WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 10, 322-373. Review Article ISSN 2277–7105

ANTICANCER ACTIVITY OF *MORUS* (MULBERRY) SPECIES WITH SPECIAL FOCUS ON MORUSIN

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Article Received on 15 April 2026,
Article Revised on 05 May 2026,
Article Published on 16 May 2026,

https://doi.org/10.5281/zenodo.20200843

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How to cite this Article: Abdullatif Azab*. (2026). Anticancer Activity of Morus (Mulberry) Species with Special Focus on Morusin. World Journal of Pharmaceutical Research, 15(10), 322–373.

ABSTRACT

In our three previous review articles about the *Morus* (mulberry) plant genus, we presented the antidiabetic, brain-related and anti-inflammatory properties-activities of its trees. In this article, anticancer and related activities of this genus will be reviewed. Literature data possess dozens of research articles about this crucial activity, since cancer is one of the major human death causes. Like in our three previous review articles about this genus, *Morus alba* (white mulberry) is the most studied and published. Also, the unique natural products that these plants contain and most of them were previously presented, will be shown in this article. Some of these, reportedly most active, will be presented separately in the discussion section. Species that were not published for anticancer activity will be highlighted in order to raise the need for research. Finally, we will present the few review articles that were published about anticancer activity of the entire *Morus* genus and show their strengths and weaknesses.

KEYWORDS: Anticancer, *Morus*, *Morus alba*, *Morus nigra*, *Morus indica*, flavonoids, Morusin, Kuwanon, Sanggenon, Nanoparticles.

Abbreviations: DCM dichloromethane, DEE diethyl ether, DMSO dimethyl sulfoxide, DNJ 1-Deoxynojirimycin, EC_{50} median effective concentration, EO essential oil, EtOAc ethyl acetate, GCC general chemical composition, HPLC high performance liquid chromatography, LPS lipopolysaccharide, NPs nanoparticles, PE petroleum ether, STZ streptozotocin, TAC total antioxidant capacity, TFC total flavonoid content, TPC total phenolic content.

1. INTRODUCTION

Cancer is the second reason of disease-mortality among humans after cardiovascular diseases: around M10 deaths, globally.[1,2] It is estimated that from 2020 to 2050 this disease, its complications, research and consequences will cost an unbelievable price of 25.2 trillion US dollars.[3] Between 2016 and 2023, cancer research global funding was an enormous number of 51.4 billion US dollars.[4]

The anticancer property of the *Morus* plant genus is extensively studied, and it was documented and published in several review articles. Surprisingly, and to the best of our literature search, no significant review articles about anticancer and related activities of the whole genus were published so far. We found four review articles about the anticancer activity of *Morus alba*[5-8] and one about *Morus nigra*.[9] For this reason, our review article is the most comprehensive.

2. Published Anticancer Activity of *Morus* Species

Morus genus plants were used by humans since the dawn of humanity for many purposes, nutritional, traditional medicine, industry and energy source. However, there is very little documentation of such use for prevention or treatment of cancer in ethnomedicine, where we found only one reliable publication.[10] Contrary to that, modern science has widely investigated the medicinal-biological and other properties-activities of these plants and thousands of articles were published. As for anticancer activity, a summary of these findings is presented as follows: ***Morus* Species; Anticancer and Other Activities, Method, Results, Reference**

Morus alba

Cortex methanolic extract had weak activity against cancer-cells injected mice. Fifteen other plants were tested in this research.[11]

Cortex aqueous extract had moderate activity against K-562, B380 human leukemia cells and B16 mouse melanoma cells. A mechanism of action is proposed.[12]

Anthocyanins-rich fruits methanolic extract was active against B16-F1 melanoma cells. Four compounds were identified in the extract: two derivatives of Cyanidin and two of Pelargonidin (**Figure 1A**).[13]

Leaves were separately extracted with *n*-butanol, methanol, 50% aqueous methanol* and

water. The extracts had significant activity against HepG2 cancer cells. A mechanism of action is proposed. Antioxidant activity and TPC are reported.[14]

* Unless stated otherwise, percentages of solvent mixtures are V/V.
Branches, fruits, leaves ad roots were separately extracted with water, and the resulting extracts were fractionized with *n*-hexane, EtOAc and methanol. The methanolic fractions were tested for anticancer activity against Calu-6 cells. The crude extracts were analyzed for organic acids resulting previously well know compounds and phosphoric acid.[15]

Roots 80% aqueous ethanolic extract had notable activity against multidrug-resistant MCF-7/Dox cancer cells. A mechanism of action is proposed.[16]

Leaves 70% aqueous methanolic extract was active against colon (HCT-15) and breast (MCF-7) human cancer cells. Mechanism of action based on downregulation of nitric oxide produced by inducible NO-synthase. The extract was chromatographed (HPLC) resulting nine previously known phenolics.[17]

Roots 70% aqueous ethanolic extract was active against neuroblastoma (B103) cells. A mechanism of action is proposed.[18]

Leaves 95% aqueous ethanolic extract ameliorated DMBA (7,12-Dimethylbenz(a)anthracene)-induced cancer in *Mesocricetus auratus* (Syrian hamster).[[19], see references]

Leaves aqueous extract inhibited *N*-Nitrosodiethylamine-induced liver cancer in rats.[20]

Leaves and 30% aqueous ethanol were used to prepare flavonoid-rich extract, and this had cytotoxic effect on HeLa cell lines.[21]

Leaves ethanolic extract was active against HCT-16 (colon cancer) cells. Antimicrobial activity is also reported.[22]

Root bark DCM extract was active against H1299, H460, and A549 cell lines. Mechanism of action is proposed, and activity is referred to Morusin (**Figure 1A**) that was detected (HPLC) in the extract.[23]

Leaves methanolic poly phenols-rich extract was active against Doxorubicin-resistant cancer

cells (liver, three types) which was induced by endoplasmic reticulum stress. A mechanism of action is proposed.[24]

Leaves were extracted with 80% aqueous ethanol, and the extract was chromatographed obtaining Isoquercetin and Rutin. The crude extract and the two compounds were tested separately and in combination with Cisplatin, against human gastric cancer cells (IBRCC10071). Results showed clear synergism between natural products and the drug.[25]

Root bark DCM extract was tested against four cell lines (H1299, HCT116, RAW264.7, THP-1) showing significant activity. A mechanism of action is proposed.[26,27,31]

Leaves and branches were separately extracted with 95% aqueous ethanol, and the extracts had moderate activity against four cancer cell lines. A mechanism of action is proposed; antioxidant activity is presented and *Peganum harmala* (highest activity) and *Melia azedarach* were also tested in this research.[28]

Fruits aqueous extract inhibited liver fibrosis in rats via suppression of inflammatory biomarkers. Authors concluded that their results indicate possible use against hepatocarcinogenesis.[29]

Leaves were exposed to γ-radiation then extracted with methanol. The resulting extract had activity against human colorectal HCT116 cancer cells. Grape leaves and seeds extracts were also studied in this research, and antioxidant activity is published.[30]

Leaves powder was administered to mice resulting significant prevention of liver cancer (STZ-induced).[32]

Ripe fruits and young leaves were separately extracted with aqueous solutions of 70% methanol, 60% ethanol and 65% acetone (6 extracts). These extracts were tested against MCF-7 breast cancer cells, resulting clear dependence on extracting solvent.[33]

Leaves and root bark were separately extracted with 80% aqueous methanol and both extracts were tested against U937 human leukemic monocytic cell line: root bark extract was more potent. A mechanism of action is proposed.[34]

Leaves were separately extracted with DCM and ethanol, and both extracts were tested against HuCCA-1, MCF-7 and A-549 cells: DCM extract was more active. Authors also

tested the activity of an active compound present in these extracts: Chlorogenic acid (higher content in ethanolic extract).[35]

Nanoemulsion prepared from fruits and leaves (separately) methanolic extracts had notable activity against MCF-7 cancer cells. Major components of fruits extract were fatty acids, while major components of leaves extract were phytosterols.[36]

Leaves 80% aqueous ethanolic extract had significant activity against HCT-116 cancer cells. Activity was dose and time dependent.[37,38]

Ripe and unripe fruits were separately extracted with 90% aqueous methanol, and both extracts were tested against A549 lung cancer cells: ripe fruits were more effective. GCC, TPC and antioxidant activities are presented.[39]

Fruits anthocyanin-rich methanolic extract was active against *N*-Nitrosodiethylamine-induced hepatocarcinogenesis in rats. Effect was verified using several biomarkers and physical parameters. The major component of the extract was Pelargonidin 3-glucoside. Antioxidant and anti-inflammatory activities are also presented.[40]

Fruits anthocyanin-rich 95% aqueous ethanolic extract was active against thyroid cancer cells: SW1736 and HTh-7. A mechanism of action is proposed.[41]

Root bark was extracted with 75% aqueous ethanol, and the extract was fractionized using several solvents, then chromatographed, affording two new and seven previously known compounds (**Figure 1A**): Alfafuran, Dioxycudraflavone A (new), 5-Hydroxyethyl moracin (new), Licoflavone C, Moracin C, Morusignin L, Morusin, Mulberrofuran G and Sanggenon V. All compounds were tested against human lung cancer cell lines (A549 and NCI-H292), but only Morusin and Mulberrofuran were active. Antidiabetic activity is published.[43]

Three donated prenylated flavonoids, Kuwanon E, Cudraflavone B, and 4'-*O*-Methylkuwanon E (**Figure 1A**), were active against THP-1 cancer cells. Antidiabetic and anti-inflammatory activities are also presented.[44]

Root bark 80% aqueous ethanolic extract was active against RAW264.7 microphages and human colorectal cancer cell line SW480. Anti-inflammatory activity is reported.[45]

Leaves tea and fermented leaves drink were active against HeLa and MCF-7 cancer cells.

Antioxidant activity is reported.[46]

Stem bark methanolic extract was partitioned with several solvents including EtOAc, and this fraction was tested against Ehrlich's ascites carcinoma cell-induced in mice. Effect was measured using several biomarkers. Antioxidant activity and antioxidative parameters are also published.[47]

Stem ethanolic extract was active against WiDr cancer cells. Antioxidant activity and GCC are also reported.[48]

Leaves hot water (95 °C) extract combined with its major component, Neochlorogenic acid (**Figure 1B**), were active against A7r5 cancer cells. Oxidative stress and diabetic conditions were induced by Glucolipotoxicity. A mechanism of action is proposed.[49]

Leaves 95% aqueous ethanolic extract was active against 7,12-dimethylbenz-anthracene-induced carcinoma in the buccal pouch of Syrian hamster, *Mesocricetus auratus*.[50]

Leaves 70% aqueous ethanolic flavonoid-rich extract had synergistic effect with Doxorubicin against HT-29 or A-172 GBM cancer cells.[51,52]

Roots hard wood was successively extracted with *n*-hexane, EtOAc and 70% aqueous acetone. The EtOAc extract was chromatographed affording a new prenylated flavonoid: 7, 2', 4', 6'-Tetrahydoroxy-6-geranylflavanone (**Figure 1B**). This compound was active against liver cancer cells (dRLh84).[53]

Leaves ethanolic extract was analyzed affording nine compounds (**Figure 1B**): Mulberrofuran F1 (new), Mulberrofuran F, Chalcomoracin, Kuwanon J, Morachalcone A, Isobavachalcone, Norartocarpetin, Kuwanon C and 6-Geranylapigenin (Albanin D). The first three compounds were active against A549, Be17402, BGC823, HCT-8 and A2780 cell lines.[54]

Leaves 70% aqueous ethanolic flavonoid-rich extract was active LL2-cells-induced cancer in mice and DNA (calf) repairing activity.[55]

