BIOLOGICAL ACTIVITIES OF KAEMPFERITRIN- A SHORT REVIEW

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Abstract

Kaempferitrin is a 3,7-diglicosilflavone, constituted by two molecules of rhamnose bound to kaempferol. Kaempferitrin is a natural product with a wide variety of biological activities including hypoglycemic, anti-inflammatory, anti-cancer, immunostimulatory, anti-depressive, anti-fungal and anti-bacterial. This flavonoid has been isolated in a widely variety of living organisms including plants and fungus. Since there are various reports on their activities, it is clear that this molecule has the potential to be used as a new drug, or as a lead to developed analogues, to treat a wide variety of diseases. In this review, we report on the available information on the various biological activities of kaempferitrin.

Keywords: Kaempferitrin, flavonoids, pharmacological activities, natural products.
Introduction

Historically, natural resources have been an important source of chemical compounds that have an impact in the development of new drugs. At least 1328 new compounds derived from natural resources have been reported from 1997 to 2014 [1]. Plants and natural products derived from them have contributed to medicine and have been important for the discovery of new drugs. Currently, several drugs used to treat different physiological disorders have little effectiveness and have been attributed a significant amount of adverse effects. Hence, further research is needed to find new drugs capable of treating a wide variety of diseases without adverse effects and more effectively. Kaempferitrin is a natural product that has shown promising biological effects and is widely distributed in fungus and plants. Plants are the most important source of this compound and has been isolated in more than thirty different species. In this kind of plants, this molecule is the major component and the bioactive metabolite. In this review we have summarized the pharmacological activities of this kind of flavonoid, kaempferitrin.

Chemical considerations of kaempferitrin

Kaempferitrin or 3,7-diglycosilflavone) belongs to a group of metabolites known as flavonoids (Figure 1). Structurally, kaempferitrin contains one kaempferol molecule and two molecules of rhamnoses bounded in position 3 and 7. In humans kaempferitrin is metabolized by the intestinal flora, resulting in structures such as 3-O-β-L-rhamnopyranoside (afzelin), kaempferol 7-O-β-rhamnopyranoside, kaempferol and p-hydroxybenzoic acid (Figure 2) [2]; also can be transformed, by deglycosylation, into afzelin when is subjected to infrared irradiation for one hour at 60°C [3]. But, irradiating the leaves (Hibiscus cannabinus) with far infrared light for 30 minutes at 60°C increased the levels of kaempferitrin in aqueous extracts. For this reason, radiation is considered in the isolation of this molecule [4].

Sources of Kaempferitrin

Kaempferitrin has been identified in several species of plants (Table 1), but it is reported as the major metabolite in the ethanolic extracts from leaves of Justicia spicigera [5]. However, concentration of kaempferitrin depends on the environment conditions where the plant grows. For example, Bauhinia forficata, commonly known as “pata de vaca” (cow paw), the concentration of kaempferitrin depends on the region where was collected, part of the plant and the solvent used [6]. In fungus species, like Annulohypoxylon boveri var. microspore an endophytic fungus, kaempferitrin is generally collected in the fraction soluble in butanol [7].

Antibacterial, antifungal and antiparasitic activity of kaempferitrin

Discovering new molecules capable of inhibiting growth and viability of pathogenic microorganisms is essential. Currently, some antibiotics are no longer functional because lost its effectiveness, the main reason has been for misuse resulting in bacterial resistance [63]. Nowadays, there is a high percentage of deaths caused by fungi or bacterial infection. For instance, Staphylococcus aureus, a pathogen strain, has caused around 7-10% of mortality [64]. Also, several fungus strains can be lethal, especially when the patients have been immunosuppressed by bacterial infections such as Candida, Cryptococcus neoformans or Salmonella typhi [64, 65]. Hence, it is important to analyze the effects of kaempferitrin to inhibit the growth of pathogenic microorganisms. In this sense, kaempferitrin have an inhibitory effect against to Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans, Shigella flexneri, Salmonella typhimurium and Acinetobacter calcoaceticus in doses ranging from 32 to 100 μg/ml. The same effect is obtained, but at lower doses 3.9, 8.5 and 16μg/ml, when used in bacteria as Enterococcus faecalis and Bacillus cereus, Candida parapsilosis and Cryptococcus neoformans [43, 66]. Kaempferitrin has also shown activity against oral bacterial strains such as Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis (MIC of 500 μM), showing a stronger effect than some mogrosides [67]. This report is an evidence that kaempferitrin can be more effective than molecules already commercialized for the treatment of oral infection. Additionally, kaempferitrin was evaluated on Dactylogyrus intermedius, a fish parasite, and was able to eradicate all the parasites housed on the gills of fish at concentration of 12 mg/ml. This research showed the potential effect of kaempferitrin as an anthelmintic drug [28]. So, it is clear that kaempferitrin have a strong antibiotic activity, and can be useful for the development of new drugs against infectious diseases.

