A Review: *Melia azedarach* L. as a Potent Anticancer Drug

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ABSTRACT

Chinaberry (*Melia azedarach* L., Meliaceae), a Mahogany family usually used as high quality timber, is native to Asia but now is found in other parts of tropical world continent. The leaves, fruits, bark, seed and root are used in traditional medicine and it has been shown to various pharmacological activities like antifungal, anti-malarial, antibacterial, hepatoprotective, anti-oxidant, anti-fertility, anethelminthic, anti-pyretic and cytotoxic. A review to its phytochemical and anticancer properties of *M. azedarach* and its sub species or varieties as an effort to analyze the literature on its developing used as anticancer agent. As results of literatures review indicates that fruit, bark, leaves, pulp and seed of *Melia azedarach* L. showed various in vitro cytotoxic activities in cancer cell lines, such as human colorectal carcinoma (HT-29), breast cancer (MCF-7, SK-BR-3), cervix hepatoma (HepG-2, SMMC-7721 and Hep3B), kidney epithelial cell (MDK8), human lung adenocarcinoma epithelial cell (A549), non-small cell lung cancer (H460), human lymphoblast lung (U937), human cancer promyelocytic leukemia (HL-60), AZ521 (stomach), human colon cancer (SW480), murine colorectal adenocarcinoma cell (CT26), human oral cancer cell (KB), human prostate cancer (PC3), liver (BEL7404), CNS (SH-SY5Y, U251, SF539), B16F10 mouse melanoma cell line; and showed various in vivo to adenocarcinoma mammary in C3H mice and mouse hepatocellular carcinoma H22 cells to BALB/c mice. Previous results suggested that cytotoxic organic compounds of *Melia azedarach* L. were supposed of flavonoids, triterpenoids (tirucallane), limonoids (meliarachin, meliatoxin B1, trichilin H, and toosendanin), steroids, and organic acids content compounds.

Key words: Anticancer, cytotoxic, *Melia azedarach*, *Melia*, triterpenoids, limonoid

INTRODUCTION

Cancer is a generic term for a large group of disease that affects any part of the body. Another definition of cancer is an uncontrolled proliferation of the cells, producing abnormal cells which invade healthy tissue and spread to other organs (malignant) and established secondary lethal tumors (metastases). Cancer is one of the major causes of morbidity and mortality worldwide. There were almost 14 million new cases in 2012, which is predicted to rise about 70% for next two decades. It is the second leading cause of death globally and is responsible for 8.8 million deaths in 2015; 18.7% of deaths are caused due to cancer; of which 70% occurs in low- and middle-income countries. Five most common causes of cancer death are lung (39.2%), liver (17.2%), colorectal (16.9%), stomach (16.5%), and breast cancers (12.5%).

Plants play a significant role in the development of anticancer during the last few decades. Ninety out of 121 prescription drugs that are being used today for cancer treatment are plant based as evidenced by the historical use and developing plants for cancer therapy. The first developing natural-derived compound in 1961 was vincristine, a vinca alkaloid class isolated from the dried leaves of periwinkle *Catharanthus roseus*. Vincristine is a chemotherapy medication to cure Hodgkine’s disease and some types of leukemia. Etoposide, an epipodophyllotoxin compound class, is another example of natural-derived compound from *Podophyllum peltatum* (the Mandrake Plant) and *Podophyllum emodi* (the Wild Chervil). Etoposide (a topoisomerase II inhibitor class) is effective against testicular cancer and small and non-small cell lung carcinoma, also to malignant lymphomas. Etoposide is a topoisomerase II inhibitor, which breaks DNA by stabilizing enzyme-DNA cleavable complexes. An additional number of natural-derived anticancer agents such as camptothecin, paclitaxel, homoharringtonine, and other compounds are shown in Table 1.

Chinaberry (*Melia azedarach* L. [MA]) is a plant species belonging to the family *Meliaceae*, a Mahogany family (*Sapindales* order). It is originally from Asia but is now found in parts of North Africa, Africa, North America, tropical South of America, and Southern part of Europe. It named as Para iso or paradise in South America and as Indian Lilac or White Cedar in the USA. *Melia*s leaves, fruits, bark, and root are used in traditional medicine. The leaves are used for anthelmintic and antifungal agent. Pharmacologically, MA

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Table 1: Plant-derived anticancer agents