Fruits 70% aqueous ethanolic extract was partitioned several times, with several solvents, then its *n*-butanol fraction was chromatographed affording 3S-(β-D-Glucopyranosyloxy)-2,3-

dihydro-2-oxo-1H-indole-3-acetic acid butyl ester (compound 1), its free acid (**Figure 1B**) and five previously known compounds. Compound 1 had activity against HeLa human cancer cells. A mechanism of action is proposed.[56]

Root bark 80% aqueous ethanolic extract was chromatographed yielding Kuwanon M (**Figure 1C**), where this compound was active against A549 and NCI-H292 cancer cell lines. A mechanism of action is proposed.[57]

Lectin that was isolated from leaves was active against MCF-7 (human breast cancer) and HCT-15 (colon cancer) cells. Effect was measured with several parameters. In the third article, a mechanism of action is proposed.[58,59,60]

Donated 4'-*O*-methylkuwanon E (**Figure 1C**) induced differentiation of THP-1 human monocytic leukemia cells and authors concluded that it is potential cyto-differentiating anticancer agent.[61]

Commercial Morusin was active against DU145, PC3, LNCaP and RWPE-1 cell lines. A mechanism of action is proposed.[62]

Follow-up of previous research using MCF-7, MDA-MB-231, MDA-MB-157, MDA-MB-453 and MCF10A cell lines. A mechanism of action is proposed.[63]

Follow-up of previous research using H1299, H292, and H460 cell lines. A mechanism of action is proposed.[64]

Mulberroside A (**Figure 1C**) was isolated from 95% aqueous ethanolic extract of twigs and branches (Ramulus Mori), and it had positive effect in HepA tumor-bearing mice.[65]

Fruits aqueous extract had synergistic effect with Paclitaxel against TSGH 8301 human bladder cancer cell line. A mechanism of action is proposed.[66]

Root bark was extracted with water, fractionized with ethanol and chromatographed affording three polysaccharides. These were analyzed for monosaccharides composition, structure and tested against SMMC7721 liver cancer cells, where one of them was active.[67]

Cortex was extracted with methanol, partitioned with EtOAc and chromatographed yielding Sanggenon G (**Figure 1C**), which was active against MDA-MB231 cell line.[68]

Twenty-eight (28) commercially available compounds from this species were tested against melanoma cells *in vitro*. Eleven compounds were active, and most potent was Sanggenon C (**Figure 1C**). A mechanism of action is proposed.[69]

Fruits 80% aqueous ethanolic flavonoid-rich extract had positive effect against colorectal tumorigenesis in mice. An extensive anti-inflammatory study is also reported.[70]

Root bark was separately extracted with water and 70% aqueous ethanol. Both extracts were tested and found active against LPS-induced proliferation in splenocytes isolated from mice. Anti-inflammatory and antioxidant activities are reported.[71]

Root bark 70% aqueous ethanolic extract was active against HeLa, HepG2 and NCI-H460 cell lines. Detailed extraction methods, TPC, antioxidant, antihyperlipidemic and three enzymes inhibition, are also reported.[72]

Raw leaves, fermented leaves, roots, branches and fruits were separately extracted with 95% aqueous methanol. The extracts were tested against HCT-116, SNU-601, Calu-6 and MCF-7 cell lines. A range of potency is reported for each extract and each cell line. Antioxidant activity and TPC are reported.[73]

Fruits were extracted with four solvent mixtures each containing 0.1% acetic acid to ensure flavonoid-rich products: water, 75% aqueous methanol/ethanol/acetone. The four extracts were tested against proliferation of HepG2 cells, resulting EC_{50} (mg/mL) 56.8, 37.8, 34.75 and 28.3, respectively.[74]

Leaves were separately extracted with water, methanol and 50% aqueous methanol. The extracts were tested against human hepatocellular carcinoma HepG2 cell line. Effect was measured with several parameters, especially cell viability, where 100% methanolic extract was most effective.[75]

Leaves were extracted with 95% aqueous ethanol, partitioned with EtOAc and methanol, and the methanolic fraction was chromatographed affording 13 compounds, one of them was new: (*2S*)-4′-Hydroxy-7-methoxy-8-prenylflavan (**Figure 1C**). All thirteen compounds were tested for inhibition of proliferation and differentiation of 3T3-L1 preadipocytes. Three known compounds were active: 2′,7-Dihydroxy-4′-methoxy-8-prenylflavan, Morachalcone B (**Figure 1C**) and Isobavachalcone.[76]

Leaves 80% aqueous methanolic extract showed highest activity among four tested plants, against P19 embryonal carcinoma cells.[77]

Leaves were separately extracted with water, 80% aqueous methanol and 70% aqueous ethanol. These extracts were tested against human breast cancer cell lines, MCF-7 and MDA-MB-231, where the three extracts had almost equal average activities. The antioxidant activity of the extracts is also published. The ethanolic extract was partially analyzed for chemical composition yielding eight known compounds including 1-Deoxynojirimycin (1-DNJ, **Figure 1C**).[78]

Root bark 75% aqueous ethanolic extract was partitioned with several solvents including *n*-butanol. This fraction was chromatographed affording 5,2'-dihydroxyflavanone-7,4'-di-*O*-*β*-*D*-glucoside (**Figure 1C**). This compound was active against HO-8910 cells.[79]

Root bark 80% aqueous ethanolic extract and its component Ursolic acid (**Figure 1D**) were active against multiple myeloma cells (RPMI8226, HEK293, Wnt3a-L). A mechanism of action is proposed.[80]

Cortex ethanolic extract was partitioned with EtOAc and water, and the organic fraction was analyzed affording Sanggenon C. This compound decreased tumor cells (H22, P388, K562) viability. A mechanism of action is proposed.[81]

Fruits aqueous extract was active against RAW264.7 and CT26 cell lines, where the effect was verified using several parameters. A mechanism of action is proposed.[82]

Leaves methanolic extract was partitioned with *n*-hexane and EtOAc, and the EtOAc fraction was chromatographed yielding eleven compounds where 3′-Geranyl-3-prenyl-2′,4′,5,7-tetrahydroxyflavone (**Figure 1D**) was new. All compounds were tested against three human carcinoma cells (HeLa, MCF-7, Hep3B) but only Morusin was active.[83]

Root bark methanolic extract was fractionized with water and EtOAc, and the EtOAc fraction was analyzed affording 15 compounds where Soroceal B and Sanggenol Q (**Figure 1D**) were new. All fifteen compounds were tested against HL-60, Hela, HepG-2, A-549 and AGS cell lines. The active compounds: Soroceal B, Sanggenol, Morusinol (**Figure 1D**); in addition to Mulberrofuran G, Mongolicin (not indicated which), Licoflavone C, Morusin and 3′-Geranyl-3-prenyl-2′,4′,5,7-tetrahydroxyflavone. Anti-inflammatory activity is reported.[84]

Leaves 95% aqueous ethanolic extracts was analyzed yielding Morachalcone C (**Figure 1D**) and Morachalcone B. Both compounds had moderate activity against HCT-8 and BGC823 human cancer cell lines.[85]

Root bark methanolic extract was fractionized with several solvents including EtOAc, and this fraction was chromatographed affording Albanol A and Mulberrofuran Q (**Figure 1D**). Albanol A was more active against human leukemia (HL60) and human melanoma (CRL1579) cell lines than Mulberrofuran Q.[86]

Root bark methanolic extract was fractionized with several solvents including EtOAc, and this fraction was chromatographed affording Albanol B (**Figure 1D**). This compound was active against human lung cancer cell lines (A549, BZR, H1975, H226) and tumor growth in Ex-3LL (Lewis lung carcinoma) tumor-bearing mice. A mechanism of action is proposed.[87]

Cyanidin-3-glucoside was isolated from fruits had inhibition effect on human breast cancer cell line, MDA-MB-453 inoculated in mice. A mechanism of action is proposed.[88]

Feeding mice 1-DNJ prevented Azoxymethane-induced colorectal cancer in mice. Effect was measured using several parameters.[89]

Donated Guangsangon E (**Figure 1E**) isolated from leaves, inhibited cell proliferation and induces apoptosis in A549 and CNE1 cells and tumor in mice after injection these cells in the animals. A mechanism of action is proposed.[90]

Commercial Kuwanon A (**Figure 1E**) had synergistic action with 5-Fluorouracil against gastric cancer cells (MKN-45, SGC-7901, HGC-27, BGC-823) and against gastric cancer in mice. Effect was measured with several biomarkers, and a mechanism of action is proposed.[91]

Commercial Kuwanon C was more active against HeLa cancer cells, compared with other natural products (commercial) found in different parts of this tree, and were used in this study. Effect was measured with several biomarkers, and a mechanism of action is proposed.[92]

Morusin was isolated from the methanolic extract of root bark, inhibited 12-*O*-tetradecanoyLphorbol-13-acetate- (TPA) and 7,12-Dimethylbenz(*a*)anthracene-induced

carcinogenesis in mice. A mechanism of action is proposed.[93]

Morusin was isolated from the ethanolic extract of branches bark (*Ramulus mori*), inhibited transplanted H_{22} hepatocarcinoma in mice.[94]

Morusin was isolated from the ethanolic extract of branches bark (*Ramulus mori*), inhibited Bel-7402 cells. A mechanism of action is proposed.[95]

Commercial Morusin was active against human hepatocarcinoma cells (HepG2, Hep3B) *in vitro*, and transplanting them in mice, *in vivo*. A mechanism of action is proposed.[96]

Commercial Morusin suppressed A549 cancer cells. A mechanism of action is proposed.[97]

Commercial Morusin inhibited human hepatocellular cancer cell lines Hep3B and Huh7. A mechanism of action is proposed.[98]

Commercial Morusin had *in vitro* activity (against MCF-10A, MCF-7, MDA-MB-231 cancer cells), and *in vivo*, against MCF-7 cancer cells-induced tumor in mice. A mechanism of action is proposed.[99]

Commercial Morusin inhibited prostate cancer cells: PC-3 and 22Rv1. A mechanism of action is proposed.[100]

Commercial Morusinol was active against A375, 293FT and MV3 cells. A mechanism of action is proposed.[101,122]

Commercial Mulberroside A inhibited A498 kidney cancer cells. A mechanism of action is proposed.[102]

Commercial Sanggenon C inhibited the growth and proliferation of glioblastoma cells. A mechanism of action is proposed.[103]

Commercial Sanggenon C had *in vitro* activity against A549 lung cancer cells, *in vivo* against tumor induced by the same cells in mice. Molecular docking was conducted. A mechanism of action is proposed.[104]

Commercial Sanggenon O (**Figure 1E**) inhibited A549 lung cancer cells and a dual action mechanism is proposed.[105]

Root bark methanolic extract was partitioned with chloroform and chromatographed yielding two new compounds Moracin P and Moracin Q, in addition to Moracin O, Moracin M, Mulberrofuran D, Mulberrofuran H, Mulberrofuran W and Albafuran A (**Figure 1E**); and Mulberrofuran G, Sanggenon O, Sanggenon C. Seven of these compounds, including the new ones, were active against Hep3B carcinoma cells. A mechanism of action is proposed.[106]

Leaves methanolic extract had weak activity against LNCaP and DU145 prostate cancer cell lines, while stems methanolic extract had significant activity against LNCaP cells and weak activity against DU145 cells.[107]

Leaves were sequentially extracted with PE, chloroform and methanol. The methanolic extract was tested against Ehrlich ascites tumor in mice, showing significant activity. GCCs of the extract were also determined.[108]

Leaves were sequentially extracted with PE, chloroform and methanol, and each extract was fractionated with several solvents. The methanolic extract was tested against MCF7 and 3T3 cancer cell lines showing significant effect. This extract was chromatographed affording Cathafuran B (**Figure 1E**), Ursolic acid and Moracin M. These compounds were also tested against the same cancer cells resulting similar effect to the methanolic extract. TFC, TPC and antioxidant activity are also reported.[109]

Leaves aqueous ethanolic extract (ratio is not indicated) with/without same extract of *M. nigra* were tested for antimutagenic activity in γ-rays irradiated rats and roots of *Vicia faba*, resulting significant positive effect in both cases.[110]

Fresh fruits 70% aqueous ethanolic extract was tested against HepG2, MCF7, HCT116 and PC3 cancer cell lines, showing moderate to significant effect. The extract was fractionized with *n*-hexane, DCM and EtOAc, and these fractions were chromatographed yielding known compounds, mainly phytosterols and fatty acids. Antioxidant activity of the crude extract is also reported.[111]

Morus atropurpurea

No published anticancer and its related activities.

