

Mangifera and Impatiens from Sumatra: Phylogenetic Positions and their Modes of Action as Anticancer Agents

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ABSTRACT

Cancer has become a growing health threat due to the emergence of multidrug resistance and the increasing diversity of cancer cells. The continuous investigation into the development of anticancer agents and treatments is crucial because the current treatments can cause adverse side effects and are often ineffective. Anticancer derived medicinal plants are a potential source of treatment. However, the abundance of medicinal plant species can cause several problems, like the adulteration. The author aims to demonstrate DNA Barcoding technique as a tool to perform phylogenetic positions of *Mangifera* and *Impatiens* species grown in Sumatra. The phylogenetic positions of the plants are supported by the review on the active secondary metabolites from *Mangifera* and *Impatiens*. The current study is based on unpublished work on DNA Barcoding technique, an established modern technique to identify the phylogenetic position and also adulteration in medicinal plants. The review on the active secondary metabolites including the mechanism of action as anticancer is based on pertinent papers that were retrieved using relevant keywords in PubMed and Science Direct. Work using DNA Barcoding technique confirmed that *Mangifera* and *Impatiens* from Sumatra are closely related to *Momordica foetida* and *Impatiens balsamina* from other areas, indicating that they may share the same anticancer traits with those species. The mechanism of action of *Mangifera* and *Impatiens* includes inhibition of the cell cycle, cytotoxicity activity, apoptosis and leading to cell death, and anti-angiogenesis activity. Further research on both species is needed to identify their relevant chemical components to potentially develop anticancer drugs, either as a single compound or as a drug combination with minimal side effects and also to determine possible adverse reactions.

Key words: Anticancer, *Impatiens*, *Mangifera*, phylogeny, Sumatra

INTRODUCTION

Cancer is one of the leading causes of morbidity and mortality worldwide. Data from the World Health Organization (WHO) suggested that there were approximately 14 million new cases in 2012^[1] and that number is expected to increase by about 70% in the next 2 decades.^[2] Data in a global level suggested that nearly 1 in 6 deaths are due to cancer and about 70% of those cases are recorded from low- and middle-income countries. Besides behavioral and dietary risk, cancer can also occur due to infections, such as hepatitis and the human papillomavirus (HPV). About 25% of cancer cases in low- and middle-income countries are due to infections.^[2]

The common cause of mortality in cancer cases is late-stage presentation and inaccessible diagnosis and treatment. In 2015, only 35% of low-income countries reported having pathology services generally available in the public sector and to date, only 1 in 5 low- and middle-income countries have the necessary data to develop cancer policy.^[2] This needs to be improved since cancer is projected to be one

of the major causes of death in this century. Globally, cancer treatment needs continuous development to keep up with this threat.

The current well-known anticancer drugs are becoming less effective due to drug resistance. Thus, new anticancer drugs, drug combinations, and chemotherapy strategies need to be developed. To achieve this, methodical and scientific research of synthetic, biological, and natural products is very important. The natural products are an abundant and well-known source for cancer treatment. There are at least 250,000 species of plants that contain thousands of chemical compounds possessing significant anticancer properties. Some of these compounds include vincristine, vinblastine, colchicine, ellipticine, lepaol, and flavopiridol, a semi-synthetic analog of the chromone alkaloid rohitukine from India and many more.^[3,4] Many naturally occurring molecules have shown promising anticancer activity, but a significant number of molecules have not yet been studied in detail.

The current article reviews the chemical structures and their mechanism of actions, along with describing the structure-function relationships of naturally derived anticancer agents at the molecular, cellular, and physiological levels of medicinal plants, the *Mangifera* species (Anacardiaceae) and *Impatiens* species (Balsaminaceae), which grow on Sumatra Island, Indonesia. To authenticate and identify both species, we performed DNA barcoding, which amplified the conserved region of the Internal Transcribed Spacer (ITS) and constructed the phylogenetic tree. This approach enables us to compare the genetic

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relationship of both species from Sumatra especially in Riau City with species grown in other countries.

Taxonomy, morphology, and phylogenetic position of *Mangifera* from Sumatra

Among the genus *Mangifera*, *Mangifera indica* is the most well-known species and one of the most important tropical plants in the world. *M. indica* has been known as several local names, such as Mabaz (Arab), Am/Um (Bengali), Mi wang (Chinese), Mango; Mangofrukt; Mangotrae (Danish), Manga; Mangga; Manja; Mangoestanboom (Dutch), Mango (English), Mango; Mangopuu (Finnish), Manguer; Manguier (French), Indischer Mangobaum; Mango (German), Magko; Mangko (Greek), Am; Ambi; Amia (Hindi), Anchar; Mangoo; Mangou (Japanese), Amb (Persian), Aamra; Ambrah (Sanskrit), Amba (Sinhalese), Mangas; Mau; Mampalam (Tamil), and Mangga (Indonesia).

The taxonomical classification of *Mangifera* can be described as follow:

- Kingdom: *Plantae*
- Subkingdom: Tracheobionta
- Superdivision: *Spermatophyta*
- Division: *Magnoliophyta*
- Class: *Magnoliopsida*
- Subclass: *Rosidae*
- Order: *Sapindales*
- Family: *Anacardiaceae*
- Genus: *Mangifera*.