<table>
<thead>
<tr>
<th>Compound</th>
<th>Class of compound</th>
<th>Plants source (Family)</th>
<th>Cancer use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vincristine</td>
<td>Alkaloid</td>
<td>Leaves of Catharanthus roseus (Apocynaceae)</td>
<td>Leukemia, lymphoma, breast, lung, pediatric solid cancers and others</td>
</tr>
<tr>
<td>Vinblastine</td>
<td></td>
<td>Bark of Taxus brevifolia, T. canadensis (Taxaceae)</td>
<td>Death and nerve cancer</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Taxanes</td>
<td>Bark of Taxus brevifolia, T. canadensis (Taxaceae)</td>
<td>Death and nerve cancer</td>
</tr>
<tr>
<td>Docetaxel</td>
<td></td>
<td>Bark and wood of Nyssaea Camptotheca acumulata (Nyssaceae)</td>
<td>Death and nerve cancer</td>
</tr>
<tr>
<td>Topotecan</td>
<td>Camptothecin derivates and Topotecan (Hycamit)</td>
<td>Amsora rohituka (leaves and stem)</td>
<td>Death and nerve cancer</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>Alkaloid rohitukine</td>
<td>Dysoxylum, Binetariferum (Meliaceae)</td>
<td>Death and nerve cancer</td>
</tr>
<tr>
<td>Flavopiridol</td>
<td></td>
<td>Anti‑oviposition, phagoinhibitor, antimolting, larvicidal, adulticidal,</td>
<td>Death and nerve cancer</td>
</tr>
<tr>
<td>Etoposide</td>
<td>Epipodophyllotoxin</td>
<td>Podophyllum peltatum and Podophyllum emodi (Berberidaceae)</td>
<td>Death and nerve cancer</td>
</tr>
<tr>
<td>Acrony cine</td>
<td>Alkaloid</td>
<td>Acronychia bauer (Rutaceae)</td>
<td>Death and nerve cancer</td>
</tr>
<tr>
<td>Brucentin</td>
<td>Alkaloid</td>
<td>Brueca antidysenterica (Simaroubaceae)</td>
<td>Death and nerve cancer</td>
</tr>
<tr>
<td>Thalcarpin</td>
<td>Alkaloid</td>
<td>Thalictrum dasycarpum (Ranunculaceae)</td>
<td>Death and nerve cancer</td>
</tr>
<tr>
<td>Homoharringtonine</td>
<td>Alkaloid</td>
<td>Cephalotaxus harringtonia (Cephalotaxaceae)</td>
<td>Death and nerve cancer</td>
</tr>
<tr>
<td>Elliptinium</td>
<td>Alkaloid</td>
<td>Bleekeria vitensis Apocynaceae</td>
<td>Death and nerve cancer</td>
</tr>
<tr>
<td>4-Ipomeanol</td>
<td>Furan</td>
<td>Ipomoeca batatas (Convolvulaceae)</td>
<td>Death and nerve cancer</td>
</tr>
</tbody>
</table>

showed various activities such as antifungal, antibacterial, antimalarial, hepatoprotective, antioxidant, antifertility, anthelmintic, antipyretic, and cytotoxic. The purpose of this article is to review phytochemical and anticancer properties of MA as an effort to give a detailed survey of the literature on its potential developing used as natural-derived anticancer source compounds. The literature reviews was carried out by analyzing journal and peer reviewed concerning of MA and Melia spp. Peer-reviewed articles were indexed by databases such as Scopus, PubMed, Google Scholar, and ResearchGate. Data selection criteria in this review are based on in vivo and in vitro cytotoxic and anticancer of Melia spp. The keywords used for search the literature in the databases: Melia cytotoxic, plants anticancer.

BOTANICAL VIEW AND ETHNOMEDICINAL USE OF MELIA AZEDARACH L.

MA belongs to Meliaceae, the mahogany family of flowering plants order of Sapindales. Meliaceae comprises 51 genera and about 575 species of trees and (rarely) shrubs or sometimes shrublets, monopodial or sympodial, usually deciduous, less often monoeocious or polygamodioecious, native to tropical and subtropical regions. Most members of the family have more leaves, with the leaflets arranged in the form of a feather, and branch flower clusters. The fruit is fleshy and colored or a leathery capsule. Genera of member of the family are differentiated according to its fruit shape and leaf rearrangement of the plants. Melia is a genus with the winged seed fruit and leaves bipinnate; margin dentate leaflet blades, crenate, or rarely entire. MA is quite a different genus to the mahogany family of flowering plants order, Meliaceae, which  ethnomedicinally use as anticancer in some regions. The purpose of this article is to review phytochemical and anticancer activities potency.

PHYTOCHEMICAL CONTENT OF MELIA AZEDARACH L.

A variety use in solvents extraction revealed the organic compound of MA. Some researchers used methanol and ethanol by reflux or by percolation. In general, organic molecule compounds of MA contain...
flavonoids, terpenoids, steroids, organic acids and anthraquinones, alkaloids, saponins, and tannins.\[12,14,15\] The most secondary metabolite contents in the parts of the plants are terpenoids and limonoid (tetranortriterpenoid), followed by steroid and flavonoids. The organic molecules are in glycoside or aglycone form, so some results confirm saponin (glycoside of triterpene or steroid).

The leaf extract contains kaempferol-3-O-β-rutinoside, kaempferol-3-L-rhamno-D-glucoside, rutin, quercetin (flavonoids); stigmasterol, campesterol (phytosterols); β-sitosterol, phytol, squalene, 3-methyldecan, heptadecane, heptadecane (alkane hydrocarbon); hexadecanoic acid, palmitic acid, pentadecanoic acid (n-alkanoids acids); β-carotene, tocopherol (Vitamin E); squalene, 1-eicosanol (triterpene); and 3,5,11,15-tetramethyl-2-hexadecan-1-ol (terpene alcohol). The leaves contain also terpenoids (α-pimene, β-pimene, α-terpinene, α-terpeneol) and limonoids (1-cinnamyl-3-acyl-11-hydroxy meliarcin, l-cinnamyl-3-methacrylyl-11-hydroxy meliarcin, deacetyl salannin, 1,3-dicinnamyl-11-hydroxy meliarcin).\[16,17\]