Morus australis

Morusin was isolated from roots ethanolic extract and was tested against TPA-induced cancer

in JB6 cells, and after their implantation in mice skin. Antioxidant activity is reported and a mechanism of action is proposed.[112]

Stem bark 95% aqueous ethanolic extract was partitioned with PE, $CHCl_3$, EtOAc, acetone, and methanol, successively. The EtOAc fraction was chromatographed yielding three new compounds Australisines A–C, in addition to Mulberrofuran E, Mulberrofuran J, Mongolicin C, Kuwanon G (**Figure 2**), Mulberrofuran F, Mulberrofuran G, Mulberrofuran Q, Chalcomoracin. Australisines A–C, Mulberrofuran G, Mongolicin C, and Chalcomoracin showed moderate cytotoxic activities against five human cancer cell lines (A549, Bel 7402, BGC 823, HCT-8, A2780).[113]

Morus bombycis

Root bark methanolic extract was partitioned with chloroform and chromatographed yielding Kuwanon Q, Kuwanon R, Kuwanon V (**Figure 3**) and Kuwanon J. These compounds were tested against Hep3B carcinoma cells, and only Kuwanon J was active. A mechanism of action is proposed.[106]

Leaves methanolic extract had weak activity against LNCaP and DU145 prostate cancer cell lines.[107]

Root bark methanolic extract was analyzed affording Kuwanon H (**Figure 3**) and Kuwanon G. These compounds inhibited specific binding of gastrin-releasing peptide (GRP) to GRP-preferring receptors in murine Swiss 3T3 fibroblasts.[114]

Morus cathayana

Leaves methanolic extract had weak activity against LNCaP and no activity against DU145 prostate cancer cell lines. In the article, this species is referred to as *Morus tiliaefolia*.[107]

Root bark 95% aqueous ethanolic extract was partitioned with $CHCl_3$ and EtOAc successively. The EtOAc fraction was chromatographed yielding two new compounds Cathayanons A and B (**Figure 4**). These compounds inhibited human leukemia HL-60 cells.[115]

Morus celtidifolia

Leaves were successively extracted with *n*-hexane and methanol, and the resulting extracts were tested against MCF-7 and HeLa cancer cell lines, showing moderate activities.

Antimicrobial and antioxidant activities are also reported.[116]

Morus ihou

No published anticancer and its related activities.

Morus indica

Leaves were successively extracted with PE, benzene, EtOAc, acetone, methanol and water. The methanolic extract was active against TPA- and DMBA-induced two-stage cancer in mice skin (*in vivo*). *In vitro* tests included inhibition of the activity and level of aryl hydrocarbon hydroxylase. The effect was measured using several biomarkers and a mechanism of action is proposed.[117]

Leaves of two varieties were separately extracted with ethanol, methanol and water. The extracts were tested against HT-29 cancer cells showing moderate effects. GCCs and antioxidant activities are also reported.[118]

Morus isingnis

No published anticancer and its related activities.

Morus japonica

No published anticancer and its related activities.

Morus laevigata

Leaves were sequentially extracted with PE, chloroform and methanol. The methanolic extract was tested against Ehrlich ascites tumor in mice, showing significant activity. GCCs of the extract were also determined.[108]

Leaves were sequentially extracted with PE, chloroform and methanol, and each extract was fractionated with several solvents. The methanolic extract was tested against MCF7 and 3T3 cancer cell lines showing significant effect. TFC, TPC and antioxidant activity are also reported.[109]

Morus latifolia

Bark and leaves were separately extracted with methanol, and the resulting extract were active against Ehrlich's ascites carcinoma in mice. The effect was measured using several parameters. Antioxidant activity is also reported.[119]

Morus liboensis

No published anticancer and its related activities.

Morus macroura

Cultured hairy roots methanolic extract was chromatographed yielding three Diels-Alder type adducts: Sorocein I (**Figure 5**), Chalcomoracin and Guangsangon E. The three compounds were tested against P-388 murine leukemia cell line, but only Guangsangon E was active.[120]

Fruits, leaves and stems were separately extracted with 80% aqueous ethanol, and the resulting extracts were partitioned with PE, DCM and EtOAc. The EtOAc fractions were analyzed affording seven previously known phenolics. These were tested against HepG-2, MCF-7, and HeLa cancer cell lines, where kaempferol-3-O-β-glucoside and quercetin-3-O-β-glucoside were found potent. Molecular docking was performed, and neuroprotective activity is also reported.[121]

Morus mesozygia

Stem bark methanolic extract was fractionated and chromatographed affording Artocarpesin, Artochamin C, Kushenol E and Moracin L (**Figure 6**), in addition to Moracin M, Moracin C and Mulberrofuran F. The crude extract and the seven isolated compounds were active against MCF-7 cancer cells, where Moracin L was most active. Structure activity relationship and antiparasitic activity are presented.[123]

Morus mongolica

Molecular docking was performed for four previously known phenolics that were isolated from this species, revealing potential of DEK oncoprotein inhibition activity.[124]

Root bark 95% aqueous ethanolic extract was partitioned with DEE and chromatographed yielding six new (2001) compounds, Sanggenol L, Sanggenol M, Mulberrofuran X, Mulberrofuran Y, Mulberrofuran Z (**Figure 7**), Mulberrofuran W, along with 10 known flavonoids. All compounds were tested and found against human oral tumor cell lines (HSC-2 and HSG), ranging from moderate to high activities.[125]

Morus multicaulis

No published anticancer and its related activities.

Morus nigra

Leaves aqueous ethanolic extract (ratio is not indicated) with/without same extract of *M. alba* were tested for antimutagenic activity in γ-rays irradiated rats and roots of *Vicia faba*, resulting significant positive effect in both cases.[110]

Leaves *n*-hexane and 70% aqueous methanolic extract were active against human cervical cancer cell line (HeLa).[126]

Fresh and dry fruits were separately extracted with 70% aqueous ethanol. The extracts was active against human breast cancer cell line (MCF-7), where the extract of the fresh fruits was more active. The effect was measured using several parameters.[127]

Fruits 80% aqueous methanolic extract was chromatographed affording leptin Morniga G and one of its components Chalcone 4 hydrate (**Figure 8**). The crude extract and its isolated ingredients were active against human colorectal adenocarcinoma cells, HT-29. The effect was measured using several parameters.[128]

Fruits juice had protective activity against genomic changes in human lymphocytes. Juice was analyzed for phenolics yielding 14 known compounds. TAC is also reported.[129]

Eleven new genotypes fruits were extracted with ethanol, and the extracts were active against MCF-7 cancer cell line. The fruits were analyzed for several general components, and their antioxidant capacities were determined. Same analysis was conducted for heartwood.[130]

Fruits 50% aqueous ethanolic anthocyanin-rich prolonged life of Diethylnitrosamine-induced hepatocellular carcinoma rats. The effect was measured using several parameters. The major components of the extract were (mg/g): Peonidin-3-glucoside 20.3 (**Figure 8**), Cyanidin-3-glucoside 14.7, Malvidin-3-glucoside 14.1 and Delphinidin-3-glucoside 11.3.[131]

Fruits juice was active against human breast cancer (MDA-MB-231) and prostate cancer (PC3) cell lines, in a dose dependant manner.[132]

Sour fruits 80% aqueous ethanolic extract was active against human colon cancer HT-29 cells. A mechanism of action is proposed. Antioxidant activity is reported.[133]

Leaves of *Morus nigra* and *Ocimum basilicum* and their mixture were separately extracted using *n*-hexane, chloroform, EtOAc and methanol. The extracts (12) were tested against

MDA-MB-231, MCF-7, HepG2, Huh-7, LoVo and HCT116 cancer cell lines. Results showed clear advantage of the combination and synergistic effect. The antioxidant activity of the combined extracts and their partial chemical compositions are also reported.[134]

Fruits were separately extracted with water and 75% aqueous ethanol, then the resulting extract were treated with high temperature for solvent removal. This resulted in decrease of anthocyanin but increase of TPC. Activity of HCT-116 cells inhibition was higher for heated extracts. Antioxidant activity is also reported.[135]

Fruits 95% aqueous ethanolic extract was analyzed resulting the isolation of twenty compounds, including two new: Moranigrine A and Morusamine (**Figure 8**). All isolated compounds were tested for inhibition of 3-Phosphoglycerate dehydrogenase, where Methyl caffeate (**Figure 8**) exhibited effective inhibition effect. Molecular docking is reported.[136]

Exosome-like lipid nanoparticles were isolated from leaves, and they were rich in glycolipids, functional proteins, and active small active molecules. The were tested *in vitro* against HepG2, CT-26, Hepa1-6, 4T1, A549, L929, and MC3T3-E1 cells; and *in vivo* against Diethylnitrosamine-induced cancer in mice. A mechanism of action is proposed.[137]

Fruits DMSO extract was active against human prostate adenocarcinoma (PC-3) cells. TPC and antioxidant activity of the extract are reported.[138]

Fruits anthocyanin-rich methanolic extract was active against HeLa and A2780 cancer cell lines. The phenolics composition of the extract is presented.[42]

Stem bark methanolic was partitioned with several solvents including EtOAc, and this fraction was chromatographed affording 15 compounds: 2',3,4',5,5'-Pentahydroxy-*cis*-stilbene, Resveratrol, Oxyresveratrol, Albafuran C, 3-Acetyl-*O*-α-amyrin, 3-Acetyl-*O*-β-amyrin and Uvaol (**Figure 8**), in addition to Norartocarpetin, Kuwanon C, Morusin, Cudraflavone A, Kuwanon G, Mulberrofuran G, Ursolic acid-3-O-acetate. Five derivatives (methylated) were prepared from the first listed three compounds, and all 19 compounds were tested against HepG2 and MCF-7 cell lines. One of these derivatives (shown in **Figure 8** as "Active Derivative") and Kuwanon C showed highest activities.[139]

Heartwood methanolic was partitioned with several solvents including EtOAc, and this fraction was chromatographed affording two compounds that were reported for the first time

from this species: Norartocarpanone and Euchrenone a7 (**Figure 8**). Both compounds were tested against P-388 leukemia cells, where Euchrenone a7 was more cytotoxic than Norartocarpanone, and their IC_{50} were 7.8 and 12.7 µg/mL respectively.[140]

Morus notabilis

No published anticancer and its related activities.

Morus papyrifera

No published anticancer and its related activities.