On the analysis of phylogenetic tree, Figure 1 shows the amplification of ITS sequences of *Mangifera* species from Sumatra for DNA barcoding and thus shows the genetic relationship with other *Mangifera* species distributed outside Indonesia (our unpublished work). The phylogenetic analysis is based on a representative sampling of the genus *Mangifera*, which includes 10 GenBank accessions. Another Anacardiaceae, *Searsia pyroides* serves as an outgroup for the phylogeny reconstruction. As shown in Figure 1, the phylogeny reconstruction reveals three major clades, supported with high bootstrap *P* values. *Mangifera* from Sumatra locally called as Ambacang clusters together with *Momordica foetida* as a sister group with high bootstrap support [Figure 1]. This is the first report showing that Ambacang represents *M. foetida* and identical to *M. foetida* that grows in Japan (Accession Number AB071680.1).

Fitmawati *et al.* (2016) also identified *Mangifera* species in Central Sumatra and suggested that evolution tree from ten *Mangifera* species

formed two clades with bootstrap *P* value 100%. Those clades are Clade I consists of *M. quadrifida* and Clade II includes *Mangifera* sp., *M. torquenda*, *M. sumatrana*, *M. foetida*, *M. odorata*, *M. zeylanica*, *M. indica*, *M. laurina*, and *M. kemanga*. In that study, Clade II developed into two subclade, namely subclade IIA consists of *Mangifera* sp., *M. torquenda* and *M. sumatrana* while subclade IIB consists of two groups were split *M. foetida* and *M. odorata* with *M. zeylanica*, *M. indica*, *M. laurina* and *M. kemanga*. Using Neighbor Joining (NJ) analysis, *Mangifera* species reconstructed three clades, which are Clade I includes *M. quadrifida* while Clade II includes monophyletic groups of *Mangifera* sp., *M. torquenda* and *M. sumatrana*, and Clade III includes *M. foetida*, *M. odorata*, *M. zeylanica*, *M. indica*, *M. laurina* and *M. kemanga* (Fitmawati *et al.*, 2016). The main difference in the NJ tree compared with the parsimonious tree was the position of Clade II and Clade III. In parsimony analysis, both clades formed a larger monophyletic group indicating they share common ancestor whereas in NJ analysis both clades were separate and resulted multifurcating tree, as has been seen in the current study.^[5] Kim and Mabry suggested some transition leaf texture relatively toward coriaceous or chartaceous such as *M. quadrifida* and *M. torquenda* indicating biparental inherited from nuclear genome.^[6] Therefore, *Mangifera* species which has transition leaf texture is a natural hybrid from different parental such as *M. odorata* hybrid from *M. foetida* and *M. indica*. Leaf structures of *Mangifera* used in this study, *M. foetida* and *M. indica* can be seen in Figure 2. Based on the morphological leaf and the genomic result as seen in phylogenetic tree [Figure 1], *Mangifera* species used in this study is closely related to *M. foetida* and share genetic compartment of *M. indica*. Thus, it indicates *Mangifera* species naturally produce nature anticancer chemicals as contains in *M. indica*. This hypothesis needs further anticancer identification and chemical structure analysis of mangiferin, the main anticancer compound of *M. indica*.

Initial tree(s) for the heuristic search were obtained automatically by applying NJ, and BioNJ algorithms to a matrix of pairwise distances estimated using the (Maximum Likelihood) ML approach and then selecting the topology with superior log-likelihood value. The analysis involved 10 nucleotide sequences. Evolutionary analyses were conducted in MEGA 6.^[7]

Taxonomy, morphology, and phylogenetic position of *Impatiens* from Sumatra

Impatiens balsamina is one of the most important tropical plants in the world that belong to the genus of *Impatiens*. The common

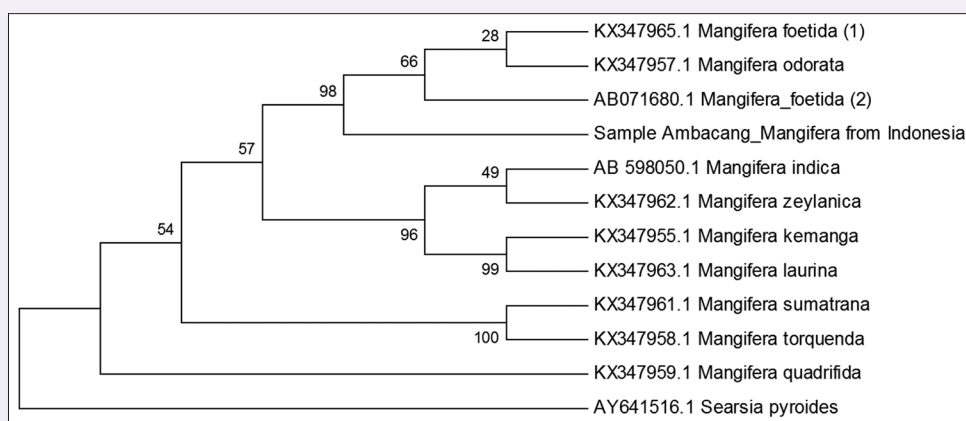


Figure 1: Molecular phylogenetic analysis of *Mangifera* from Sumatra, called Ambacang as compared to related *Mangifera* species generated from NJ analysis