The seeds of MA contain terpenoids and limonoid glycosides, viz., 6,11-diacetoxy-7,10-oxo-14 beta-epoxymeliacarin (1,5-diene-3-O-β-D-glucopyranoside), 3β-[6,2-hydroxy-21,23,24-apo-triteracila-14,24-dien e-21-one, meldenin and melianol, meliacarpin, meliartiernin vanillin, vanillin and steroids (β-sitosterol, campesterol, cholesterol, daucosterol, stigmasterol), hydroxy-3-methoxycinnamaldehyde and (±) pinoresinol. The seeds also contain organic acids (linoleic acid, linolenic acid, oleic acid, 9-octadecenoic acid), benzoic acid, vanillic acid.\[12,18\]

Melia's stem contains terpenoids (α-pimene, β-pimene, a-terpinene, a-terpeneol) and limonoids (7a-acetoxy-14 β,15β-epoxygedun nan-ene-3-O-β-D-glucopyranoside, 12-acetoxymelarostatin, amorarostatin, fraxinellone, 12-hydroxymeloarostatorene, 3-hydroxyep-7,24-diene-21,16-olide, kulactone, kulinone, kulkolate, methylkulanate, including melianin-A and melianin-B). They also contain flavonoids (4', 5-dihydroxy flavone-7-O-L-rhamnopyranosyl-(1-4)-β-D-glucopyranoside), antraquinone (1,3,5,8-tetrahydroxy-2-methylanthraquinone; 8-β-Me-ether, 3-O-α-L-rhamnopyranosyl, 1,5-dihydroxy-8-methoxy-2-methylanthraquinone-3-O-α-L-rhamnopyranoside, 1,8-dihydroxy-2-methyla nthraquinone-3-O-β-D-galactopyranoside).\[16,17]

The roots of Melia contain terpenoids and limonoids (6-acetoxy-7a-hydroxy-3-oxo-14β, 15 β-epoxymeliacin, 5-diene, 6-acetoxy-3β-[6,2-hydroxy-7,10-oxo-14 β,15β-epoxydelinan-ene-3-0-β-D-glucopyranoside, azeclaracin-1, azeclaracin-2, azeclaracin-3, azeclaracin-4) and flavonoids (apigenin-5, 8-O-β-D-galactopyranoside), steroids (24-methylencycodoarolan, 24-methylencycodoartanone, 4-stigmastanene-3-one, 4-campestene-3-one β-sitosterol, β-sitosterol-B-D-glucoside); acids (trans-cinnamic acid, vanillic acid [4-hydroxy-3-methoxybenzoic acid]). Root bark contains terpenoids and limonoids (12-O-acetyl azedarachin-A, 12-O-acetyl azedarachin-B, 1-acetyltigloyl-11-methoxy meliarcinpin, 12-O-acetylchirchelin-B, 20-acetyl-29-deacetyl-29-isobutyl yrylsendanin, azedaracin-A, azedarachin-A, azedarachin-C, 1-cinnamyl-3-acyl-11-hydroxy meliarcinpin, 1-cinnamyl-3-hydroxy-11-methoxy meliarcinpin, 1-deoxy-3-methacrylyl-11-methoxy meliarcinpin, 1-deacetylnimbolinin-B, 1,12-diacetyltiglolin, B, 7,12-diacetyltiglolin-B, 29-isobutylsendanin, meliarcin pin E, nimbolidin-B, salannin, 1-tigloyl-3-11-methoxy meliarcinpin, 1-tigloyl-3,20-diacyl-11-methoxyneilmelarapin, 3-tigloyl-1,20-diacyl-11-methoxyneilmelarapin, trichilin-B, trichilin-D, trichilin-H) and steroids (6-β-hydroxy-4-campestene-3-one, 6-β-hydroxy-4-stigmastene-3-one, azedarachol).\[12]

Melia's fruits contain terpenoids and limonoids (6-acetoxy-14,15,15-epoxy-3, 11-dihydroxymeliacarla-l, 5-diene-7-one, amorarostatin, amarostatone, azedarachin-A, l-cinnamyl-3, 11-dihydroxy-meliarcinpin,
These may be hydroxyl cinnamic acid derivatives, phenols or phenolic acid, tannins, lignins, coumarins, quinones, stilbenes, etc.\(^{[25]}\)

Huang et al.\(^{[26]}\) presented a comprehensive review about terpenoids and their potential effects against cancer. A variety of terpenoids have been identified that are effective against proliferation of cancer cell. Anticancer terpenoids include sesquiterpenoids, monoterpenoid, diterpenoid, and triterpenoid or tetraterpenoids. It was triterpenoids which most extensively studied as anticancer research. The mechanism of terpenoid as anticancer cellular target remains unclear. A proposed mechanism of action of monoterpenic D-limonene might inhibit 3-hydroxy-3-methylglutaryl coenzyme reductase, which leads to obstructing p21, and its membrane localization by inhibiting small G proteins isoprenylation. This mechanism is believed in contributing to the chemoprevention on the cancer therapy. P21 is a cyclin-dependent kinase inhibitor protein, has functions in cell cycle regulator (progression at G, and S phase), but does not appear to be applicable to all cancer types. Other findings suggest that D-limonene is primarily involved in the mitochondrial death pathway by apoptosis-upregulated Bax protein expression, with the mitochondria release of cytochrome c, and by the cleavage of caspase-3 and -9 but not caspase-8.

