a rat model. Sterols, the major bioactive components of ASU, have also shown anti-inflammatory activity in particular chondrocytes.

Other studies have combined ASU with bioactive compounds such as epigallocatechin gallate (EGCG), and α-lipoic acid (LA). Interestingly, contrary to previous research, Heinecke et al. reported a slight inhibition of COX-2 expression and $PGE_2$ production in activated chondrocytes. However, when ASU was combined with EGCG, both mediators were more significantly inhibited than their mono treatments. Another study by Ownby et al. demonstrated that this combination inhibited the gene expression of interleukin-1 beta (IL-1β), tumor necrosis factor- α (TNF-α), IL-6, COX-2, and IL-8 in activated chondrocytes. The combination of ASU with LA showed a more significant suppression of $PGE_2$ production in activated chondrocytes than ASU or LA.

The implementation of ASU in the treatment of other inflammatory diseases has also been explored. ASU has shown efficacy against periodontal disease by modulating the expression of transforming growth factor beta 1 (TGF-β1), TGF-β2, and bone morphogenetic protein 2 (BMP-2). Additionally, a recent study demonstrated that ASU can repair periodontal disease within seven days. These results underline the significant anti-inflammatory properties of avocado mediated via multiple signal transduction pathways and their role in the potential treatment of various inflammatory diseases.

**Additional Health Benefits of MT VARJA**

Cardiovascular Health and Diabetes
Clinical have shown a positive effect on cardiovascular health and lipid profiles with the presence of avocado in the diet. It has been observed that the intake of avocado in a balanced diet had a great impact on preventing cardiovascular diseases because of the low cholesterol levels. Grant in 1959 conducted the first avocado clinical trial where 0.5 to 1.5 avocados were incorporated in the diet of 16 male patients and showed a significant decrease or the same total serum cholesterol level with no increase in weight. Avocado phytosterols were found to inhibit cholesterol absorption and synthesis by mimicking its molecular structure, which resulted in lowered total cholesterol levels in the body.

The indigestible carbohydrates abundantly found in avocado are reported to prevent diabetes and regulate blood cholesterol. The glycemic index can be defined as a comparative ranking of carbohydrate in foods according to their effect on blood sugar levels. Despite its carbohydrate content, the glycemic index rating of avocado is quite low. In rats, various aqueous concentrations of avocado seed extract exhibited hypoglycemic and antihyperglycemic effects by significantly decreasing the blood glucose levels, highlighting its potential in the management of diabetes mellitus. Another study conducted to investigate the effect of avocado paste on rats with a hypercholesterolemic diet with high fructose showed lower levels of blood sugar and significant reduction of fat accumulation in the liver, which was attributed to the presence of bioactive compounds (polyphenols, fiber, and carotenoids). Investigation on the inhibitory effects of phenolic extract from the avocado pulp, leaves, and seed on various type 2 diabetes enzymes (α-amylase and α-glucosidase) was also performed. The peel extract exhibited the highest inhibition against α-amylase and α-glucosidase, while the leaf extract significantly inhibited the α-glucosidase.

**Anti-Viral Properties of MT VAJRA**
Mettitech and Global Pharm's R&D first discovered the link between fatty acids in avocadoes and their effect on viral capsid over two years ago. Subsequently, these enterprises patented their discovery **(US patent no. US 10,888,105 B2),** leading to the commercialization of **MT VAJRA**

Given COVID19's type of aerial transmissibility, COVID19 is significantly contagious. Moreover, the Centers for Disease Control's (CDC) COVID19 guidelines indicate that respiratory droplet remains suspended in the air and can travel up to six feet. Additionally, the virus itself can survive on clothing and surfaces for up to 48 hours.

Fortunately, **MT VAJRA** helps combat the SARS-COV2's capsid content utilizing its avocado-based therapy. The treatment aims to inhibit the viral capsid, thereby reducing

the likelihood of infection and severe illness. MT VAJRA are 100% organic and can be a natural alternative to various types of viral treatments.

Specifically, **MT VAJRA** consists of enzymes (cellulase, amylase, protease, lipase, pectinase, xylase, beta-gluconae, mannase, phytase), mineral salts, organic acids, molasses, and water. This composition makes **MT VAJRA** completely devoid of artificial chemical components.

## *Conclusion:*

Several preclinical studies performed in the last few decades lay emphasis on the unique nutritional and phytochemical composition of avocado and their potential in the treatment and prevention of different diseases. The cumulative effects of avocado components in the prevention and treatment of oxidative stress and age-related degenerative diseases are also indicated in a few studies. However, more comprehensive in vitro, in vivo, and clinical investigations are fundamental to significantly expand the understanding of the molecular mechanisms of action of its phytochemicals for developing subsequent therapeutic and nutritional interventions against cancer, diabetes, inflammatory, microbial, and cardiovascular diseases.

Utilizing the link between organic extract fatty acids and their effects on viral capsids, **MT VAJRA's** extract can reduce the spread of infection and become an effective nutraceutical means to combat current and future pathogens. Thus far, **MT VAJRA** has been successful in over 22 countries. Hopefully, more countries will authorize **MT VAJRA's** distribution. Given preliminary results on its effectiveness, there is a great chance that this product will soon become available in various countries around the world.

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## *Conflicts of Interest*

As a medical research institute, the Metti Global Pharm R&D receives research grants and donations from foundations, universities, government agencies, individuals, and industry. Sponsors and donors also provide untied funding for work to advance the vision and mission of the institute. The authors declare no conflict of interest.

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