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Diosmetin and tamarixetin (methylated flavonoids): A review on their chemistry, sources, pharmacology, and anticancer properties

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ABSTRACT

This review begins with an introduction to the basic skeleton and classes of flavonoids. Studies on flavonoids have shown that the presence or absence of their functional moieties is associated with enhanced cytotoxicity toward cancer cells. Functional moieties include the C2–C3 double bond, C3 hydroxyl group, and 4-carbonyl group at ring C and the pattern of hydroxylation at ring B. Subsequently, the current knowledge on the chemistry, sources, pharmacology, and anticancer properties of diosmetin (DMT) and tamarixetin (TMT), two lesser-known methylated flavonoids with similar molecular structures, is updated. DMT is a methylated flavone with three hydroxyl groups, while TMT is a methylated flavonol with four hydroxyl groups. Both DMT and TMT display strong cytotoxic effects on cancer cell lines. Studies on the anticancer effects and molecular mechanisms of DMT included leukemia and breast, liver, prostate, lung, melanoma, colon, and renal cancer cells, while those of TMT have only been reported in leukemia and liver cancer cells. These findings suggest that flavones lacking the C3 hydroxyl group at ring C are more cytotoxic than flavonols having the C3 hydroxyl group. The *in vitro* and *in vivo* cytotoxic activities of DMT and TMT against cancer cells involve different molecular targets and signaling pathways. From this study, it is clear that little is known about the pharmacology and anticancer properties of DMT and TMT. The potentials for further research into these aspects of the two lesser-known methylated flavonoids are enormous.

INTRODUCTION

Flavonoids represent the largest family of phenolic secondary metabolites from plants with more than 9,000 compounds reported (Wang *et al*., 2011). They occur in most herbs, fruits, and vegetables (Kopustinskiene *et al*., 2020; Panche *et al*., 2016). These polyphenols have a molecular structure consisting of two benzene rings A and B that are joined by a heterocyclic pyran ring C forming the benzopyrone (C6-C3-C6) moiety (Raffa *et al*., 2017; Singh *et al*., 2014). Rings A and C are composed of the chroman (C6-C3) nucleus (Kanadaswami *et al*., 2005). The basic skeleton along with the functional moieties is shown in Figure 1.

Flavonoids are subdivided into classes including aurones, chalcones, flavonols, flavones, flavanones, flavan-3-ols, anthocyanins, and isoflavones (Kar Mahapatra *et al*., 2015, 2019). The majority of the flavonoids have the B ring linked in position 2 to the C ring (Fig. 1), and they include aurones, chalcones, flavones, flavonols, flavanones, and flavanols (e.g., Guven *et al*., 2019; Panche *et al*., 2016; Raffa *et al*., 2017; Singh *et al*., 2014). Aurones are a subclass of flavones (Boumendjel, 2003), while chalcones are precursors of flavonoids and isoflavones (Kar Mahapatra *et al*., 2015). Flavones (e.g., apigenin and luteolin) have a C2–C3 double bond and a 4-carbonyl group but they lack the C3 hydroxyl group at ring C. Flavonols (e.g., fisetin, quercetin, morin, and myricetin) possess all the three functional moieties (Fig. 1). Flavanones (e.g., naringenin, hesperitin, and taxifolin) lack the C2–C3 double bond, while flavanols (e.g., catechin and epicatechin) lack the C2–C3 double bond and the 4-carbonyl group (Guven *et al*., 2019; Panche *et al*., 2016). Flavonoids in which the B ring is linked at positions 3 and 4 to the C ring are called isoflavones (e.g., genistein and

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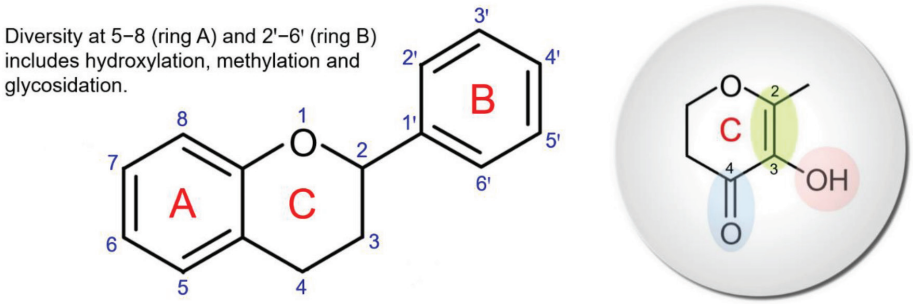

Figure 1. Basic skeleton of flavonoids (left) showing the C2–C3 double bond, 4-carbonyl group, and C3 hydroxyl group of flavonols at ring C (right).

daidzein) and neoflavonoids (e.g., calophyllolide), respectively (Panche *et al*., 2016). In nature, flavonoids occur as aglycones, glycosides, and methylated derivatives.