Terpenoid is mostly produced in vegetative parts of plants, flowers, but occasionally in roots.\(^{[27]}\) These anticancer compounds are isolated from a very limited number of plants, while still large numbers of reported plants are phytochemical unexplored.\(^{[28]}\)

Kim et al.\(^{[29]}\) isolated a limonoid compound, 28-deacetylsendanin, from the fruit of M. toosendan Sieb. et Zucc. and examined on anticancer activity against eight human cancer cells from six organs lines and SRB assay as compared to adriamycin. The most sensitive cell of 28-deacetylsendanin (dose–response) was against SF-339 (central nervous system [CNS]) and PC-3 (prostate). All the cell lines responded similarly to adriamycin to give rise to nearly identical dose-response profiles. This result showed that 28-deacetylsendanin had more sensitive and selective inhibitory effects on in vitro growth of human cancer cell lines in a comparison with adriamycin.

Other two new limonoid compounds (toosendanal and 12-O-methyl volkensin), along with three known limonoids (meliatoxin B1, trichilin H, and toosendanin [TSN]), were isolated from the fruits of M. toosendan Sieb. et Zucc. Trichilin H and TSN were highly cytotoxic against KB cells in vitro, while 12-O-methyl volkensin, toosendanal, and meliatoxin B1 did not show any significant level of toxicity. Limonoid meliatoxin B1 and TSN showed cytotoxic activity against KB cells (IC\(_{50}\) 10 µg/mL and 3.82 µg/mL). The results also suggesting structure-cytotoxic activity relationship of C-14/C-15-epoxide and C-15-keto structures against KB cells (cytotoxic requires the C-14/C-15-epoxide structure such structure of trichilin H and TSN compared to C-15-keto structures of toosendanal and meliatoxin B1).\(^{[29]}\)

Wu et al.\(^{[30]}\) isolated 6 steroids from the leaves of MA. The compounds were elucidated and tested to A549, H460, U251 cell line. The result obtained 3 compounds have cytotoxic effect (IC50 12.0-30.1 µg/mL). Ntalli et al.\(^{[31]}\) isolated three known tirucallanes and a new tirucallane triterpenoid, 3-α-tigloylmelianol, from the dichromomethanol-soluble part of the methanol extract obtained from the fruits of MA 21β-acetoxymelianol, 3α-tigloylmelianol, and melianone were cytotoxic while 21β-acetoxymelianol and 3-α-tigloylmelianol showed an additional moderate antiproliferative effect against the A549 (human lung adenocarcinoma epithelial) cell line. The structure of tirucallane triterpenoid and limonoid is shown in Figure 1.

In vitro research on anticancer activity of the extract and some compounds being isolated from MA showed the promising results. Jafari et al.\(^{[32]}\) reported that extract from Melia’s seed kernel produced IC\(_{50}\) range of 8.18–60.10 µg/mL, as the highest cytotoxic activity and selectivity to cancer cell lines by MTT assay compared to A. indica. At the study, the leaves, pulps, and seeds as well as three main fractions of the leaf extracts were determined against five cell line (HT-29, A-549, MCF-7, HepG-2, and MDBK). Four flavonol 3-O-glycosides (rutin, kaempferol-3-O-robinobioside, kaempferol-3-O-rutinoside, and isoquercetin), purine nucleoside, and β-adenosine were isolated in phytochemical analysis. The leaves of MA have plenty content of flavonoids, well-known secondary compounds which supposed to be accountable for many medicinal uses in the traditional exploited. The results also showed that in terms of cytotoxicity methanol leaf fraction of MA to be safer compared to other solvent fractions.

Flavonoids as phenolic compounds have important effects on cancer chemoprevention and chemotherapy. In many molecular mechanisms of chemoprevention, they play a major role by interacting between different types of genes and enzymes. Many mechanisms of action have been identified, including antioxidation, carcinogen inactivation, antiproliferation, cell cycle arrest, apoptosis induction, angiogenesis inhibition, and reversal of multidrug resistance or a combination of these mechanisms.\(^{[33-35]}\) Proposed mechanism of inhibition carcinogenesis of flavonoid is shown in Figure 2.\(^{[35]}\)
He et al.\[36\] tested in vivo and in vitro of TSN, a triterpenoid derivative isolated from M. toosendan Sieb. et Zucc. in the in vitro experiment by means of TSN (0.1–0.9 µM) to human hepatocellular carcinoma cell lines (SMMC-7721 [a p53+] and Hep3B [a p53–]). Dose- and time-dependent manner of antiproliferation effects was observed. The IC₅₀ of TSN against SMMC-7721 and Hep3B cells was 0.5 µM and 0.9 µM, respectively (after treated 72 h). Caspase activity was also shown from annexin V staining (morphological observation). Ratio of Bcl-2/Bax and Fas was associated with the induction of apoptosis in mitochondria-dependent pathway in p53– and p53 + hepatocellular carcinoma cells. In vivo experiment used BALB/c mice which were subcutaneous inoculated with mouse hepatocellular carcinoma H (22) cells. TSN intraperitoneal high dose (0.69 mg/kg) and low dose (0.173 mg/kg) resulted in strongly suppressive effects on the tumorigenicity and apoptotic response. Results from the immunohistochemistry for Bcl-2, Bax, as well as Fas, showed that the anticancer effects of TSN were induced via apoptosis in a mitochondria-dependent manner. In vitro–in vivo confirmed results of TSN have induced mitochondria-dependent apoptosis in hepatocellular carcinoma cells.