Morus rotunbiloba

Leaves EO (hydrodistillation) had activity against Hep2 and SW620 cell lines. The reprted major component of this EO (10.48%) is Benzyl alcohol. Antimicrobial and antiviral activities are also reported.[141]

Leaves hot aqueous extract was fractionized with DEE and this fraction was analyzed yielding six identified compounds, where the three major components were (**Figure 9**, %): Tannic acid 37.9, Epigallocatechin-3-*O*-gallate 21.1 and Caffeic acid 11.2. The DEE fraction was active against mutagenic *Salmonella typhimurium* strain TA 98 induced by a mutagen Trp-P-1. Antiviral activity is also reported.[142]

Morus rubra

Fresh fruits 70% aqueous ethanolic extract was tested against HepG2, MCF7, HCT116 and PC3 cancer cell lines, showing moderate to significant effect. The extract was fractionized with *n*-hexane, DCM and EtOAc, and these fractions were chromatographed yielding known compounds, mainly phytosterols and fatty acids. Antioxidant activity of the crude extract is also reported.[111]

Fruits DMSO extract was active against human colon cancer (WiDr) cells. The effect was measured using several parameters and a mechanism of action is proposed.[143]

Phenolics-rich fruits DMSO extract was active against human prostate (PC-3) and lung (A549) cancer cells, with human normal foreskin fibroblast (CRL-2522) cells and Cisplatin as references. The extract was analyzed resulting the isolation of seven known phenolics. TPC and antioxidant capacity are also reported.[144]

Morus serrata

Leaves were sequentially extracted with PE, chloroform and methanol, and each extract was fractionated with several solvents. The methanolic extract was tested against MCF7 and 3T3 cancer cell lines showing significant effect. TFC, TPC and antioxidant activity are also reported.[109]

Morus trilobata

No published anticancer and its related activities.

Morus wittiorum

Stem bark 95% aqueous ethanolic extract was fractionized with PE, $CHCl_3$ and EtOAc, and this fraction was chromatographed affording nine known compounds: 4'-Prenyloxyresveratrol (**Figure 10A**) Quercetin, 5,7,3',4'-Tetrahydroxy-3-methoxyflavone, Norartocarpanone, Dihydrokaempferol, Euchrenone a7, Morachalcone A, Resveratrol and Oxyresveratrol. These compounds were tested against human ovarian cancer (A2780) and human gastric cancer (BGC-823), where two of them were active. Anti-inflammatory activity is also reported.[145]

Follow up of previous research yielded three new compounds: Wittifuran H, Wittifuran I and Wittifuran U (**Figure 10A**). These compounds were tested against human gastric cancer cell line BGC-823, but only Wittifuran I was active. Anti-inflammatory activity is reported.[146]

Another study by the same group of previous two publications with the same isolation methods. In this study, four new compounds were isolated, Wittiorumin G, Wittifurans P–R, along with Sorocein A, Mulberrofuran O (**Figure 10A**) Albafuran C, Mulberrofuran E, Mulberrofuran F. These compounds were tested and some of them were active against five human cancer cell lines (A549, Bel-7402, BGC-823, HCT-8, A2780). Antioxidant activity is reported.[147]

Fourth research by the same group using same methods resulted the isolation of Wittifurans O, N, K and L (**Figure 10B**). These compounds were tested against human ovarian cancer cell line A2780, but only the first two were active.[148]

Morus yunnanensis

No published anticancer and its related activities.