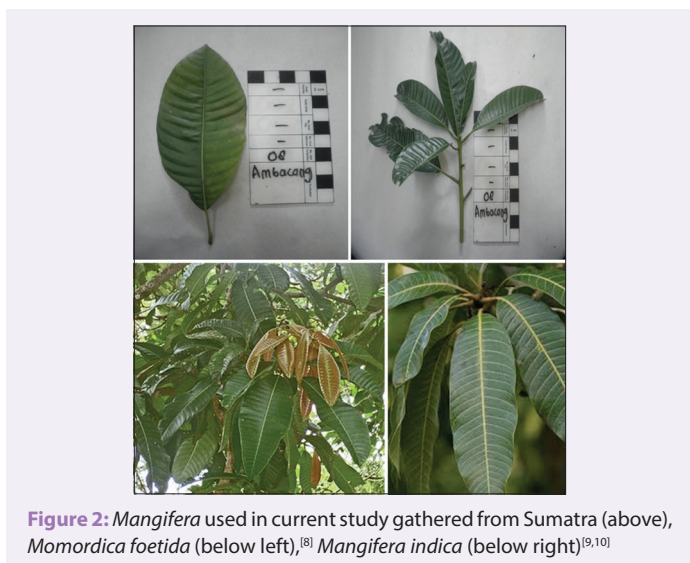


Figure 2: *Mangifera* used in current study gathered from Sumatra (above), *Momordica foetida* (below left),^[8] *Mangifera indica* (below right)^[9,10]

names of *I. balsamina* include Garden balsam, rose balsam, spotted snapweed, touch-me-not (English), Gul-mehndi (Hindi), Basava paadadagida (Canada), Tilo-onapu (Malay), Khujang lei (Manipuri), Chrido; Terada (Marathi), Tiuree (Nepal), Tairini (Sanskrit), Aivartenkittumpai; Aivartyenki (Tamil), Chilaka mukka puvvu; kaasithummi; Kasi tummi (Telugu), Gul-mehndi (Urdu), and Pacar air (Indonesia).

The taxonomical classification of *I. balsamina* can be described as follow:

- Kingdom: *Plantae*
- Division: *Magnoliophyta*
- Class: *Magnoliopsida*
- Order: *Ericales*
- Family: *Balsaminaceae*
- Genus: *Impatiens*
- Species: *I. balsamina*.

Figure 3 shows the amplification of ITS sequences of *Impatiens* gathered from Sumatra for DNA barcoding and thus shows the genetic relationship with other *Impatiens* species distributed outside Indonesia (our unpublished work). The phylogenetic analysis is based on a representative sampling of the genus *Impatiens*, which includes 28 GenBank accessions. Another *Balsaminaceae*, *Anagallis arvensis* serves as an outgroup for the phylogeny reconstruction. As shown in Figure 3, the phylogeny reconstruction reveals two major clades, supported with high bootstrap *P* values. *Impatiens* from Riau, Sumatra clusters together with *I. balsamina* from Thailand (KC 905466.1) as a sister group with high bootstrap support [Figure 3]. This is the first report showing that *Impatiens* from Riau [Figure 4], Sumatra genetically similar to several *Impatiens* species that grows in other countries [Figure 3].

Initial tree(s) for the heuristic search were obtained automatically by applying NJ and BioNJ algorithms to a matrix of pairwise distances estimated using the ML approach and then selecting the topology with superior log-likelihood value. The analysis involved 28 nucleotide sequences. Evolutionary analyses were conducted in MEGA 6.^[7]

Anticancer activity of *Mangifera* and *Impatiens*

Extended studies reported that *M. indica* possesses anticancer activity as well a few numbers of studies suggest *I. balsamina* also performed

the activity. The prominent anticancer compound from the *Mangifera* species is mangiferin [Figure 5], and the prominent anticancer compound from the *Impatiens* species is balsaminone [Figure 6]. This review also describes other promising chemical compounds that might be beneficial to the development of new drug combinations, as well as chemical modifications for enhanced administration of anticancer drugs. The following paragraph describes the chemical structures of the anticancer compounds and their modes of action.

Biological activity and the active compounds from genus *Mangifera*

Mango (*M. indica* L.), one of the most popular fruits in the tropics have been studied widely for their pharmacological activity. Most parts of the mango, such as the stem, bark, leaves, and pulp are known for various medical applications, including their antioxidant,^[11,12] anti-inflammatory,^[13] and anticancer properties,^[14] as we will discuss further in the current review.

The anticancer activity of *Mangifera* is found not only in the leaves, flesh, and stem but also in the peel. Kim *et al.* suggested that the antioxidant and antiproliferative activities of mango peels might be due to the synergistic actions of its bioactive compounds. This phenomenon needs further investigation, especially because mango peel can be further processed and has shown potential as a functional food and a valuable food ingredient.^[15] Several studies have been performed to investigate the pharmacological potential of the *Mangifera* species both *in vitro* and *in vivo*. *In vitro* studies have described several mechanisms of action of the anticancer compounds found in *Mangifera*.