Tang et al.\[39\] evaluated in vitro and in vivo of M. toosendan fruit ethanol extract of (EMTF) against human colon cancer (SW480) and murine colorectal adenocarcinoma cells (CT26). Chromatin condensation and DNA fragmentation of EMTF treated were proved that cell proliferation of SW480 and CT26 was inhibited by EMTF. Apoptosis of the tumor cells is resulted by increasing mitochondrial membrane permeability and cytochrome c release from mitochondria. EMTF induced caspase-9 activity which further activated caspase-3 and poly-(ADP-ribose) polymerase cleavage. EMTF also confirmed in vivo result of tumor volume reduction and apoptotic effects, while EMTF did not induce the side effects. All results suggested that EMTF may be a chemotherapeutic agent effective to treat colon cancer.

Kim and Kang\[38\] studied toxicity and anticancer activity of the hexane layer of MA var. japonica Makino's bark extract by hollow fiber (HF) assay and 28-day repeated toxicity. As a result, highest cytotoxicity was observed at 200 mg/kg body weight of hexane layer with 4 mg/kg body weight of cisplatin treated group. The toxicity results showed no significant changes in body weight gain and general behavior, while cisplatin-treated group showed significantly decreased compared to the control group but regained weight with hexane layer treated (100 and 200 mg/kg body weight). The biochemical parameters (alanine aminotransferase, total bilirubin, aspartate aminotransferase, creatinine, and blood urea nitrogen) showed significant increase in cisplatin-treated groups; however, in the group cotreatment of hexane layer (200 mg/kg b.w), these parameters decreased. In white blood cells and neutrophils analysis, cisplatin was reduction, but co-treatment with hexane layer improved these toxicities caused by cisplatin. The eosinophil foci cell in the central vein and portal triad of the liver showed in cisplatin-treated mice. These results showed that hexane layer of MA might have anticancer activity and improve the toxicity effect of cisplatin anticancer drug.

Akihisa et al.\[39\] isolated limonoid and triterpenoid from the fruits of MA (Meliaceae). All isolated compounds (31 limonoids and one tirucallane-type triterpenoid) examined the cytotoxic activities against HL60, A549, AZ521, and SK-BR-3 human cancer cell lines. The results showed that meliarachin C (IC₅₀ 0.65 IM) and 3-O-deacetyl-40-demethyl-28-oxosalannin (IC₅₀ 2.8 IM) produced highest cytotoxic activity and exhibited high selective toxicity against HL60 cells (leukemia). This was demonstrated mostly due to the induction of apoptosis via the mitochondrial and death receptor-mediated pathways.\[30\] Apoptosis-inducing activity was measured by flow-cytometry (annexin V–propidium iodide (PI), while western blot analysis to obtain apoptotic mechanism (evaluate activation of caspases-3, -8, and -9 by which compounds induce apoptotic cell death). One of the earliest markers of apoptotic cell death is exposure of the membrane phospholipid phosphatidylserine to the external cellular environment.\[40\] Annexin V is a protein calcium-dependent phospholipid binding. It has high affinity for phosphatidylserine, which locates on the cell surface. PI does not enter intact membranes cells. By observing annexin V and PI result, apoptotic processes mechanism can be determined. Annexin V positive and PI negative are observed at early apoptotic process, annexin V and PI double-positive are observed at late apoptotic, while annexin V negative and PI positive will be observed on necrotic cell death.
levels of procaspases-8, -9, and -3 reduced (almost in a time-dependent manner).\(^{[41]}\) Caspases are known to mediate the apoptotic pathway.\(^{[42,43]}\) Initiator caspases including caspases-8 and -9 seem as apical caspases will be activated in death receptor and apoptotic cell death by mitochondrial stress-induced mechanism. These initiator caspases are accountable (directly or indirectly) for activating various effector caspases (including caspases-3, -6, and -7, which have short prodomains). Many of the apoptotic features, which are nuclear and cytoplasmic condensation, DNA fragmentation, cell membrane decomposition, and others, are directly responsible by effector caspases cleave and a number of structural and regulatory proteins.\(^{[44]}\)