Flavonoids are endowed with health-promoting properties including nutraceutical, pharmaceutical, and cosmeceutical applications (Panche *et al*., 2016). Pharmacological properties include antioxidant, antimicrobial, antiallergic, anti-inflammatory, anticarcinogenic, and antidiabetic effects (Guven *et al*., 2019; Raffa *et al*., 2017). The medical applications of flavonoids involve protection against cancer and other diseases, such as cardiovascular, rheumatic, obesity, high cholesterol, hypertension, and neurological disorders (Ballard and Junior, 2019; Havsteen, 2002).The anticancer effects of flavonoids operate during the stages of initiation, promotion, and progression of carcinogenesis. In the initiation and promotion stages, flavonoids can inhibit cell proliferation (Abotaleb *et al*., 2019; Ballard and Junior, 2019). At the stage of progression, flavonoids can inhibit proangiogenesis, regulate metastasis, induce cytotoxicity and apoptosis, promote cell cycle arrest, and reverse multidrug resistance (MDR) or a combination of these mechanisms (Abotaleb *et al*., 2019; Chahar *et al*., 2011; Raffa *et al*., 2017). The antitumor activities of flavonoids include the induction of apoptosis, suppression of protein tyrosine kinase activity, antiproliferation, antimetastasis, anti-invasive effects, and antiangiogenesis (Kanadaswami *et al*., 2005). Many studies have provided scientific evidence for the anticancer properties of flavonoids *in vitro* and *in vivo* (Ren *et al*., 2013; Wang, 2000). Flavonoids such as quercetin and flavopiridol are now in phase II human clinical trials for different cancers.

When tested against different cancer cells, cytotoxicity of various classes of flavonoids based on IC_{50} values was ranked as flavones > flavonols > flavanones > isoflavones ~ flavanols (Kuntz *et al*., 1999; Li *et al*., 2008; Plochmann *et al*., 2007; Sak, 2014). Flavones have the strongest cytotoxicity over the other groups of flavonoids due to the presence of the C2–C3 double bond, compared to the 4-carbonyl (4-oxo or 4-keto) group and the C3-hydroxyl group at ring C (Fig. 1). Disparity exists as stronger cytotoxicity has been reported in flavonols than flavones, for example, quercetin > kaempferol > apigenin (Wang *et al*., 2018).

The pattern of hydroxylation in ring B influences the degree of cytotoxicity; for example, the *ortho*-hydroxylated quercetin (3′ and 4′) is three times more cytotoxic than the *meta*-hydroxylated morin (2′ and 4′). Other factors influencing cytotoxicity are *O*-methylation and glucuronidation in the A ring which are associated with enhanced cytotoxicity, while a higher number of hydroxyl residues and solubility are inversely correlated with cytotoxicity (Plochmann *et al*., 2007).

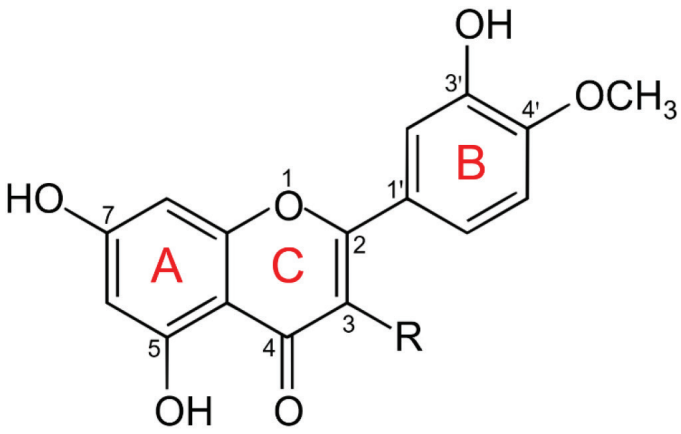

Figure 2. Molecular structures of diosmetin (R = H) (left) and TMT (R = OH) (right).