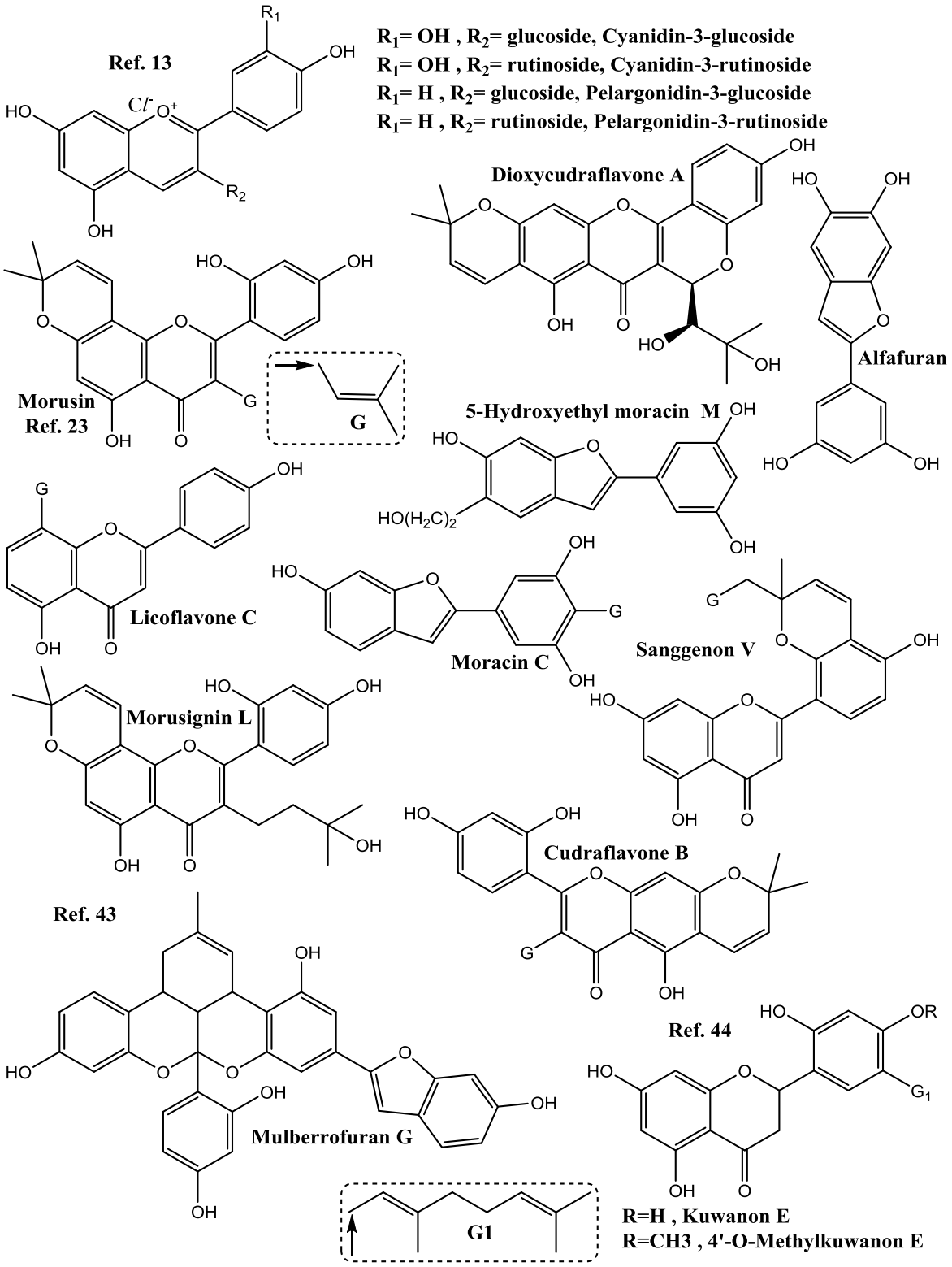

Figure 1AL Natural products isolated from *Morus alba.*

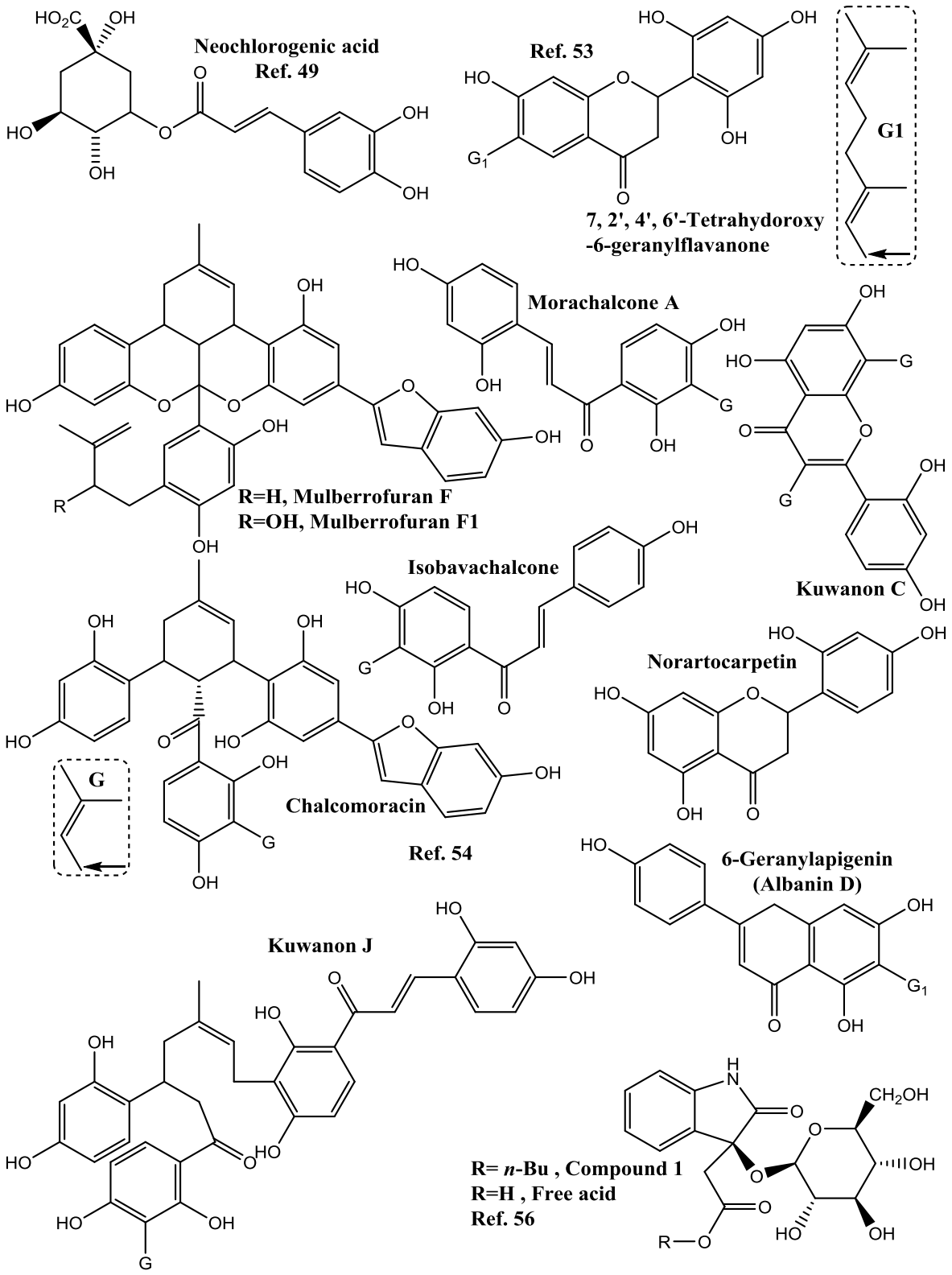

Figure 1B: Natural products isolated from *Morus alba.*

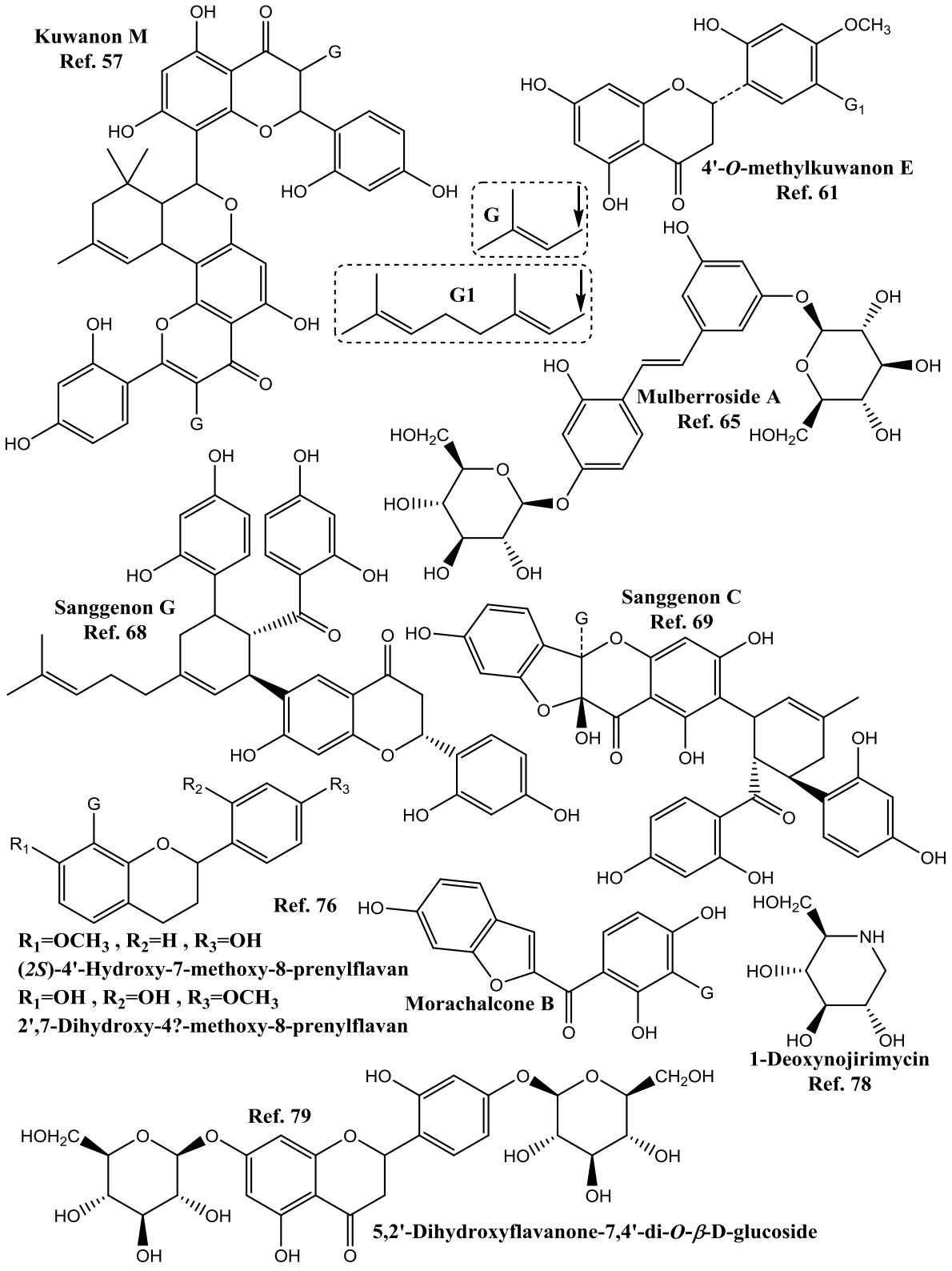

Figure 1C: Natural products isolated from *Morus alba.*

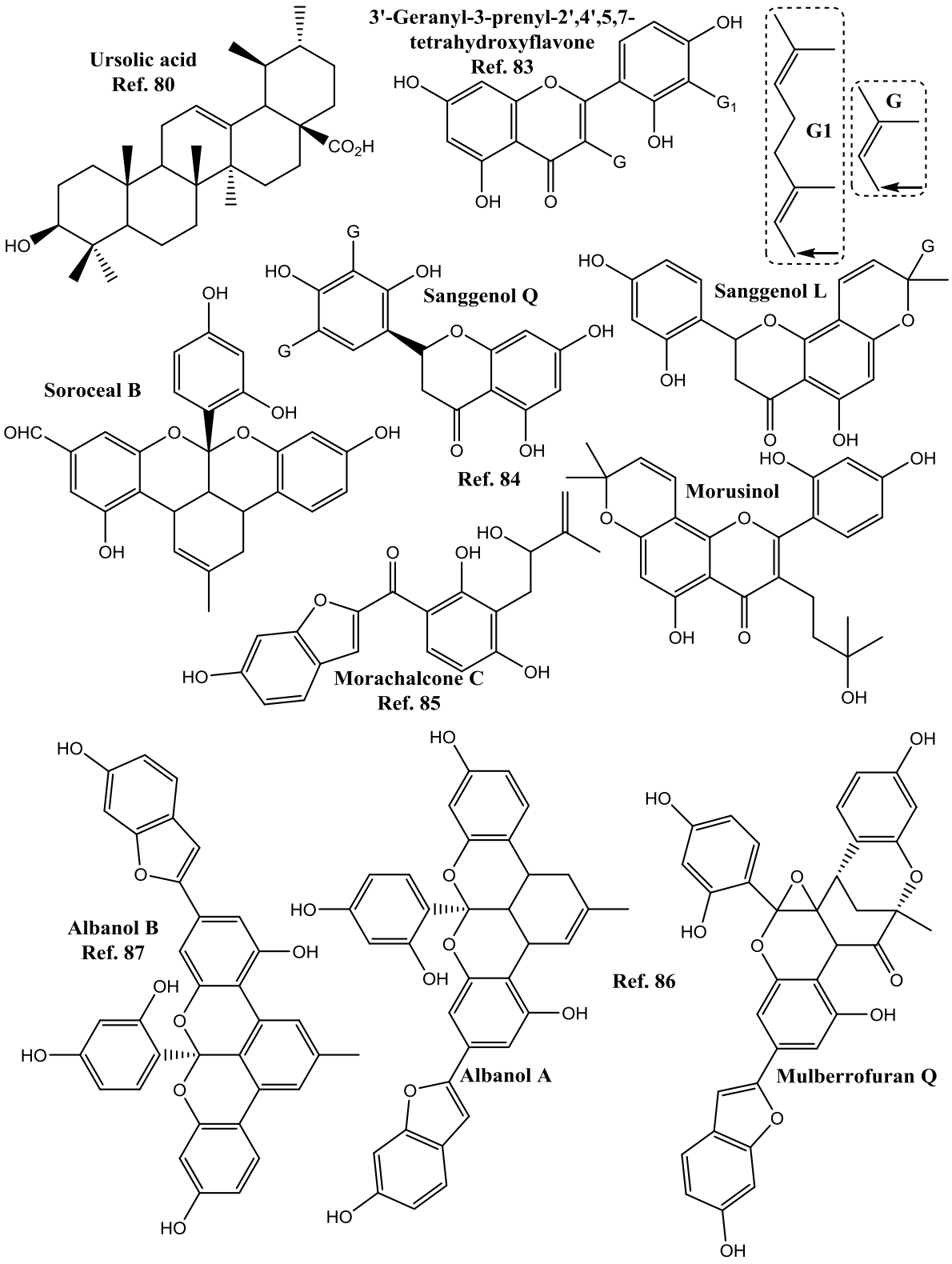

Figure 1D: Natural products isolated from *Morus alba.*

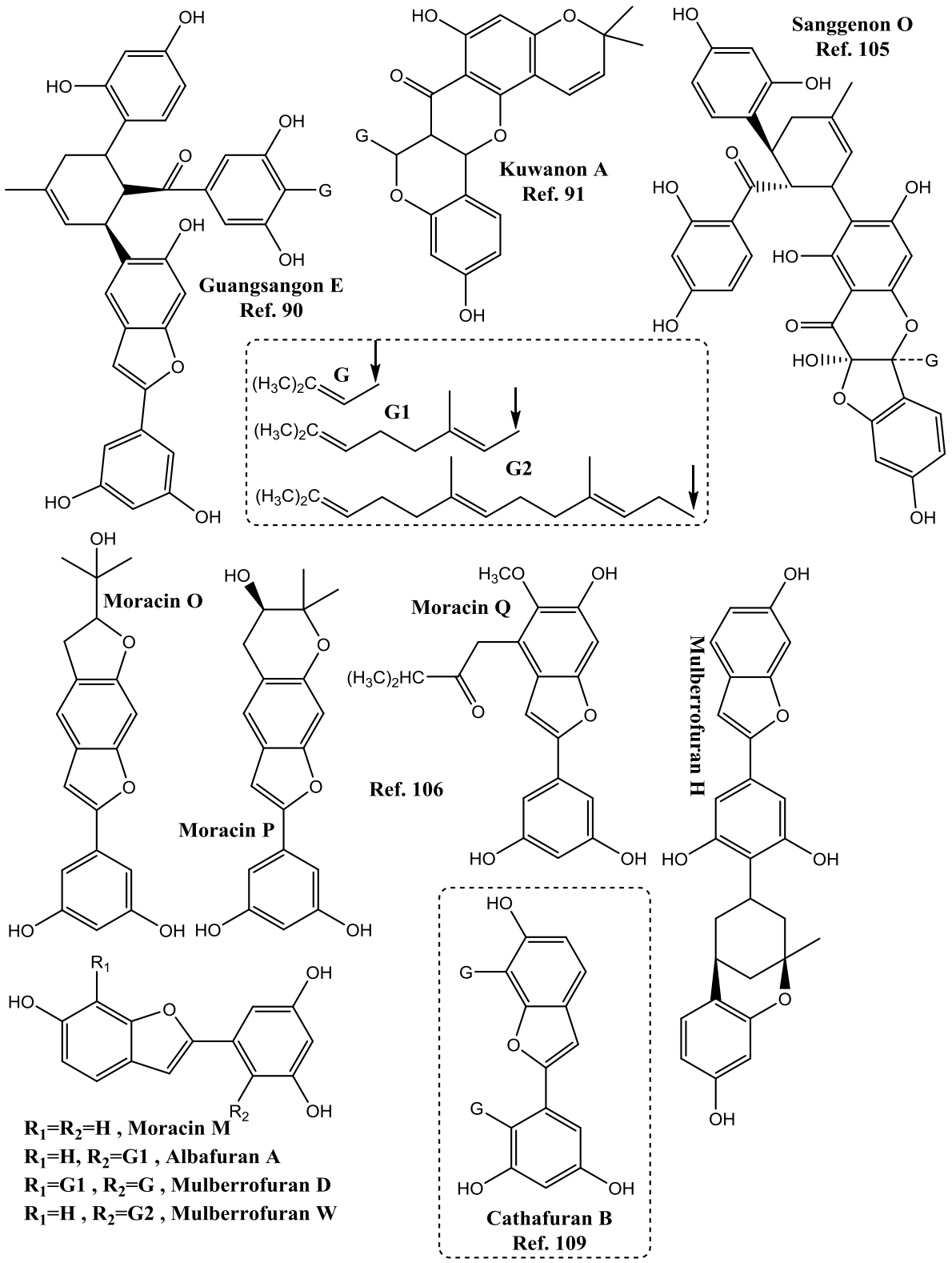

Figure 1E: Natural products isolated from *Morus alba.*