Furthermore, potential antitumor-promoting effects for 25 compounds were further examined by inhibitory effects on the induction of Epstein–Barr virus early antigen (EBV-EA) by TPA induced. All compounds tested exhibited moderate inhibitory effects (IC\(_{50}\) 347–530 mol ratio/32 pmol TPA) with preservation of high viability (60%) of Raji cells. Among the compounds tested, three compounds, 1, miliarachin C, 12-dehydro-29-exo-neoazedarachin D, and mesendanin E, exhibited more potent than or almost equivalent inhibitory effects (IC\(_{50}\) 347–401 mol ratio/32 pmol TPA) with the reference compound, compared to \(\beta\)-carotene (IC50 397 mol ratio/32 pmol TPA), a vitamin A precursor studied widely in cancer chemoprevention animal models. Since the inhibitory effects against EBV-EA activation have been demonstrated to closely parallel to those against tumor promotion in vivo, compounds miliarachin C, 12-dehydro-29-exo-neoazedarachin D, and mesendanin E (nimbin type limonoid) could be valuable antitumor promoters.\(^{[45]}\)

Acetylazedarachin B as induced apoptotic cell compound, activates caspases-3, -8, and -9 w, cleaved caspases-3, -8, and -9 of HL-60 for 12, 24, and 36 h, respectively. It would decrease the level of Bid and increase of tBid in a time-dependent manner, in which could activate the mitochondrial pathway. It would also decrease the level of Bcl-2 and increased the level of Bax. The Bax/Bcl-2 ratio is one of the intrinsic mechanisms of apoptosis in mitochondria. These results involved Bax/Bcl-2 signal transduction death in leukemia cells both by the mitochondrial and death receptor-mediated pathways.

TSN, a triterpenoid derivative isolated from the barks of \(M.\) toosendan Sieb et Zucc, shown stronger cytotoxicity on U937 cells than VP-16 (etoposide), a clinical anticancer drug. The apoptosis activity of TSN was subsequently associated as evidenced by the typical condensed and fragmented nuclei, DNA and exposure of phosphatidylserine on the outer leaflet of plasma membrane of the cells.\(^{[46]}\)

Furthermore, Zhang et al.\(^{[47]}\) isolated 12-O-acetylatedzadarachin B from the fruit extract from \(M.\) dubia leaf extract of defatted MA fruit by silica gel column chromatography and RP preparative HPLC. Kikuchi et al.\(^{[48]}\) tested the cytotoxic activity of compound 1 and two anticancer drugs, cisplatin and 5-fluorouracil (5-FU), against four human cancer cell lines, HL-60 (leukemia), AZ521 (stomach), A549 (lung), and SK-BR-3 (breast), with MTT assay. The result showed potent cytotoxicity of the extract against leukemia (HL-60) (IC\(_{50}\) 0.016 μM) and stomach (AZ521) (IC\(_{50}\) 0.035 μM) cancer cell lines; and 100 times higher than those of cisplatin (IC50 4.2 μM [HL-60] and 9.5 μM [AZ521]). 12-O-acetylatedzadarachin B, however, did not show cytotoxicity against SK-BR-3 cells. The potent cytotoxicity of 1 against HL-60 and AZ521 and nonactivity against SK-BR-3 might be due to the presence of a cell-specific receptor that differentiates one tumor type from another or receptor activated the antiapoptotic signaling pathway. 12-O-acetylatedzadarachin B exhibited induction of apoptosis detected by the observation of membrane phospholipid exposure and DNA fragmentation in flow-cytometry and western blot analysis showed that compound markedly reduced the levels of procaspases-3, -8, and -9; while the levels of cleaved caspases-3, -8, and -9 are increased. 12-O-acetylatedzadarachin B increased significantly the Bax/Bcl-2 ratio. These results suggested that compound 1 induced apoptotic cell death in HL-60 via both mitochondrial-mediated and death receptor-signal transduction-mediated pathways. It was also reported that Fas receptor, a known death receptor which induces apoptosis, can activate the necrotic pathway. The necrotic cell death of AZ521 by compound 1 was, therefore, supposed to be induced by the participation of Fas receptor signaling although it is not certain whether caspase-8 is activated by Fas receptor. Therefore, 12-O-acetylatedzadarachin B may be a promising lead compound for developing an effective drug for the treatment of leukemia. Flow-cytometric analysis suggested that the cytotoxicity of compound 1 against AZ521 is due to inducing apoptosis as well as necrosis and may be a promising lead compound for developing an effective drug for leukemia.

Yao et al.\(^{[49]}\) tested of 70% ethanol extract of MA on melanogenesis of mouse melanoma cell line (B16F10). The melanin content increased after treatment of the cells with the MA extract (10, 20, and 40 μg/ml in a concentration-dependent manner without cytotoxicity at 24 h) while did not influence tyrosinase activity and the protein levels of tyrosinase and tyrosinase-related protein-2 (TRP-2). In conclusion, that the MA extract increases melanogenesis via upregulation of TRP-1 posttranscriptional control protein expression in B16F10 cells and supposed MA extract acts as melanogenesis rapid inducer. This result showed that MA extract has potential treat the hypopigmentation diseases.