Mechanisms of action of *Mangifera* based on *in vitro* studies

Inhibition of the cell cycle

In past studies, researchers proved that the juice and fruit extract from *M. indica* L showed inhibition for the cell cycle in G (0)/G (1) phase in the *in vitro* model in BALB/3T3 cells and HL-60 cells.^[14] Several polyphenols compounds contained in *M. indica* have been studied and exhibited increasing mRNA expression of pro-apoptotic biomarkers and cell cycle arrest, which has been tested in several cancer cell line such as leukemia, lung, breast, prostate, and colon.^[16] The compound from *Mangifera* species that is responsible for the activity to arrest the cell cycle (delay the S phase, arrest G2/M phase) is called mangiferin.^[17] Some studies revealed that mangiferin induces G2/M phase arrest in HL-60 cell strains, and increases CDC2, Cyclin B1, Cyclin A, Wee1, CDC25C, and Chk1 mRNA expression level of HL-60 cells. The G2/M phase arrest activity by mangiferin indicates that it possesses anti-leukemia properties.^[18,19] Abu Bakar *et al.* proved that a crude extract of *M. panjang* is capable of inducing G2-M arrest, resulting in substantial sub-G1 apoptosis arrest and apoptosis.^[20]

Cytotoxicity activity

Several compounds contained in *M. indica* L, such as butylated hydroxytoluene, 4,6-di (1,1-dimethylethyl)-2-methyl, fumaric acid, butylated hydroxytoluene, 4,6-di (1,1-dimethylethyl)-2-methyl, fumaric acid, 2-decyl undecyl ester, isoheptadecanol (1-Hexadecanol, 2-methyl), Apigenin 7-glucoside, cis-5 Dodecenoic acid, and (3-cyanopropyl) dimethylsilyl ester showed cytotoxicity to breast cancer cell lines with IC₅₀ values of 30 and 15 µg/mL.^[21] Cycloartane-type triterpenes, mangiferolate, mangiferolate B, and isoambolic acid from *M. indica* L inhibit the growth of human pancreatic cancer cell lines, such as the PANC-1 line.^[22]

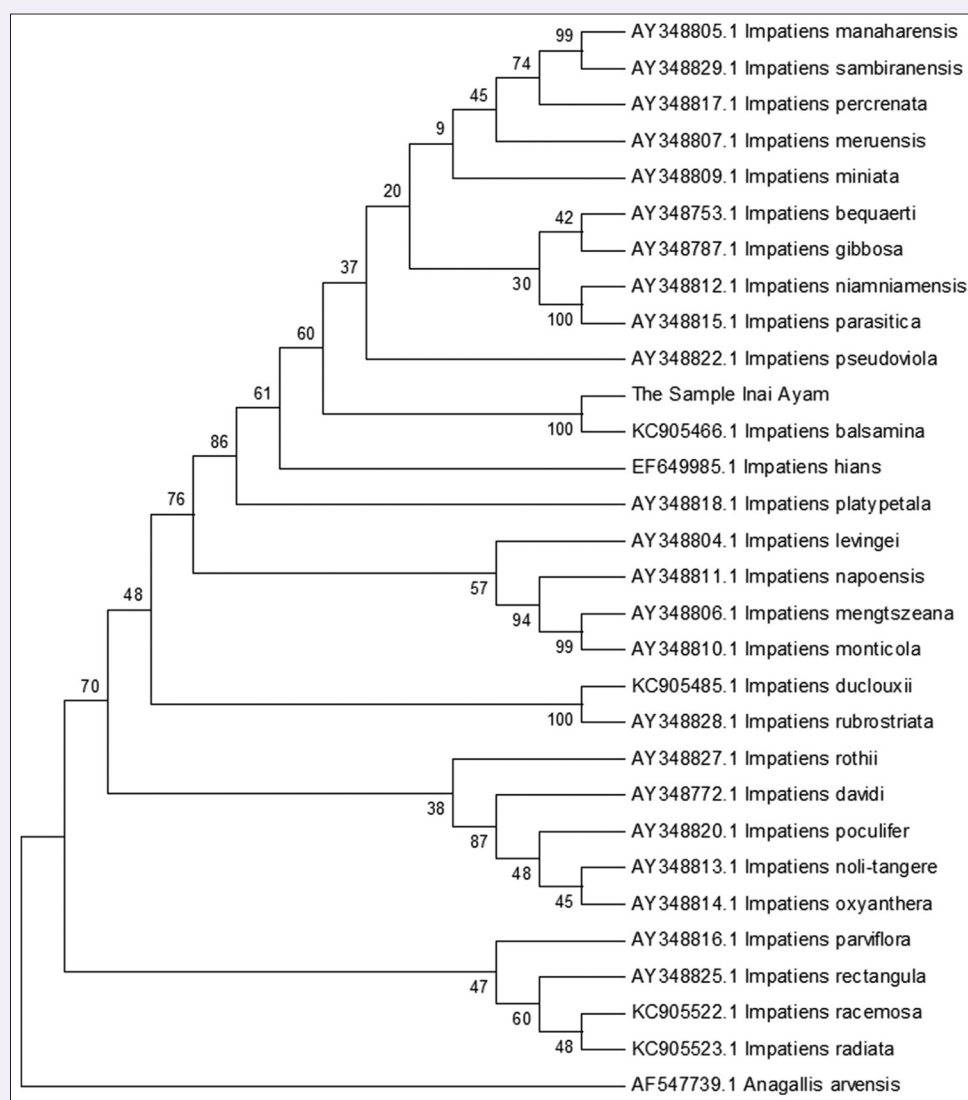


Figure 3: Molecular Phylogenetic analysis of Pacar Air as compared to related *Impatiens* species generated from NJ analysis