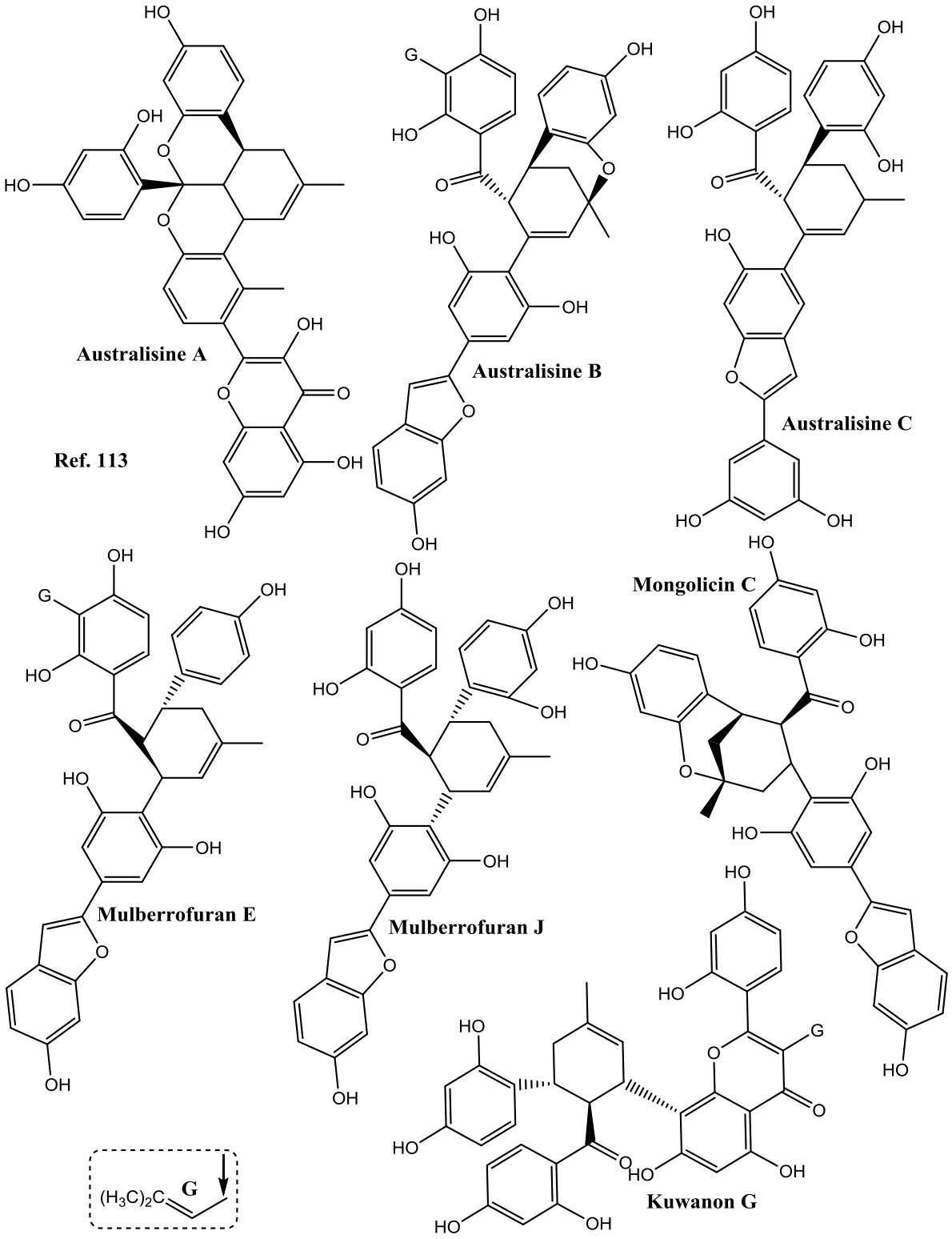

Figure 2: Natural products isolated from *Morus australis.*

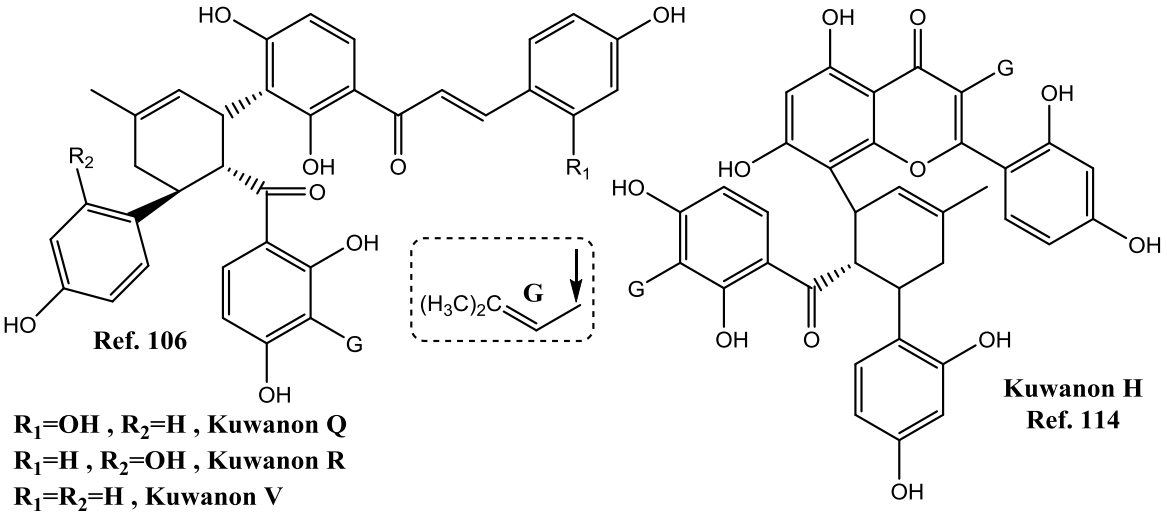

Figure 3: Natural products isolated from *Morus bombycis.*

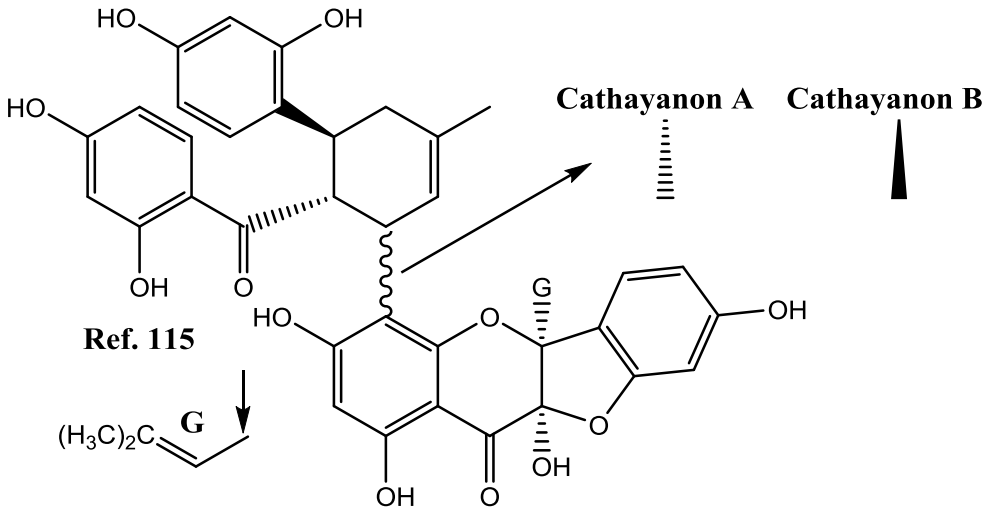

Figure 4: Natural products isolated from *Morus cathayana.*

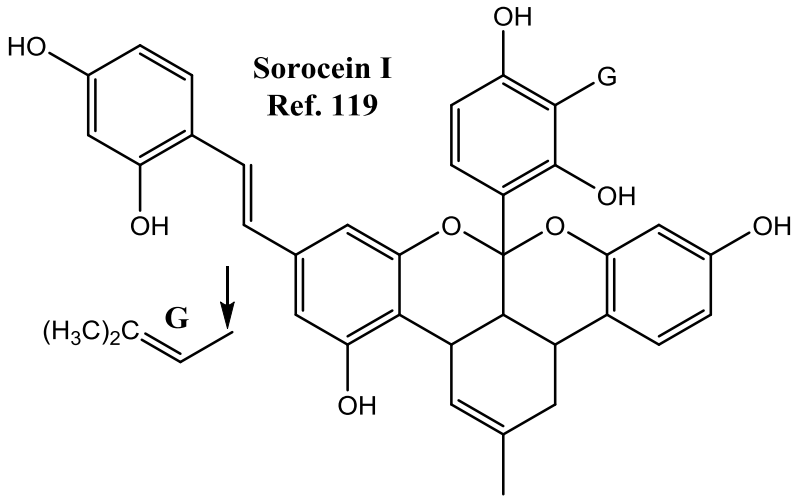

Figure 5: Natural products isolated from *Morus macroura.*

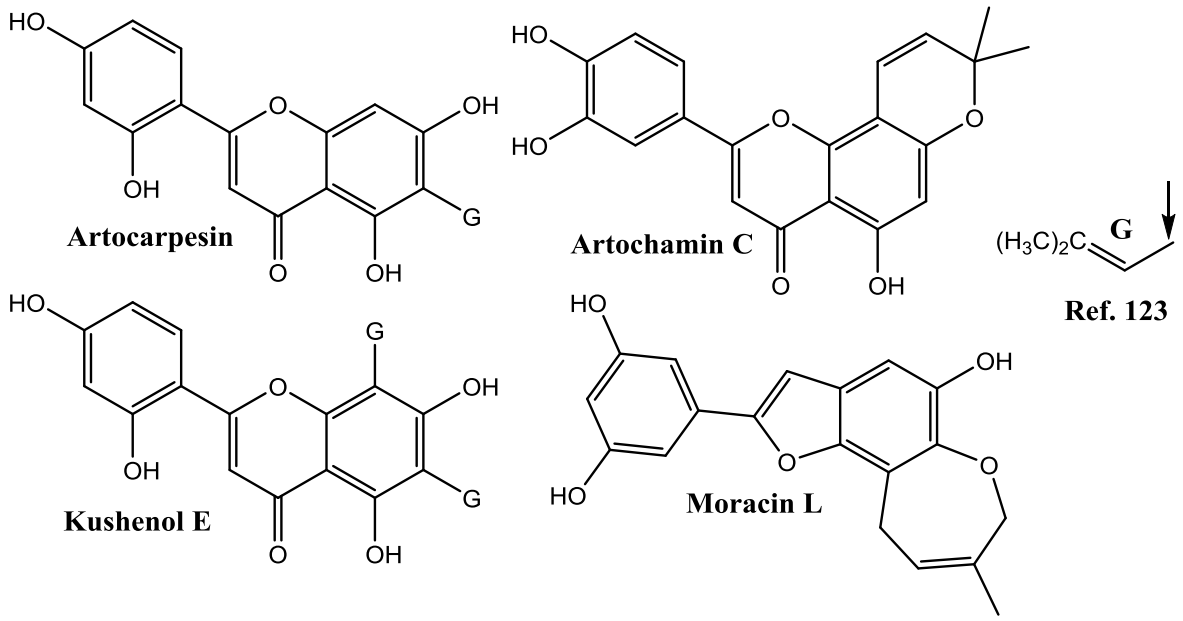

Figure 6: Natural products isolated from *Morus.*