Sumarawati et al.\(^{[50]}\) showed a significant decrease in tumor volume of adenocarcinoma mammary in C3H mice of combination group of MA extract, doxorubicin, cyclophosphamide. The mechanism supposed via increasing BAX expression and decreasing AgNOR expression. Liu et al.\(^{[51]}\) also tested an alcohol-chloroform extraction of the bark of \(M.\) toosendan Sieb. et Zucc in vitro against human hepatocellular carcinoma cell lines (SMMC-7721) and Hep3B and in vivo by inoculated subcutaneous H22 cells (mouse hepatocellular carcinoma) to BALB/c mice. As the results showed that TSN has potent anti-cancer effects, supposed through suppressing proliferation and inducing apoptosis of cancer cells in vivo and in vitro. The mechanism anticanancer of MA via apoptosis emphasized also involves in mitochondrial and death receptor pathway. The summary of in vitro–in vivo anticancer research, including cytotoxic isolated compounds such as limonoid, triterpenoid, and steroid derivatives of \(M.\) dubia, is depicted in Table 2.

Accumulating results and evidence indicate that the anticancer potency effects of neem extracts are mediated by free radical scavenging, by DNA repair and cell cycle alteration, by programmed cell death (apoptosis) and autophagy, by increasing immune surveillance, anti-inflammatory, anti-angiogenic, anti-invasive and anti-metastatic activities as well as its ability to modulate several dysregulated oncogenic signaling pathways. Neem and its constituents including limonoids that target multiple signaling pathways aberrant in cancer are promising candidates for anticancer drug development.\(^{[52]}\) Recent developing research trends are optimizing cytotoxicity of the plant extracts by developing it to nanoparticle form. Sukirtha et al.\(^{[53]}\) and Kahiravan et al.\(^{[54]}\) provided green route synthesis of silver nanoparticles of Melia dubia leaf extract. The results of Kahiravan showed that the silver nanoparticles (7.3 nm) of leaf extract of the plants active against KB cell.

**CONCLUSION**

Review of literature indicates that fruit, bark, leaves, pulp, and seed of MA showed various \textit{in vitro} cytotoxic activities in cancer cell lines, such as human colorectal carcinoma (HT-29), breast cancer (MCF-7, SK-BR-3), cervix hepatoma (HepG-2, SMMC-7721 and Hep3B), kidney epithelial cell (MDBK cell lines), human lung adenocarcinoma epithelial (A549),
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nonsmall cell lung cancer (H460), human lymphoblast lung (U937), human cancer promyelocytic leukemia (HL-60), AZ521 (stomach), human colon cancer (SW480), murine colorectal adenocarcinoma cell (CT26), human oral cancer cell (KB), human prostate cancer cell (PC3), liver (BEL7404), CNS (SH-SY5Y, U251, SF539), and B16F10 mouse melanoma cell line and showed various in vivo to to adenocarcinoma mammary in C3H mice and mouse hepatocellular carcinoma H22 cells in BALB/c mice.