When tested against five different cancer cell lines, flavonoids can be categorized into those with strong and those with weak *in vitro* cytotoxic effects (Chang *et al*., 2008). Apigenin, luteolin, and fisetin of the strong category are characterized by having two hydroxyl groups in rings AC, while myricetin and morin of the weak category have three hydroxyl groups in rings AC (Fig. 1). Both naringenin and apigenin share the same molecular structure. Naringenin without the 2,3-double bond displayed weak cytotoxic effects suggesting the importance of the double bond between C2 and C3 (Chang *et al*., 2008). Genistein and daidzein are isoflavones in which ring B is attached to ring C at C3 instead of C2.

For polymethylated flavonoids (e.g., natsudaidain), a methoxy group at C8 and a hydroxyl group at C3 are essential for their antiproliferative activity of the flavonoids (Kawaii *et al*., 1999). Isoflavones (e.g., genistein and daidzein) are flavonoids in which the B ring is linked in position 3 of the C ring (Chang *et al*., 2008; Lopez-Lazaro, 2002; Lopez-Lazaro *et al*., 2002). Generally, isoflavones have weaker cytotoxicity than the other flavonoids linked in position 2. In addition, the sugar moiety of flavonoids (e.g., rutin and isoquercetin) reduces their cytotoxic activity (Lopez-Lazaro, 2002; Lopez-Lazaro *et al*., 2002). In flavonoids, the ring B catechol moiety of flavonoids (e.g., 3′,4′-diOH) and the –OMe group at 5′ are beneficial toward their cytotoxicity, while glycosylation at C5 of ring A has adverse effects on cytotoxicity

(Wang *et al*., 2018).This review begins with an introduction to the basic skeleton and different classes of flavonoids. Subsequently, the current knowledge on the chemistry, sources, pharmacology, and anticancer properties of diosmetin (DMT) and tamarixetin (TMT), two lesser-known methylated flavonoids with similar molecular structure, is updated. Sources of information cited were from Google Scholar, PubMed, PubMed Central, Science Direct, J-Stage, PubChem, and Directory of Open Access Journals.

CHEMISTRY AND SOURCES

Diosmetin

DMT (4′-methylluteolin, luteolin 4′-methyl ether or 5,7,3′-trihydroxy-4′-methoxyflavone) is a natural methylated flavone. Its molecular formula is $C_{16}H_{12}O_6$ and its molecular weight is 300 g/mol (Patel *et al*., 2013). DMT has three hydroxyl groups at 5, 7, and 3′ positions (Fig. 2). Being a flavone, the molecule has a C2–C3 double bond and a 4-carbonyl group but lacks the C3 hydroxyl group at ring C. The DMT molecule is structurally similar to that of luteolin with the exception of the 4′-methoxy group in DMT and the 4′-hydroxyl group in luteolin. DMT is an aglycone of diosmin or DMT 7-*O*-rutinoside (Chen *et al*., 2019a).

DMT has been isolated from many plant species. They include the aerial parts of *Soroseris hookeriana* (Hooker's Soroseris) (Meng *et al*., 2000) and *Petroselinum crispum* (parsley) (Yoshikawa *et al*., 2000), *Citrus* fruit juices (Abad-Garcia *et al*., 2014), and flowers of *Chrysanthemum morifolium* (chrysanthemum) (Lin and Harnly, 2010; Xie *et al*., 2009). From the flowers and leaves of *Origanum vulgare* (oregano), the contents of DMT have been reported to be 0.18 and 0.04 DW: mg/g dry weight (Radušienė *et al*., 2008). From the ethyl acetate fraction of the methanol extract of *Eleocharis dulcis* (water chestnut) peel, the content of DMT (30 mg/g) ranked second to that of fisetin (32 mg/g) (Zhan *et al*., 2016). Glycosides of DMT are commonly found in *Citrus* fruit juices, notably those of *Citrus medica* and *Citrus bergamia* (Caristi *et al*., 2006; Hostetler *et al*., 2017).