Kaempferitrin as hypoglycemic agent.

Diabetes is a chronic metabolic disorder due to lack of insulin and is characterized by hyperglycemia. Kaempferitrin and its metabolites have been widely studied for its effect in the control of hyperglycemia. Recent study, showed that kaempferitrin (4 mg/kg) enhance the glycolytic effect of enzyme 6-phosphofructo-1-kinase (PFK) and reduce blood glucose levels after 2 hours on skeletal muscle, liver and adipose tissue in diabetic mice, suggesting that the molecule is able to reduced blood glucose levels via stimulation of PFK. Although it is not known how exactly acts, this mechanism seems to involve the sugars contained in their chemical structure of kaempferitrin, which gives an

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insulin-like effect [16; 68]. Additionally, in alloxan induced diabetic rats, kaempferitrin also showed hypoglycemic effect at doses of 50, 100 and 200 mg/kg one hour after administration, while blood glucose levels in non-diabetic rats remained unaffected. In the same study the antioxidant properties of kaempferitrin was also evaluated, obtaining a IC₅₀ = 84.0 ± 7.8 mM) [69]. Obesity is linked to diabetes due to a low secretion of adiponectin, a hormone that regulates glucose and fat metabolism. The plasma levels of adiponectin are inversely correlated with the insulin resistance. Kaempferitrin is able to activate the insulin pathway and stimulate secretion of adiponectin in 3T3-L1 cells, up-regulating level of phosphorylation on ser473 site by Protein kinase B (PKB/akt) on insulin receptor beta and insulin receptor substrate 1, and translocation of GLUT4, that is an important molecular process to maintain blood glucose homeostasis. However, exist a little controversy about of the effect of kaempferitrin on glucose transporter. On the one hand, Tzeng et al (2009) showed the potential effect of kaempferitrin to translocate GLUT4 to membrane and increasing GLUT4 protein levels; also, was able to stimulate a sustained adiponectin secretion compared to insulin [70]. Nevertheless, in the other hand, Prasad et al (2009) conclude that kaempferitrin has an inhibitory effect on the GLUT4 translocation on 3T3-L1 cells. This could be due to a competition for the binding site with insulin. For this reason, further studies are needed in order to evaluated the real effect of kaempferitrin on GLUT4 [71].

**Kaempferitrin and cancer**

Cancer is a disease characterized by the excess of cell proliferation. Some authors have described the hallmarks of this disease as overgrowth, resistance to cell death, induction of angiogenesis, invasiveness, metastasis and evasion of suppress growth factors [72]. Effects of kaempferitrin has been evaluated in different cancer cell lines including HeLa (cervical cancer), SKOV-3 (ovarian cancer), DU-145 (prostate cancer cell line), SW-480 (colorectal carcinoma), MDA-MB-231 (breast cancer) and HepG2 (liver cancer), but only with the HeLa cells had the highest cytotoxic effect (IC₅₀ = 4.5 μM), as a result of DNA fragmentation and externalization of phosphatidylserine, major features of apoptosis, but also induces cell arrest in the G1 phase regulating proteins involved in the cell cycle as p21, p16 and cyclin D1 on HeLa cells [73]. Kaempferitrin also demonstrated in vivo activity in mice with tumors induced by HeLa cells, resulting in tumor size reduction of up to 78% compared with the control group at a dose of 25 mg/kg [73]. The cytotoxicity of compounds bound to kaempferitrin has also been evaluated. Four kaempferitrin metal ion complexes Cu, Zn, Co and Cd were synthetized and then evaluated for its ability to inhibit Topoisomerase I enzyme (DNA TOPO I) on MCF-7, SGC-7901, SK-OV-3 and HepG2 cell lines. The results found that the four complexes affected the performance of DNA TOPO I. However, the copper complex was the best proliferation inhibitor, showing an inhibition rate of 44.19 and 44.58% on SGC-7901 and SK-OV-3 cell lines, respectively [74].

**Kaempferitrin and inflammation**

Inflammation is a biological process induced by infection or tissue injury. The main goal of this process is to repair tissue damaged and eradicate infection. Chronic inflammation is associated with many diseases such as atherosclerosis, diabetes, neurodegenerative diseases and cancer [75]. Myeloperoxidase (MPO) is a heme-enzyme present in human neutrophils and monocytes and it is reported as one of the most important enzymes implicated in both, inflammation and infection. MPO converts hydrogen peroxide and chloride into potent oxidizing agents, playing important biological roles. It is well established that an excessive MPO activity is involved in many diseases including atherosclerosis, cancer and Alzheimer’s disease.