Apoptosis and leading to cell death

Mangiferin from the pulp, peel, seed, bark, and the leaf of the *Mangifera* species shows decreasing expression of PARP, caspase 9, 7, and 3, leading to apoptosis. The loss of mitochondrial membrane potential is also an indicator of the death of cells.^[17,23] Mangiferin also shows an activity to downregulate bcr/abl expression, leading to an apoptosis induction.^[23] Mangiferin also induced apoptosis in K562 cell lines through reduced bcr/abl fusion protein P210, B-Cell Lymphoma-2 (Bcl-2) and surviving mRNA gene expression, and increased bax gene expression.^[24] Pan *et al.* suggests that there is downregulation of Bcl-2 expression, while those of Bax were up-regulated.^[25] Padma *et al.* (2015) discovered that mangiferin was found to induce apoptosis by increasing caspase-3 activity and DNA fragmentation.^[26] Mangiferin also mediated down-regulation of nuclear factor-kappaB (NF- κ B) and showed potential for chemotherapeutic agent-mediated cell death^[27] especially because it does not change the expression of other survival signal-regulated kinase $\frac{1}{2}$, protein kinase B, and p38 mitogen-activated protein.^[26] The mechanism of mangiferin in inducing cell death is also by increasing the release of lactate dehydrogenase leakage and nitric oxide. This has been observed in embryonic rhabdomyosarcoma and in the most

prevalent types of cancer among children.^[27] Mangiferin also caused cell shrinkage and nuclear condensation, along with the occurrence of a late event of apoptosis.^[27] The crude extract from *M. panjang* has the capability of inducing apoptosis dependent on the caspase-2 and-3 in MCF-7 cells, and on caspase-2,-3 and-9 in MDA-MB-231 cells.^[20] Kim *et al.* (2012) observed that mangiferin inhibits cell proliferation. The mechanism of effect is possibly related to inducing apoptosis and the expression of the Fas protein.^[28]

Anti-inflammatory activity

Mangiferin is capable of modulating several key inflammatory pathways that induce carcinogenesis by decreasing several responsible genes and pathways such as NF- κ B, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (I κ B- α), Janus Kinase 1/2 (JAK-1/2), Signal transducer and activator of transcription 3 (STAT3), AKT, Mitogen-Activated Protein Kinase, and I κ B kinase (I κ K).^[17] NF- κ B down-regulation is essential for regulating the expression of cyclooxygenase-2, intercellular Adhesion Molecule-1 (ICAM-1), Bcl-2, interleukin-6 (IL-6), IL-8, C-X-C Chemokine Receptor type-4 (CXCR4), X-linked Inhibitor of Apoptosis Protein, and Vascular Endothelial Growth Factor (VEGF),

which is involved in inflammation, metastasis, cell survival, and angiogenesis.^[17,29,30] Mangiferin acts as a down-regulator of NF- κ B, resulting in the reduction of the genes listed above, and increased apoptosis. Mangiferin was also observed to downregulate IL-6 and IL-8 inflammatory cytokines production when stimulated by tumor necrosis factor (TNF), thus resulting in reducing the inflammatory response.^[31]

Anti-angiogenesis activity

Mangiferin also reduces the expression of VEGF, TNF- α , and fibroblast growth factor.^[31] The progression of cancer cells to tumor formation requires the ability to produce a blood supply containing nutrients and oxygen. This is known as tumor angiogenesis. It has been widely known that the VEGF-A protein stimulates angiogenesis.^[32] García-Rivera *et al.* demonstrated an inhibitory effect of mangiferin extract on TNF-induced

transcription of VEGF-A in MDA-MB231 cells. This finding needs further long-term investigation and evidence from *in vivo/ex vivo* studies.^[31]

Antioxidant activity

Mangiferin (C-glycosylated xanthone) from the pulp, peel, seed, bark, and leaf possess the antioxidant property to decrease oxygen free radicals, thereby reducing the DNA damage.^[17] Moreover, the antioxidant activity of the Mangiferin species is enhanced by the presence of its polyphenols compounds.

In addition to mangiferin, other polyphenols found in *Mangifera* that have potential antioxidant activity include mangiferin gallate, isomangiferin, isomangiferin gallate, quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-xyloside, quercetin 3-O-arabinopyranoside, quercetin 3-O-arabinofuranoside, quercetin 3-O-rhamnoside, kaempferol 3-O-glucoside, rhamnetin 3-O-galactoside/glucoside, and quercetin.