Tamarixetin

TMT (4′-*O*-methylquercetin, quercetin 4′-methyl ether or 3,5,7,3′-tetrahydroxy-4′-methoxy flavonol) is a natural methylated flavonol with a molecular formula of $C_{16}H_{12}O_7$ and molecular weight of 316 g/mol. TMT has four hydroxyl groups at 3, 5, 7, and 3′ positions (Fig. 2). Being a flavonol, the molecule has a C2–C3 double bond, a 4-carbonyl group, and a C3 hydroxyl group at ring C. It is structurally similar to isorhamnetin (3′-*O*-methylquercetin) and quercetin. TMT has been isolated from the leaves of *Tamarix ramosissima* (salt cedar) (Sultanova *et al*., 2001), *Azadirachta indica* (neem) (Yadav *et al*., 2017), and *Psidium guajava* (guava) (Shao *et al*., 2014).

PHARMACOLOGY

Diosmetin

The anti-inflammatory, antioxidant, and hepatoprotective effects of DMT have been reported (Yang *et al*., 2017). Other pharmacological properties of DMT include antimicrobial (Meng

Table 1. Anticancer effects and molecular mechanisms of diosmetin (DMT).

Cancer cell line and type	Anticancer effect and molecular mechanism of diosmetin (reference)
MDA-MB-468 breast	Inhibits cell proliferation, causes G1 cell cycle arrest, and exerts cytostatic effects *via* CYP1 enzyme-mediated conversion to luteolin (Androutsopoulos *et al*., 2009a)
MCF-7 breast	Inhibits cell proliferation and its cytotoxic effects are dependent on CYP1 enzyme conversion to luteolin (Androutsopoulos *et al*., 2009b)
MDA-MB-231 breast	Exerts antiproliferative and proapoptotic activities *via* cell cycle arrest and the mitochondria-mediated intrinsic apoptotic pathway (Wang *et al*., 2019)
HepG2 liver	Exerts synergistic cytostatic effects and arrest G2/M cell cycle when applied with luteolin *via* CYP1A-catalyzed metabolism, activation of c-jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK), and P53/P21 upregulation (Androutsopoulos and Spandidos, 2013)
HepG2 liver	Induces cell apoptosis by upregulating p53 *via* the transforming growth factorβ (TGF-β) signal pathway (Liu *et al*., 2016a)
SK-HEP-1 liver	Inhibits cell metastasis by downregulating the expression levels of MMP-2 and MMP-9 *via* the protein kinase (PKC)/ mitogen-activated protein kinase (MAPK)/metalloproteinase (MMP) pathways (Liu *et al*., 2016b)
HepG2 liver	Inhibits cell proliferation and induces apoptosis by regulating autophagy *via* the mammalian target of rapamycin (mTOR) pathway (Liu *et al*., 2016c)
HepG2 liver	Triggers apoptosis by activation and inactivation of the p53/Bcl-2 pathway and the Notch3/nuclear factor-kappa B (NF-κB) pathway, respectively (Qiao *et al*., 2016)
HepG2 liver	Inhibits cell proliferation and promotes cell apoptosis and cell cycle arrest by targeting chk2 (Ma and Zhang, 2020)
PC-3 and LNCaP prostate	Suppresses cell proliferation *via* induction of apoptosis and cell cycle arrest (Oak *et al*., 2018)
NSCLC lung	Induces apoptosis by producing reactive oxygen species (ROS) and reducing Nrf2 stability *via* suppression of the PI3K/Akt/ glycogen synthase kinase 3 beta (GSK-3β) pathway (Chen *et al*., 2019b)
B16F10 melanoma	Suppresses tumor progression and metastasis by inducing cell death and inhibiting angiogenesis (Choi *et al*., 2019)
HCT-116 colon	Induces apoptosis, inhibits cell proliferation, and arrest G2/M cell cycle mediated by the membrane death receptor (Koosha *et al*., 2019a)
HCT-116 colon xenograft	Reduces tumor growth in nude mice by downregulation of Bcl-2 and overexpression of Bax (Koosha *et al*., 2019b)
ACHN renal	Induces apoptosis and cytotoxicity by reducing protein kinase B (AKT) phosphorylation *via* p53 upregulation (Qiu *et al*., 2020)
K562 leukemia	Induces apoptosis *via* activation of caspases 8 and 3/7 and the death-inducing cytokine tumor necrosis factor alpha (TNFα) (Roma *et al*., 2019)