Another protein that plays a relevant role in inflammatory response is the tumor necrosis factor alpha (TNF-α), that belongs to the cytokines family. TNF-α is released by the immune system in response to an infection or a tissue injury. Elevated TNF-α synthesis has been associated with the development of diabetes, septic shock, tumorogenesis, rheumatoid arthritis, psoriasis, arthritis and inflammatory bowel disease [76]. Using a spectrophotometric method, kaempferitrin was evaluated as an inhibitor of MPO (IC₅₀ = 15.8 nM). It has been found that the inhibitory effect of kaempferitrin depends on its chemical structure; for example, flavonoids which have a catechol derivate on B-ring exhibited a better inhibitory effect on MPO [51]. At a dose of 100 mg/kg, kaempferitrin inhibits leukocytes and neutrophils migration. This compound also inhibited infiltration of mononuclear cells to about 65% in the model of carrageenan-induced pleuritis in rats. In addition, kaempferitrin inhibits the activity of MPO and adenosine deaminase (ADA) by 90.6 and 77.1%, respectively, and increases interleukin-1β (IL-1β) levels by 61.1%, showing a higher activity than dexamethasone, a widely used anti-inflammatory [77]. In another study, kaempferitrin was evaluated as an inhibitor of molecules produced by macrophages in the inflammatory response, showing an ability to inhibit the production of nitric oxide (NO), TNF-α and interleukin (IL)-12 in a dose-dependent manner [78]. Koelzer J. et al 2009 demonstrated that kaempferitrin was able to inhibit both activity of MPO, ADA, leukocyte migration, and levels of NO and IL-17A in the mouse pleural cavity. In writhing test induced by administration of p-benzoquinone in mice, kaempferitrin at doses of 50 mg/kg, had a potent antinociceptive and

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anti-inflammatory effect without inducing any apparent acute toxicity or gastric damage [58].

**Immunostimulatory effect of kaempferitrin**

*Justicia spicigera*, one of the plants most used in Mexican traditional medicine, had an immunostimulating effect in *in vitro* conditions, and the reason is because of its high content of Kaempferitrin. Also, at a dose of 25 μM, kaempferitrin had an effect on the proliferation of murine splenocytes and macrophages, as well as human peripheral blood mononuclear cells. Kaempferitrin showed also to increase lysosomal activity and pinocytosis in murine macrophages RAW 264.7 cell line in a dose dependent manner. The effects of kaempferitrin on the natural killer cell (NK) activity were also analyzed. The NK activity increased by 10.7%. It is important to note that stimulation and activation of the immune system has been considered a novel strategy for the prevention and the treatment of infectious diseases and cancer [79].

**Effects of kaempferitrin on the Central Nervous System**

Depression and anxiety disorders represent the second most common health problems worldwide. Many studies have found that depression is related to alterations in the central serotonergic system impacting on the activity of the hypothalamic-pituitary-adrenal axis. Kaempferitrin was evaluated as an anti-depressant in two models of depression in mice reducing immobility time in the forced swimming test and tail suspension test, without affecting the locomotor activity. In this study the authors suggesting that the effect of kaempferitrin is mediated through the serotonergic system, mainly through its interaction with pre-synaptic 5-HT1A receptors [80].

Epilepsy is a disease characterized for the presence of chronic and non-chronic seizures. It is reported as the most common neurological disease affecting 50 million people worldwide. This disease represents an important health problem because it affects the daily life style of the patients [81]. Administration of kaempferitrin via intracerebroventricular (i.c.v., 40 ventricle, 1 μg/μL) modified the convulsive behavior and significantly delayed partial seizures stage II and stage III, and protected from generalized seizures stage IV and V [82].

**Kaempferitrin and renal injury**

One of remarkable features of cellular injury is leakage of lactate dehydrogenase (LDH), a soluble cytosolic enzyme that catalyzes the conversion of lactate to pyruvic acid, from inside the cells. This leakage is due largely to increased membrane permeability, which is modified by certain factors such as anticancer drugs, radiation, and attack by oxygen free radical species. The protective effect of kaempferitrin on the LLC-PK1 cell line, which has similar characteristics of the proximal tubular cells of the kidney, was evaluated. Kaempferitrin showed the ability to reduce the released of LDH when it was cocultured with the LLC-PK1 cell line. These results suggest that the strong effect as a membrane protector, could be due to the sugar in its structure. Since this was able to vary the radical scavenger activity by changing the electron distribution and participating in electron delocalization on the structural backbone, this has also been observed in similar flavonoids like afzelin, hyperin, isoquercitrin, kaempferol-7-glucoside, quercitin and rutin [83].