During mango fruit development, phenol concentration has been found to be higher in the peel than in the flesh at all stages.^[33] In general, ripe peels contain higher total polyphenols than raw peels.^[11] Berardini *et al.* found that, while mangiferin contents slightly decreased at elevated temperatures, the contents of the other xanthone derivatives significantly increased.^[34]

Berardini *et al.* established the antioxidative activity of mango peel extract and suggested that the antioxidative capacity of the extract was higher than that of standard mangiferin and quercetin 3-O-glucoside. The result indicates that the antioxidative capacity of the peel extract works synergistically with the other compounds.^[34]

The most prominent compounds that displayed antioxidant activity are mangiferin, methyl gallate, gallic acid (pro-oxidant), penta-O-galloyl-glucoside, ascorbic acid (pro-oxidant), and Trolox. Penta-O-galloyl-glucoside and mangiferin, when tested individually, were potent inhibitors of xanthine oxidase, while gallic acid and ascorbic acid displayed pro-oxidant activity. Penta-O-galloyl-glucoside



Figure 4: *Impatiens balsamina* gathered from Riau, Sumatra

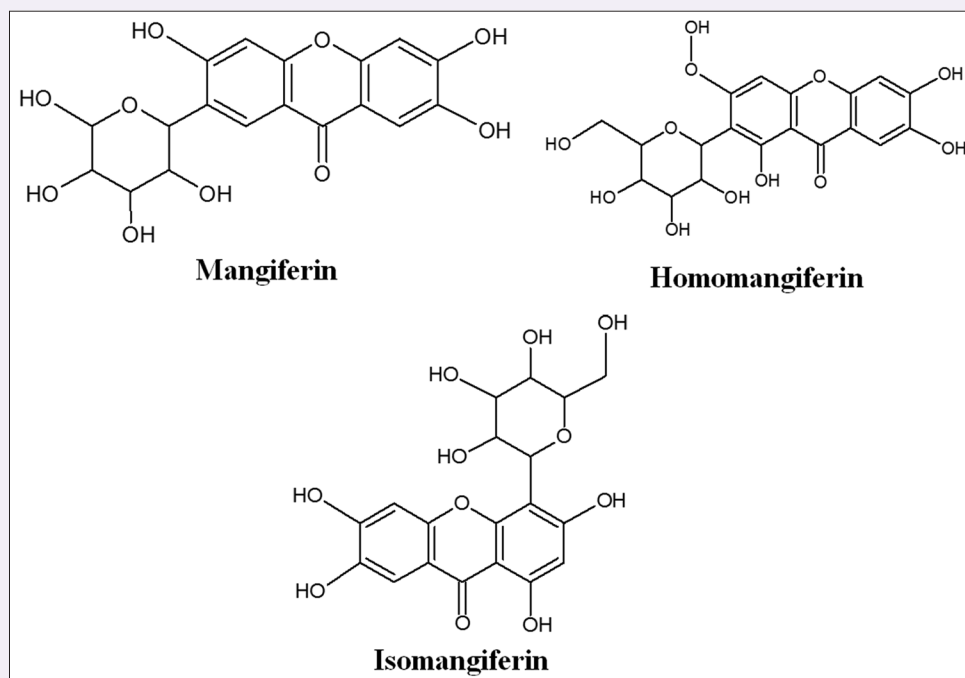


Figure 5: Chemical structures of secondary metabolites from *Mangifera* species

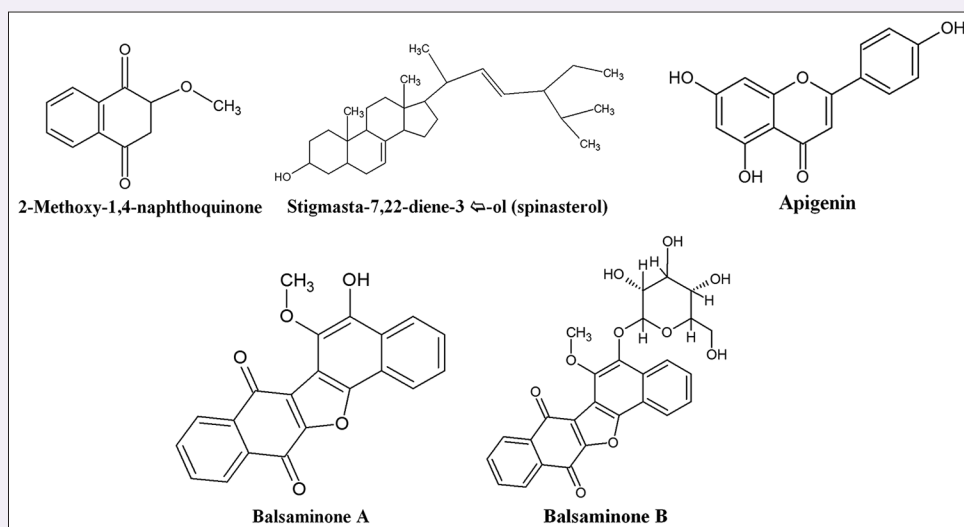


Figure 6: Chemical structures of secondary metabolites from *Impatiens balsamina*

is the major compound detected in the peels and kernels of the mango by products, with higher concentrations found in the kernels when compared to the peels. In the bark and young leaves, the predominant compound detected was mangiferin an important molecule with potential pharmacologic activities.^[35,36]