Bax = Bcl-2 associated X protein; Bcl-2 = B-cell lymphoma 2; chk2 = checkpoint kinase 2; CYP = cytochrome P450; JNK = c-jun N-terminal kinase; ERK = extracellular signal-regulated kinase; GSK-3β = glycogen synthase kinase 3 beta; MAPK = mitogen-activated protein kinase; MMP = metalloproteinase; mTOR = mammalian target of rapamycin; NF-κB = nuclear factor-kappa B; Nrf2 = nuclear factor erythroid 2–related factor 2; PI3K = phosphoinositide 3-kinase; PKC = protein kinase C; ROS = reactive oxygen species; TGF = transforming growth factor-β; and TNFα = tumor necrosis factor alpha.

et al., 2000), oestrogenic (Yoshikawa *et al*., 2000), neuroprotective (Bhatt and Benzeroual, 2013), drug-drug interaction (Bajraktari and Weiss, 2020), osteoblastic (Hsu and Kuo, 2008), and MDR protein inhibitory (van Zanden *et al*., 2005) activities.

Tamarixetin

TMT displays anti-inflammatory (Lesjak *et al*., 2018; Park *et al*., 2018), cardioprotective (Fan *et al*., 2019; Hayamizu *et al*., 2018), gastroprotective (Yadav *et al*., 2017), and MDR protein inhibitory (van Zanden *et al*., 2005) activities.

ANTICANCER PROPERTIES

Diosmetin

Against Caco-2 and HT-29 and colon cancer cells, the EC_{50} values of DMT were 108 and 204 µM, respectively (Kuntz *et al*., 1999). The cytotoxicity of DMT was 1.2 and 1.8 times weaker than those of luteolin. Against COLO 205 colon cancer cells, the IC_{50} value of DMT (82.9 µM) was slightly stronger than luteolin (96.9 µM) while diosmin (>200 µM) did not show any activity (Xie *et al*., 2009). Against A549 lung cancer cells, the IC_{50} value of DMT (101 μg/mL) was weaker than luteolin (59.6 μg/ml) and fisetin (86.5 μg/ml) (Zhan *et al*., 2016). Besides the structural features of flavonoids, for example, flavones versus flavonols, these results show that cytotoxicity also depends on the type of cancer cells tested. Cytotoxic effects of DMT toward HCT-116 colon cancer cells (3.6 µg/ml) were 14 times more potent than toward CCD-841 normal colon cells (52 µg/ml) (Koosha *et al*., 2019a).

The anticancer effects and molecular mechanisms of DMT toward different cancer cell lines are listed in Table 1. Against MCF-7 and MDA-MB-468 breast cancer cells, DMT inhibits cell proliferation, arrests G1 cell cycle, and exerts enhanced cytotoxic or cytostatic effects *via* CYP1 enzyme-mediated conversion to luteolin (Androutsopolous *et al*., 2009a, 2009b). DMT displays antiproliferative and proapoptotic activities against MDA-MB-231 breast cancer cells *via* cell cycle arrest and the mitochondria-mediated intrinsic apoptotic pathway (Wang *et al*., 2019).

When used in combination against HepG2 liver cancer cells, DMT and luteolin exhibit cytostatic effects and arrest G2/M cell cycle *via* CYP1A-catalyzed metabolism, P53/P21 upregulation, and JNK and ERK activation (Androutsopolous and Spandidos, 2013). When tested with HepG2 and SK-HEP-1 liver cancer cells, DMT induces cell apoptosis by upregulating p53 *via* the TGF-β signal pathway (Liu *et al*., 2016a); inhibits cell metastasis by downregulating the expression levels of MMP-2 and MMP-9 *via* the PKC/MAPK/MMP pathways (Liu *et al*., 2016b); inhibits cell proliferation by inducing apoptosis and by regulating autophagy *via* the mTOR pathway (Liu *et al*., 2016c); triggers apoptosis by activation of the p53/Bcl-2 pathway and inactivation of the Notch3/NF-κB pathway (Qiao *et al*., 2016); suppresses cell proliferation; and enhances cell apoptosis and cell cycle arrest by targeting chk2 (Ma and Zhang, 2020).