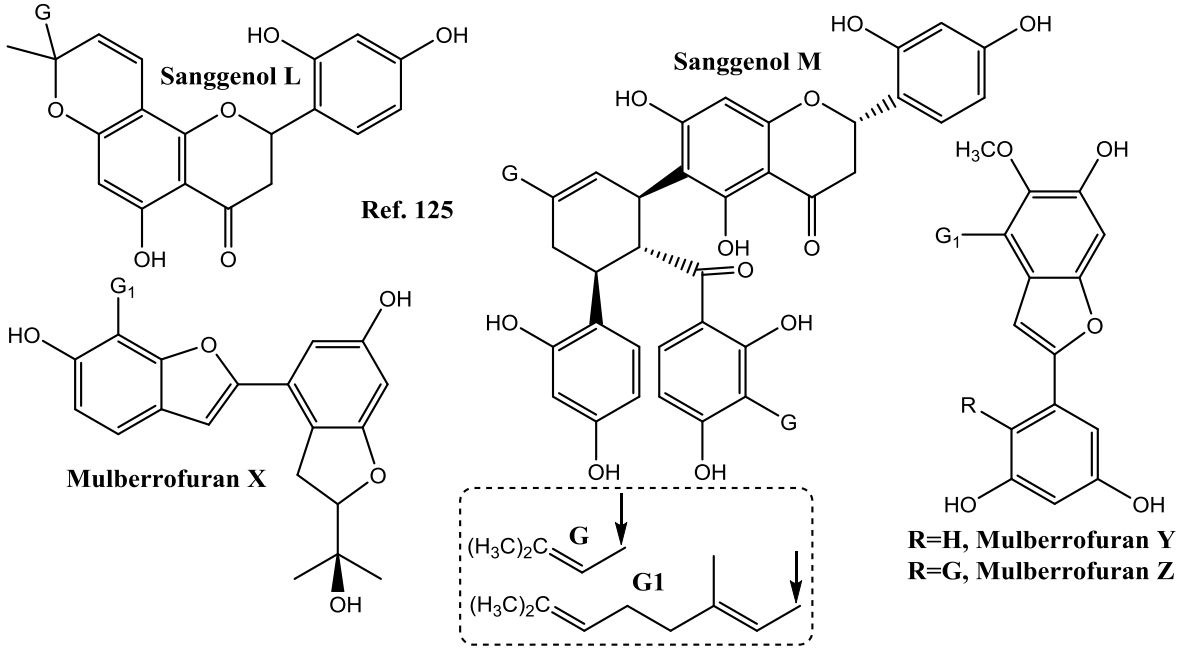

Figure 7: Natural products isolated from *Morus mongolica.*

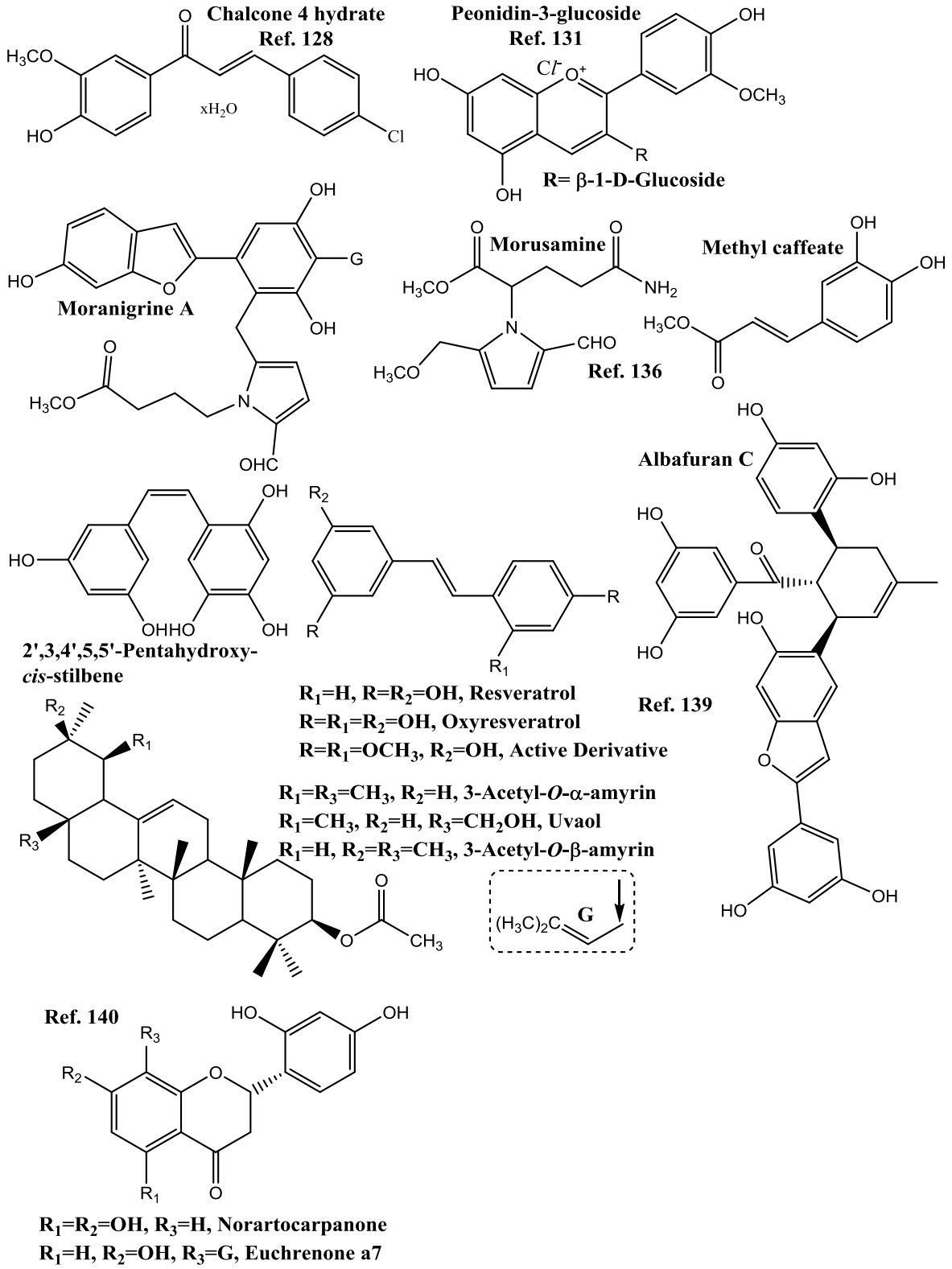

Figure 8: Natural products isolated from *Morus nigra.*

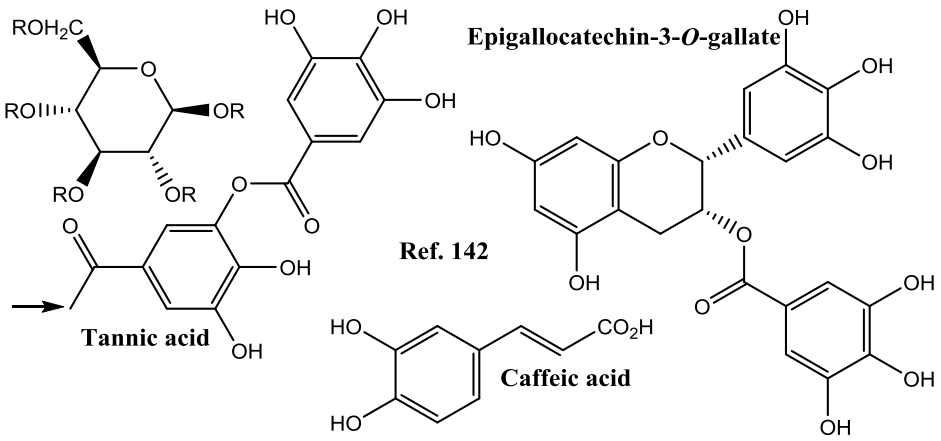

Figure 9: Natural products isolated from *Morus rotunbiloba.*

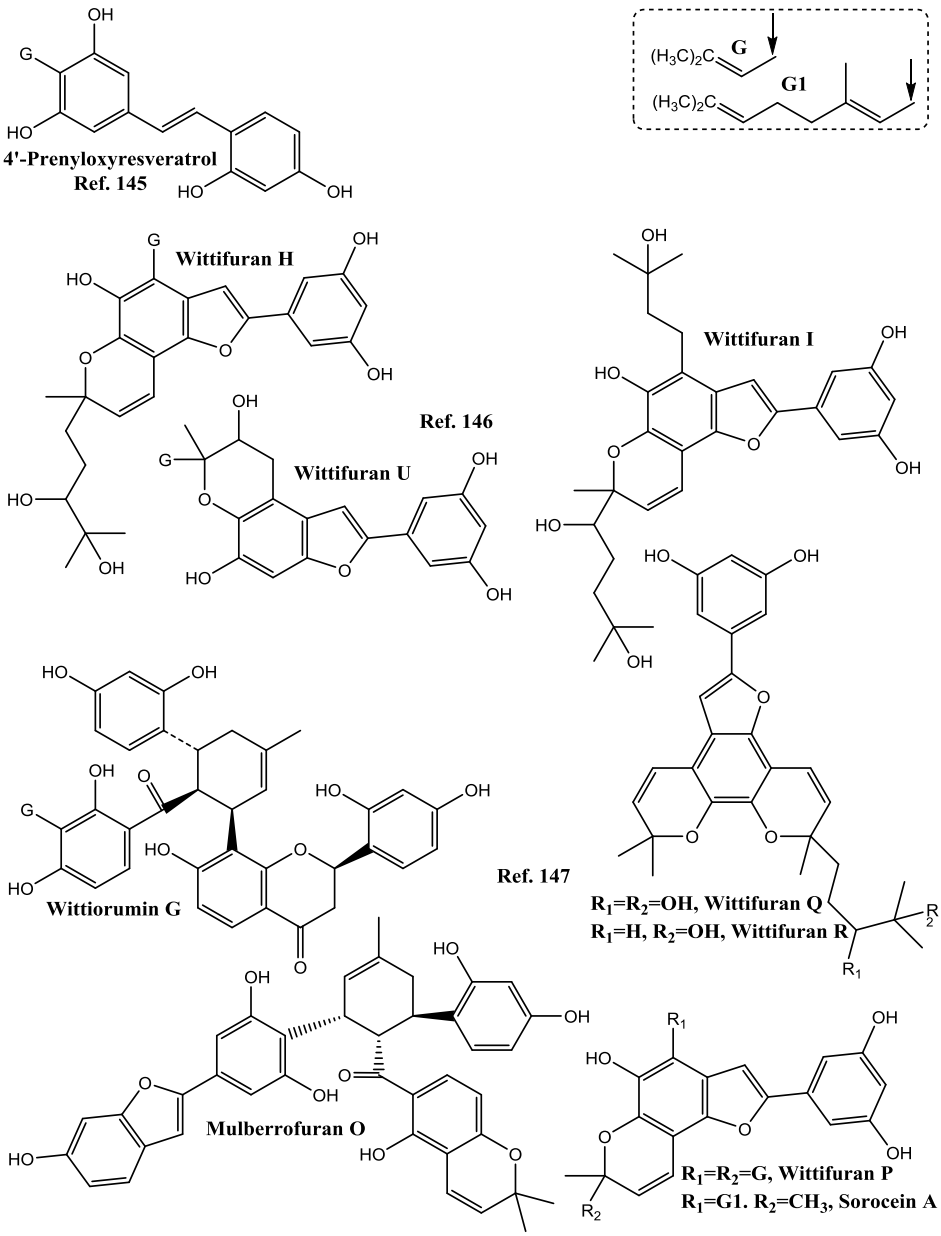

Figure 10A: Natural products isolated from *Morus wittiorum.*

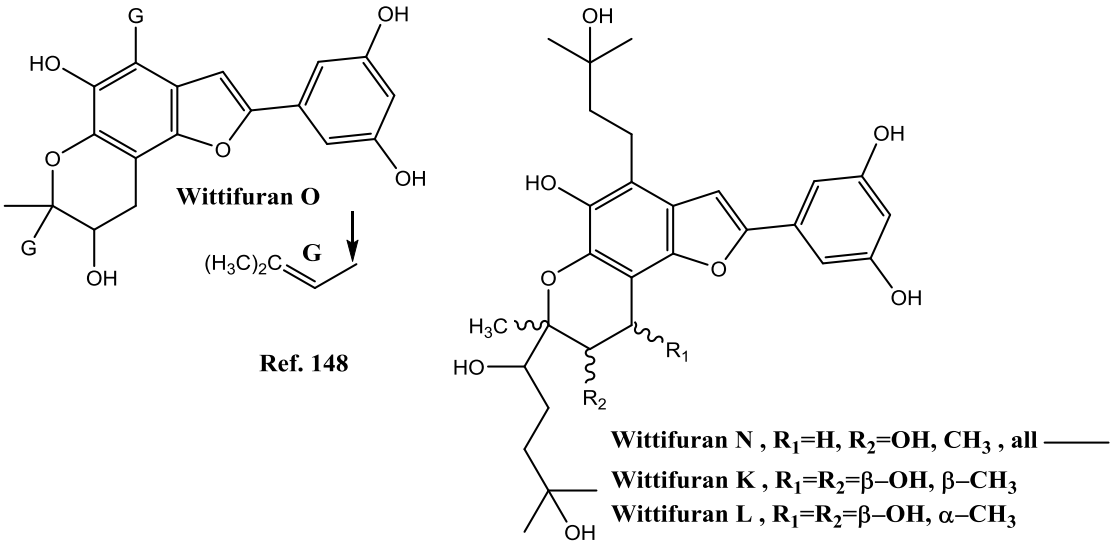

Figure 10B: Natural products isolated from *Morus wittiorum*.