Drug combination of mangiferin with other chemotherapeutic drugs

Combinations of mangiferin with other chemotherapeutic drugs resulted in superior effects. Mangiferin combined with oxaliplatin shows promising activity by reducing oxaliplatin IC_{50} in HT29 (3.4-fold) and HeLa (1.7-fold) *in vitro*. This activity is also aided by increasing the activity of caspase-3 and DNA fragmentation delay in the S-phase of the cell cycle. Mangiferin can also be combined with oxaliplatin. This study indicates these combinations favor apoptotic cell death.^[27] Combinations of mangiferin with other natural products, such as gallic acid, can inhibit NF- κ B activation by I κ K- γ' , resulting impaired I κ B degradation, NF- κ B translocation, and NF- κ B/DNA binding.^[33] Combinations of mangiferin with other chemotherapeutic drugs with various targets and modes of action reduces side effects while improving nutrition levels.

Mechanisms of action of *Mangifera* based on *in vivo* studies

Several *in vivo* studies of mangiferin have been conducted. Li *et al.* (2013) observed decreased tumor volume, weight, and proliferation, and increased apoptosis in mangiferin-treated MDA-MB-231 xenograft mice. It decreased the expression of MMP-7 and -9, vimentin, activated β -catenin, and increased the expression of E-cadherin.^[37] More recent publications have shown that in A549 xenograft mice *in vivo*, mangiferin exhibited anti-tumor properties and markedly decreased the volume and weight of subcutaneous tumor mass, which lengthened lifespan. Moreover, in combination with cisplatin, mangiferin enhanced its antiproliferative effects, thus indicating the potential for a combined therapy.^[38] *In vivo* tests have also been conducted in various animal models of cancer. In one such test, mangiferin exhibited chemopreventive effects in Swiss Albino mice treated with the compound (50 mg/kg body weight) for 6 weeks.^[38,39] Rajendran *et al.* also reported that cancer-bearing animals pretreated with mangiferin exhibited reduced alveolar damage with a nearly normal architecture. Animals posttreated with mangiferin showed slightly reduced alveolar damage. Mice treated with mangiferin

alone showed no significant change in lung histology from that of the control animals.^[39]

Leiro *et al.* studied the immunomodulatory activity of mangiferin on the expression of several genes related to the NF- κ B signaling pathway using activated mouse macrophages. The inhibition of gene expression by mangiferin at a concentration 10 μ M includes (1) the genes Rel/NF- κ B/I κ B family, RelA and RelB (=I-rel) indicates an inhibitory effect on NF- κ B-mediated signal transduction, (2) TNF receptor (TNF-R)-associated factor 6 (Traf6), resulting in probable blockage of the activation of the NF- κ B pathway by lipopolysaccharide, TNF, or IL-1, (3) proteins involved in responses to TNF and apoptotic pathways triggered by DNA damage includes TNF-R, the TNF-receptor-associated death domain, and the receptor-interacting protein, (4) extracellular ligand IL-1a, indicating likely interference with responses to IL-1, (5) the pro-inflammatory cytokines IL-1, IL-6, IL-12, TNF- α , regulated on activation, normal T-cell expressed and secreted known as chemokine (C-C motif) ligand 5 (CCL5), and cytokines produced by monocytes and macrophages including granulocyte colony-stimulating factor (CSF), granulocyte-macrophage CSF, macrophage CSF, (6) other toll-like receptor proteins including c-Jun N-terminal kinases (JNK1, JNK2) and Tab1 gene, (7) Scya2 (small inducible cytokine A2); and (8) various ICAMs, as well as the Vascular Cell-Adhesion Molecule in high concentrations in atheromas.^[40] The inhibition of JNK1, together with stimulation of c-JUN like the Jun oncogene suggests that mangiferin may protect cells from oxidative damage and mutagenesis and it has been also previously proved in term of the antioxidant activity.

The administration of mangiferin to rats with D-galactosamine-induced hepatotoxicity alters all adverse effects. This indicates that mangiferin has a hepatoprotective role due to the induction of antioxidant defense via the NRF2 pathway and the reduction of inflammation pathways via the inhibition of NF- κ B activity.^[41] Guha *et al.* (1996) also showed mangiferin to have *in vivo* growth inhibitory activity against ascetic fibrosarcoma in Swiss mice.^[42] In murine splenocytes and thymocytes, mangiferin activated the splenocytes of tumor hosts at early and late stages of tumor growth. The phytohemagglutinin and Con A unresponsive splenocytes of advanced tumor bearers proliferated extensively in response to mangiferin. Mangiferin, when used with Con A, produced additive stimulatory effect and induced heightened DNA synthesis of normal and advanced tumor bearers' splenocytes.^[42]