Table 2: Cytotoxic activity of Melia spp

<table>
<thead>
<tr>
<th>Melia species</th>
<th>Part of the plant used</th>
<th>Extract</th>
<th>Cytotoxic/AntiCa Method</th>
<th>Result</th>
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</tr>
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<tbody>
<tr>
<td><em>Melia toosendan</em> Sieb. et Zucc.</td>
<td>Fruit</td>
<td>-</td>
<td>Eight human cancer cell from six different organs lines and SRB assay compare to adriamycin</td>
<td>28-deacetyl sendanin had more sensitive and selective inhibitory effects on in vitro. The most sensitive cell of 28-deacetyl sendanin (dose-response) was against SF-539 (CNS) and PC-3 (prostate), six cell lines were more sensitive to 28-deacetyl sendanin and two were more resistant</td>
<td>Kim, <em>et al.</em> [28]</td>
</tr>
<tr>
<td><em>Melia toosendan</em> Sieb. et Zucc.</td>
<td>Fruits</td>
<td>Aqueous solution of the 90% methanolic extract was partitioned with diethyl ether and 1-butanol</td>
<td>bioassay-guided fractionation against KB cells.</td>
<td>IC₅₀ (mg/ml) of Limonoids: Toosendanal &gt;10, 12-O-Methylvolkensin 8.72, Meliatoxin B1 &gt;10, Trichilin H 0.11, Toosendanin 3.82; and Adriamycin HCl 0.066 6 steroids</td>
<td>Tada, <em>et al.</em> [29]</td>
</tr>
<tr>
<td><em>Melia azedarach</em> M. azedarach L.</td>
<td>Leaves</td>
<td>Extract</td>
<td>3 human cancer cell line (A549, H460, U251) IC₅₀ 12.0-30.1 µg/ml</td>
<td>6 steroids</td>
<td>Wu <em>et al.</em> [30]</td>
</tr>
<tr>
<td><em>Melia azedarach</em> and <em>A indica</em></td>
<td>Leaves, pulps and seeds</td>
<td>MeOH extraction</td>
<td>HT-29, A-549, MCF-7 and HepG-2 and MDBK cell lines; MTT assay</td>
<td>Flavonol 3-O-glycosides including rutin, kaempferol-3-O-robinobioside, kaempferol-3-O-rutinoside and isoquercetin along with a purin nucleoside, β-adenosine</td>
<td>Jafari <em>et al.</em> [31]</td>
</tr>
<tr>
<td><em>Melia toosendan</em> Sieb. et Zucc.</td>
<td>Toosendanin, a triterpenoid derivative isolated from bark</td>
<td>in vivo of toosendan (0.1–0.9 µM) add to SMMC-7721 (p53+) and Hep3B (p53-) (human hepatocellular carcinoma cell lines); in vivo to Balb/c mice s.c. inoculated with mouse hepatocellular carcinoma H (22) cells. i.p TSN high-dose (0.69 mg/kg) and low-dose (0.173 mg/kg)</td>
<td>The IC₅₀ of TSN treated after 72 h for SMMC-7721 and Hep3B cells was 0.5 µM and 0.9 µM, respectively. Morphological observation results show of caspases activity. Ratio of Bcl-2/Bax, and Fas were associated with induction of apoptosis via the mitochondria-dependent pathway in p53- and p53 + hepatocellular. In the <em>in vivo</em> experiment, BALB/c mice were resulted in strongly suppressive effects on the tumorigenicity and apoptotic response. Results from the immunohistochemistry for Bcl-2, Bax, as well as for Fas showed that the anticancer effects of toosendanin were induced via apoptosis in a mitochondria-dependent manner. <em>In vitro</em>-<em>in vivo</em> confirmed results of anticancer of TSN</td>
<td>He <em>et al.</em> [32]</td>
<td></td>
</tr>
<tr>
<td><em>Melia toosendan</em></td>
<td>Fruit</td>
<td>ethanolic extract (EMTF)</td>
<td>in vitro and <em>in vivo</em> against human colon cancer (SW480) and murine colorectal adenocarcinoma cells CT26. The results showed that EMTF inhibited cell proliferation of SW480 and CT26 by promoting apoptosis. The <em>in vivo</em> results confirmed reduction of tumor.</td>
<td>Remarkable toxic to KB cell</td>
<td>Tang <em>et al.</em> [33]</td>
</tr>
<tr>
<td><em>Melia azedarach</em> L. var. japonica Makino’s</td>
<td>Bark extract</td>
<td>hexane layer</td>
<td>hollow fiber (HF) assay and 28-day repeated toxicity study to confirm the anti-cancer effect and safety of the hexane layer against A549 carcinoma cells KB cell</td>
<td>Hexane layer might have an anti-cancer activity and could improve the toxicity of cisplatin</td>
<td>Kim &amp; Kang [34]</td>
</tr>
<tr>
<td><em>Melia dubia</em></td>
<td>Extract</td>
<td>Silver nanoparticle</td>
<td></td>
<td></td>
<td>Kathiravan, <em>et al.</em> [35]</td>
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<tr>
<td><em>M. azedarach</em></td>
<td>Fruit</td>
<td>MeOH extract of n-hexane defatted, fractionated and isolated</td>
<td>Cytotoxic activities against HL60, A549, AZ521, and SK-BR-3 human cancer cell lines. Meliarachin C (IC₅₀ 0.65 μM) and 3-O-deacetyl-4-O-demethyl-28-oxosalannin (IC₅₀ 2.8 μM) exhibited potent cytotoxic activity against HL60 cells, and was mainly due to the induction of apoptosis</td>
<td>Thirty-one limonoids and one tirucallane-type triterpenoid</td>
<td>Akhihiza et al. [39]</td>
</tr>
<tr>
<td><em>Melia azedarach</em></td>
<td>Mature Fruits</td>
<td>aqueous of MeOH extract of defatted n-hexane extract and partitioned, column chromatograph</td>
<td>Cytotoxicity against leukemia (HL-60) (IC₅₀ μM) and stomach (AZ521) (IC₅₀) cancer cell lines</td>
<td>Toosendanin, a triterpenoid derivative</td>
<td>Zhang et al. [41]</td>
</tr>
<tr>
<td><em>Melia azedarach</em></td>
<td>extract</td>
<td>70% ethanol extract of MA</td>
<td>Melanogenesis of a B16F10 mouse melanoma cell line</td>
<td></td>
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<tr>
<td><em>Melia toosendan</em></td>
<td>Seed</td>
<td>Aqueous of EtOH Soxhletation extract</td>
<td>Volume tumor, AgNOR measurement and Bax protein expression into C3H mice inoculated with adenocarcinoma mammary, axilla i.p.</td>
<td>Tannin, flavonoidsquersetin, saponin. Combination of MA‑Dox‑Cyclo decrease volume, Bax increase and decrease AgNOR</td>
<td>Sumarawati et al. [47]</td>
</tr>
<tr>
<td><em>Melia azedarach</em></td>
<td>Bark</td>
<td>alcohol-chloroform extraction</td>
<td>In vivo and in vitro studies (human hepatocellular carcinoma cell lines SMMC-7721 and Hep3B and BALB/c mice inoculated s.c) mouse hepatocellular carcinoma H22 cells</td>
<td>Toosendanin extract has potent anti-cancer effects via suppressing proliferation and inducing apoptosis of cancer cells in vivo and in vitro. The mechanism of apoptosis involves in mitochondrial pathway and death receptor pathway</td>
<td>Liu et al. [46]</td>
</tr>
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<td>Melia dubia</td>
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</table>

Previous results showed that cytotoxic organic compounds of MA were supposed of flavonoids, triterpenoids (tirucallane), limonoids (meliarachin, melatoxin B1, trichiliin H, and TSN), steroids, and organic acids content compounds.

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**Conflicts of interest**

There are no conflicts of interest.

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