3. DISCUSSION

In our previous three articles about the medicinal activities of *Morus* genus we reviewed the nutritive and antidiabetic[149], the brain-related protective[150], and the anti-inflammatory properties.[151] Based on the rich literature data of anticancer activity publications of this genus, this review article is the fourth out of five in this series, where the last will be about antioxidant activity. In this discussion, special attention is given to the very active natural product Morusin.

But as we mentioned in the introduction (section 1), review articles about the anticancer activity were published per species, and the whole *Morus* genus was not published in significant articles. Contrary to that, Morusin (see below) and a few other natural products were reviewed and published. A.H. Pratama ahc reviewed the possible potential of Morin (**Figure 11**), isolated from *Morus alba*, as anti-breast-cancer agent.[152]

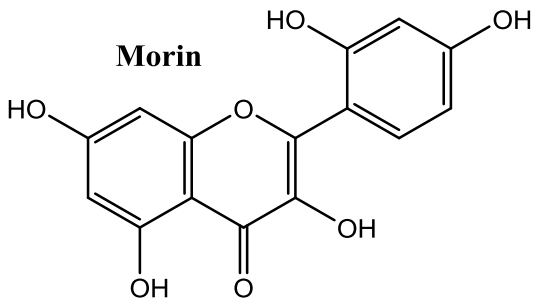

Figure 11: The structure of Morin (Flavonol).

Due to the extensive influence of the extraction method on the quantity and the quality of its

outcome, especially its natural products composition, many studies were conducted to optimize this process. In the previous section we cited the works of F. Li ahc[74] and A.K. Srivastava ahc.[8] But these efforts are continuous and S. Fenderya ahc carried out extractions with ethanol-water mixtures, where some of them were ultrasound assisted, and others were not.[153] They discovered that the use of ultrasound assistance did not improve the anticancer activity of the extract, against Caco-2 cells.

Three of the publications that we encountered during the writing of this review article were of special interest. We cited N.T. Dat ahc that reported 3′-Geranyl-3-prenyl-2′,4′,5,7-tetrahydroxyflavone (**Figure 1D**) was new but inactive anticancer compound[83]. This finding contradicts the results of J. Qin ahc that reported that this compound was active anticancer agent.[84] S.R. Deshmukh ahc reported the antioxidant and antiproliferative activities of the plant commonly known as Noni or Indian Mulberry.[154] In fact, this plant does not belong to the *Morus* genus and not even to the Moraceae family. Its scientific name is *Morinda citrifolia*, it is part of the *Morinda* genus, which is part of the Rubiaceae (Coffee) family. But it is very important to notice the very close appearance of its fruits and leaves to those of *Morus alba*.

Numerous research and review articles were published about the preparation of nanoparticles (NPs) using *Morus* extracts, and the uses of these NPs for various, mainly medicinal purposes. For example, M.F. Baran ahc prepared silver nanoparticles (AgNPs) using *Morus alba* leaves extract, and these NPs were active against aging-related infectious diseases.[155] R. Nazario-Naveda ahc also synthetized AgNPs) using *Morus nigra* fruits, and the resulting NPs were highly active against two bacterial strains.[156]

But in this article will focus on NPs with anticancer and related activities. Summary of the published articles is presented in **Table 1**.

Table 1: NPs with anticancer activity, prepared using *Morus* materials.

NPs	*Morus* Species	Testing Method [Reference]
Ag	*alba*	Potato disc assay[157]
Ag	*alba*	Against MCF-7 and MCF-10A cells[158]
Ag	*alba*	Against MCF-7 cells[159]
Ag	*indica*	Against HT-29 cells[160]
Ag	*indica*	Against HepG2 cells[161]
Ag	*nigra*	Against MCF-7 cells[162]
Ag/Cu	*nigra*	Against HeLa and HCT116 cells[163]

Ag/ZnO/Ag-ZnO	*macroura*	Against HepG2 cells[164]
Au	*nigra*	Against MCF-7, HT-29, OVCAR3, HeLa cells[165]
Se	*rubra*	Against MDA-MB-231 cells[166]
ZnO	*laevigata*	Against HT-29 cells[167]
ZnO	*nigra*	Against AGS gastric cancer cells[168]

Of all the compounds isolated from *Morus* species, Morusin (**Figure 1A**) was the most studied, most published and most mentioned in this article. Its studies were so extensive and frequent, so it was published in many research articles, but also in review articles. For this part of our review article, we will cite more publications that are related to this natural product, which were not cited above. These publications will be summarized in **Table 2** below.

Table 2: Additional Publication about Morusin (References 1-168 are not included).

Author(s)	Article Type	Major Topic(s), Reference
S.W. Cho ahc	Research	The roles of Morusin and autophagy in possible anticancer activity[169]
S. Agarwal ahc	Research	Morusin loaded niosomes for cancer therapy[170]
H.J. Park & S.H. Park	Research	Mechanism of action is proposed for cytoprotective autophagy by Morusin[171]
J. Wang ahc	Research	Mechanism of action is proposed for autophagy and apoptosis induced by Morusin[172]
C. Bailly	Research	Structural relationships of Artonin E and Morusin (see **Figure 12**) and molecular docking for anticancer activity[173]
S. Sarmoko ahc	Research	Theoretical investigation of the mechanisms of action of Morusin as anti-breast cancer, and molecular docking[174]
Y. Ran ahc	Research	Morusin-Cu(II)-indocyanine green nano-assembly for chemo-photothermal tumor therapy[175]
D.W. Choi ahc	Review	Various activities of Morusin including anticancer. Very illustrative, well organized and informative. Mechanism of action are presented.[176]
A. Panek-Krzysko, M. Stompor-Goracy	Review	Effect on human health. Mechanisms of action, *Morus* sources, metabolites, synthesis, various activities with focus on anticancer. Excellent.[177]
A. Hafeez ahc	Review	General activities focusing on anticancer. Very illustrative, mechanisms of action, sources, structurally close compounds. Excellent.[178]
M. Fatima ahc	Review	General for bioactive compounds of *Morus alba*, including Morusin. Very informative for history and number of publications.[179]
E.W. Chan	Review	General: chemistry and pharmacological properties of Morusin and Morusinol.[180]

Z. Zoofishan ahc	Review	Phenolic antioxidants of *Morus nigra* roots, and antitumor potential of Morusin. Very rich with natural products structures.[181]
H.N. Azzam ahc	Review	Morusin as drug candidate. Short version of the biosynthesis is presented (**Figure 13**).[182]

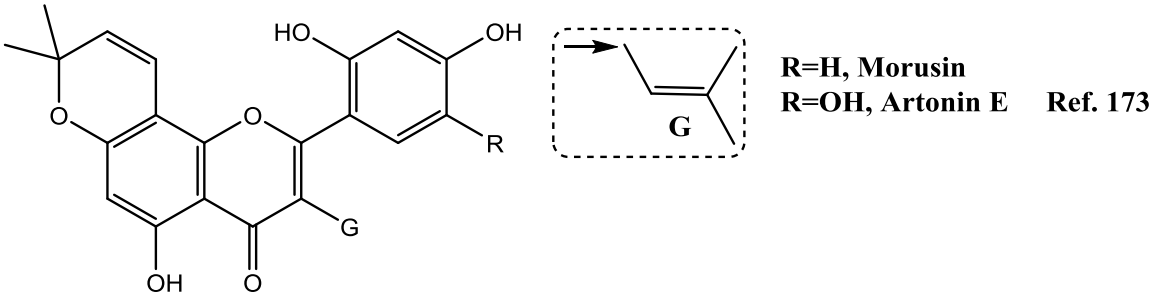

Figure 12: Structures of Artonin E and Morusin.

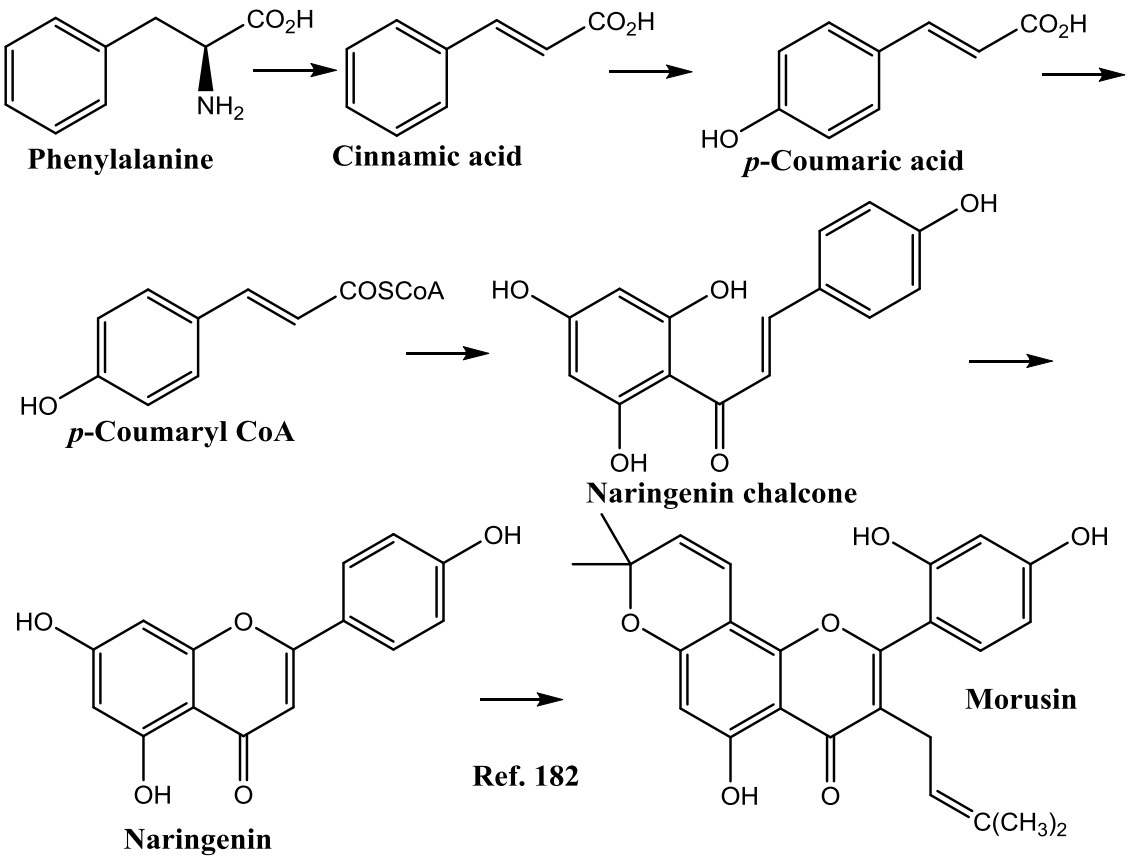

Figure 13: The Biosynthesis of Morusin.

Finally, several research groups published successful laboratory synthesis of Morusin. Among these, the work of T.H. Tseng ahc is the most significant.[183]

4. CONCLUSIONS

1) *Morus* species, especially *Morus alba* possess high anticancer potential.

2) While *Morus alba* was extensively studied and published, some other species were very limitedly or not published at all for anticancer activity.

3) Based on item 2, significant research efforts are needed.

4) *Morus* species contain vey active anticancer natural products like Morusin.

5) Some other natural products were also investigated for anticancer activity, but the published results indicate that more compounds should be tested.

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