Table 1: Several compounds contained in *Impatiens balsamina* possess anticancer activities

| Number | Species | Compounds | Part of plant | Mode of action | Literatures |
|--------|-------------------------------|--|---------------|--|------------------------------|
| 1 | <i>Impatiens balsamina</i> | dinaphthofuran-7,12-dione derivative, balsaminone C, dinaphthofuran-7,12-dione derivatives, balsaminone A, balsaminone B | Seed | The compounds have a potent cytotoxicity activity against cancer cell lines A549, Bel-7402 and Hela | Pei <i>et al.</i> , 2012 |
| 2 | <i>Impatiens balsamina</i> | Apigenin, flavonoid, naphthoquinone, glycosides, kaempferol, rhamnosyl glycoside, kumarins | | Anticancer activity against HeLa and NIH3T3 cell lines | Baskar <i>et al.</i> , 2012 |
| 3 | <i>Impatiens glandulifera</i> | Glanduliferins A and B | Dried stem | Inhibit the growth of human cancer cells with IC ₅₀ value of glanduliferins A 30 µM. Video microscopy analysis showed that the effects of compounds have a cytostatic rather than a cytotoxic activity in U373 GBM cells | Cimmino <i>et al.</i> , 2016 |
| 4 | <i>Impatiens balsamina</i> | Methanol extract (N/A) | | Extract decrease the cell viability of HSC-2 cells and activated AMPK signaling, but inactivated mammalian target of rapamycin signaling. Extracts induce apoptosis by the activation of caspase-3, poly (ADP-ribose) polymerase cleavage and nuclear condensation. Moreover, AMPK activation by two known activators (5-aminoimidazole-4-carboxamide-1-β-ribofuranoside and metformin) decrease cell viability and induce apoptosis. Extracts also increased the expression levels of mitochondria-related proteins (t-Bid, Bak, and Bad), which contributed to the disruption of mitochondrial membrane potential, cytochrome C release, and the activation of caspase-9 | Shin <i>et al.</i> , 2015 |

AMPK=AMP-activated protein kinase, GBM=Glioblastoma, ADP=Adenosine diphosphate, HSC=Human oral squamous cell carcinoma, N/A=Not available, IC=Inhibitory concentration

Biological activity and the active compounds from genus *Impatiens*

I. balsamina L. is one of the medicinal plants widely distributed and used as an indigenous medication in Asia for the treatment of rheumatism, fractures and fingernail inflammation. Lobstein *et al.* isolated anti-*Helicobacter pylori* compounds from the pods, roots, stems and leaves of *I. balsamina* L, called 2-methoxy-1,4-naphthoquinone and stigmasta-7,22-diene-3 β-ol (spinasterol).^[43] The activity of 2-methoxy-1,4-naphthoquinone was equivalent to that of amoxicillin. The compound exhibits great potential to be developed as an agent for the eradication of *H. pylori* infection and exhibits thermal and pH stability.^[43,44] Compounds of 2-methoxy-1,4-naphthoquinone have also shown antifungal and antibacterial activities.^[45,46]

Several quinone compounds were observed, such as natural bisnaphthoquinone, methylene-3,3'-bilawsonone, which was isolated from root cultures of *I. balsamina*, along with two naphthoquinones (lawsone and 2-methoxy-1, 4-naphthoquinone), two coumarin derivatives (scopoletin and isofraxidin), and a sterol (spinasterol).^[47,48]

Ethanol extracts of *I. balsamina* were also investigated for anticancer and *in vitro* cytotoxic activities against transplantable tumors and human cell lines. The extract was examined using two methods, *in vitro* cytotoxicity using Hela and NH3T3, and *in vivo* using Dalton's Ascites Lymphoma (DLA) tumor-bearing mice. The extract showed less toxicity against normal cells. The results indicated significant antitumor and cytotoxic effects against DLA and human cancer cell lines.^[48]

Ding *et al.* (2008) showed that extracts of ethanol or chloroform from *I. balsamina* exhibit anti-tumor activity against the human hepatocellular carcinoma cell line HepG2. From those extracts, 2-methoxy-1,4-naphthoquinone was discovered to be an active anticancer compound.^[49,50] Shin *et al.* investigated the efficacy of a methanol extract from *I. balsamina* L. against HSC-2 human oral cancer cells. The results also suggest that *I. balsamina* extract is promising as an oral cancer treatment through the mechanism of action of

adenosine monophosphate-activated protein kinase and t-Bid.^[51] Several compounds contained in *I. balsamina* possess anticancer activities, and that have been summarized in Table 1. Other potential chemical compounds are described in Figure 6.

CONCLUSION

Taken together, phylogenetic analysis showed that the *Mangifera* and *Impatiens* species from Sumatra share a close relationship with *M. foetida* and *M. indica* and *I. balsamina* from other countries. The results indicate a modulation of the expression of genes that are critical for the regulation of apoptosis, anti-tumorigenesis, and anti-inflammation. This raises the possibility that they may be of value for the treatment of inflammatory diseases and/or cancer as a single active compound, as an active fraction, or in combination with chemotherapeutic drugs. Further investigation is urgently needed, one of them to identify the abundance of mangiferin and other anti-cancer compounds contained in *Mangifera* from Sumatra and anticancer compounds of *Impatiens*.

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Conflicts of interest

There are no conflicts of interest.

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