The anticancer properties of DMT were studied using other cancer cells such as PC-3 and LNCaP prostate [**1**], NSCLC lung [**2**], B16F10 melanoma [**3**], HCT-116 colon [**4**], ACHN renal [**5**], and K562 leukemia [**6**] cell lines (Table 1). DMT suppresses cell proliferation of [**1**] *via* induction of apoptosis and cell cycle arrest (Oak *et al*., 2018); induces apoptosis of [**2**] by producing ROS and reducing Nrf2 stability *via* suppression of the PI3K/Akt/GSK-3β pathway (Chen *et al*., 2019b); and suppresses tumor progression and metastasis of [**3**] by inducing cell death and inhibiting angiogenesis (Choi *et al*., 2019). DMT promotes apoptosis, inhibits cell proliferation, and arrests G2/M cell cycle of [**4**] mediated by the membrane death receptor (Koosha *et al*., 2019a); reduces tumor growth of [**4**] in nude mice *via* downregulation of Bcl-2 and overexpression of Bax (Koosha *et al*., 2019b); promotes apoptosis and cytotoxicity of [**5**] by reducing AKT phosphorylation *via* p53 upregulation (Qiu *et al*., 2020); and induces apoptosis of [**6**] *via* activation of caspases 8 and 3/7 and the death-inducing cytokine TNFα (Roma *et al*., 2018).

Tamarixetin

Against A549 and HCC44 lung cancer cells, cytotoxicity of TMT was 19.6 and 20.3 µM, respectively (Sak *et al*., 2018). Its cytotoxicity was 3.7 and 5.3 times stronger than that of quercetin. The IC_{50} values of TMT were comparable to those of isorhamnetin (3′-*O*-methyl quercetin) with values of 26.6 and 15.9 µM, respectively. Cytotoxicity of TMT against four different leukemia cell lines, Based on IC50 values, cytotoxicity of TMT against four different leukemia cell lines were 5.5 µM for U937 cells, 7.5 µM for HL-60 cells, 7.5 µM for Molt-3 cells and 24 µM for K562 cells (Nicolini *et al*., 2014). For Molt-3 and HL-60 leukemia cells, IC_{50} values were both 7.5 µM. In a study on the antiproliferative effects of quercetin and catechin metabolites in IC_{50} values, the cytotoxicity of TMT (82 µM) was comparable to quercetin (85 µM) when tested against Caco-2 colon cancer cells (Delgado *et al*., 2014). Against MCF-7 breast and BxPC-3 pancreatic cancer cells, cytotoxicity of TMT was 1.5 and 3.0 times weaker than quercetin, respectively. When tested against AGS gastric, B16F10 melanoma, C6 glioma, and HeLa cervical cancer cells using quercetin, 7-*O*-methylated quercetin, and 3-*O*-methylated quercetin, TMT exhibited the strongest cytotoxic activity (Darsandhari *et al*., 2020).

There are only two studies on the anticancer effects and molecular mechanisms of TMT (Table 2). Against doxorubicin-resistant K562/ADR leukemia cells, TMT inhibits cell proliferation, arrests G2/M cell cycle, and induces apoptosis (Nicolini *et al*., 2014). In another study, the cytotoxicity of TMT toward HepG2 and PLC/PRF/5 liver cancer cells and nude mice tumor xenograft was reported (Xu *et al*., 2019). In liver cancer cells, TMT suppresses cell viability *via* apoptosis, lactate dehydrogenase (LDH) release, caspase-3 activation, ROS accumulation, and decreased mitochondrial membrane potential. In liver tumor xenograft, TMT enhances the expression levels of proapoptotic proteins, including Bax and cleaved caspase-3, and inhibits the expression levels of antiapoptotic proteins. Both *in vitro* and *in vivo* studies showed that TMT significantly suppressed the phosphorylation of ERK and AKT in liver cancer cells and tumors (Xu *et al*., 2019).

Structure–activity relationship (SAR) studies

There are very few structure–activity relationship (SAR) studies on DMT and TMT related to anticancer activities. In a study of the inhibitory effects of MDR proteins 1 (MRP 1), an important mechanism in MDR during cancer treatment, methylated flavonoids are among the best inhibitors with IC_{50} values ranging from 2.7 to 14.3 µM (van Zanden *et al*., 2005). Inhibition at 25

Table 2. Anticancer effects and molecular mechanisms of TMT.

Cancer cell line and type	Anticancer effect and molecular mechanism of TMT (reference)
K562/ADR leukemia	Inhibits cell proliferation in a concentration- and time-dependent manner, induces apoptosis, and arrests G2/M cell cycle (Nicolini *et al*., 2014)
HepG2 and PLC/PRF/5 liver	Suppresses cell viability *via* enhanced cell apoptosis, LDH release, caspase-3 activation, and ROS accumulation and decreases mitochondrial membrane potential (Xu *et al*., 2019). Phosphorylation of ERK and AKT in liver cancer cells is significantly suppressed
Nude mice with HepG2 and PLC/PRF/5 liver xenograft tumor	Enhances the expression levels of proapoptotic proteins (including Bax and cleaved caspase-3) and inhibits the expression levels of antiapoptotic proteins after 14-day administration (Xu *et al*., 2019). Phosphorylation of ERK and AKT in xenograft liver tumors is significantly suppressed

AKT = protein kinase B; Bax = Bcl-2 associated X protein; Bcl = B-cell lymphoma; ERK = extracellular signal-regulated kinase; LDH = lactate dehydrogenase; ROS = reactive oxygen species.

μM and IC_{50} values was 84% and 2.7 μM for DMT and 68% and 7.4 μM for TMT. DMT was the strongest, while TMT ranked third. Values of DMT and TMT were stronger than luteolin and quercetin, suggesting that the 4′-methyl ether moieties of DMT and TMT contribute to their inhibitory effects. In another study on the inhibitory effects of flavonoids on NF-κB signaling in MDA-MB-231 breast cancer cells, DMT (3.7%) displayed stronger inhibition than TMT (2.4%) (Amrutha *et al*., 2014). Inhibitory values of DMT and TMT were stronger than luteolin (3.0%) and much weaker than quercetin (3.7%), respectively.

CONCLUSION

Flavonoids are the largest family of phenolic secondary metabolites from plants. They have a molecular structure consisting of two benzene rings (A and B) joined by a pyran ring (C) forming a benzo-pyrone (C6-C3-C6) moiety. The majority of the flavonoids have the B ring linked in position 2 to the C ring, and they can be further divided into classes such as flavones, flavonols, flavanones, and flavanols. Studies have shown that the presence or absence of some functional moieties is associated with enhanced cytotoxicity toward cancer cells. They include C2–C3 double bond, a 4-carbonyl group, and a C3 hydroxyl group at ring C and the pattern of hydroxylation (*ortho* or *meta*) at ring B.

DMT and TMT are methylated flavonoids. DMT is a methoxyflavone having three hydroxyl groups, while TMT is a methoxyflavonol with four hydroxyl groups. This review on the anticancer properties of DMT and TMT supported the view that flavones without the C3 hydroxyl group are stronger in cytotoxicity against cancer cells than flavonols with the C3 hydroxyl group. However, further investigations are needed to confirm the role of the C3 hydroxyl group in cytotoxicity toward cancer cells.

Further clinical research on DMT and TMT is warranted to evaluate their safety and chemopreventive efficacy when used alone or in combination with other chemotherapy agents. Current knowledge of their pharmacokinetics, bioavailability, and SAR studies is meager. Further research on the structural modifications of DMT and TMT is needed for the synthesis of novel derivatives with enhanced inhibitory effects against different cancer cells and reduced cytotoxicity toward normal cells. For lesser-known bioactive compounds, such as DMT and TMT, their use in purified and standardized extracts containing chemical constituents that have the desired pharmacological activity may be the most practical approach. While Western medicine employs pure and single compounds, Chinese medicine (CM) has long used different combinations of compounds in the form of medicinal herbs to treat, ameliorate, and relieve the symptoms of different diseases. CM may have fewer and less severe side effects than single pure drugs, making them especially attractive to consumers. The development and clinical usage of different formulations of DMT and TMT with synergistic anticancer effects, reduced side effects, and acceptable quality control remain a major challenge. Little is known about the pharmacology and anticancer properties of DMT and TMT. The potentials for further research into these aspects of the two lesser-known methylated flavonoids are enormous. This will generate much research interest among medicinal chemists and researchers who are keen on lesser-known flavonoids.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

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