**Osteoporosis and kaempferitrin**

Osteoporosis is one of the most common bone diseases. This is a skeletal condition characterized by decreased density of normally mineralized bone. This reduction of bone density leads to decreased mechanical strength, thus making the skeleton more likely fracture [84]. Unfortunately there are few treatments to treat or prevent this disease. Nevertheless, kaempferitrin has shown preventive properties on ovariectomized rat model of osteoporosis as well as *in vitro* osteoblast and osteoclast cell lines. Kaempferitrin is capable to improve the bone mass and microarchitecture in the ovariectomized rats and also exhibits stimulatory effect on osteoblastic cells and inhibitory action on osteoclastic cells [85].

**Conclusion**

Kaempferitrin has been shown to have a wide range of important pharmacological activities, which could be useful to treat diseases with high incidence in the world. Currently, drugs for some diseases like cancer are known to have several adverse effects. The use of natural bioactive metabolites like kaempferitrin could help in solving this problem, since the synthesis of analogues of kaempferitrin could enhance the efficiency and/or potency of this natural product. It is know that the addition of some functional groups in specific positions on the molecule can improve the biological effect by increasing the affinity to the ligand or also improve the formulation of a drug. For instance, the addition of a polar chemical group on the molecule improve aqueous solubility. This characteristic is important due to the difficulty in some cases to get a molecule with little affinity to a vehicle in the medicine formulation. Further research is needed to synthesize safe and efficient analogues of kaempferitrin. The current technologies in drug discovery research could provide the necessary data to consider kaempferitrin and its analogues as safe drug candidates to evaluate in humans.

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**References**


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Table 1. Plant species where kaempferitrin has been isolated.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Part of the plant used</th>
<th>Extract</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthopanax sciadophyloides</td>
<td>Leaves</td>
<td>Methanol</td>
<td>[8]</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>Leaves</td>
<td>Methanol: Acetone 1:1</td>
<td>[9-10]</td>
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<tr>
<td>Asplenium trichomanes</td>
<td>Fronds</td>
<td>Ethanol 95 %</td>
<td>[11]</td>
</tr>
<tr>
<td>B. forficata subsp. pruinosa</td>
<td>Leaves</td>
<td>Water and ethanol</td>
<td>[12]</td>
</tr>
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<td>Balauinia forficata</td>
<td>Leaves</td>
<td>Water/ethanol: Water/ethanol/aqueous</td>
<td>[6], [13-16]</td>
</tr>
<tr>
<td>Bryophyllum pinnatum</td>
<td>Whole plant</td>
<td>Ethyl acetate</td>
<td>[17]</td>
</tr>
<tr>
<td>Camellia sinensis</td>
<td>Leaves</td>
<td>Water</td>
<td>[18]</td>
</tr>
<tr>
<td>Cardamine leucantha</td>
<td>Aerials parts</td>
<td>Ethanol</td>
<td>[19]</td>
</tr>
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<td>Celastrus orbiculatus</td>
<td>Leaves</td>
<td>Boiling water</td>
<td>[20]</td>
</tr>
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<td>Cheilanthes glauca</td>
<td>Stem</td>
<td>Methanol: Water</td>
<td>[21]</td>
</tr>
<tr>
<td>Chenopodium murale</td>
<td>Aerials parts</td>
<td>Ethanol 70%</td>
<td>[22]</td>
</tr>
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<td>Chromolaena subscandens</td>
<td>Leaves</td>
<td>Methanol</td>
<td>[23]</td>
</tr>
<tr>
<td>Cinnamomum insularimontanum Hayata</td>
<td>Stem</td>
<td>Methanol</td>
<td>[24]</td>
</tr>
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<td>Cinnamomum osmophloeum</td>
<td>Leaves</td>
<td>Hot water Methanol</td>
<td>[25]</td>
</tr>
<tr>
<td>Dicliptera chinensis</td>
<td>Leaves</td>
<td>EtOAc-soluble portion of 95% ethanol extract</td>
<td>[26]</td>
</tr>
<tr>
<td>Dorycnium intermedium Ldb.</td>
<td>Aerials parts</td>
<td>Methanol 80%</td>
<td>[27]</td>
</tr>
<tr>
<td>Dryopteris crassirhizoma</td>
<td>Root</td>
<td>Methanol</td>
<td>[28]</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Part</td>
<td>Solvent(s)</td>
<td>Notes</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---------------------</td>
<td>---------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Dwarf Ginseng</td>
<td>Leaves</td>
<td>Methanol</td>
<td>[29]</td>
</tr>
<tr>
<td>Epimedium acuminatum</td>
<td>Aerials parts</td>
<td>EtOH, n-BuOH soluble portion.</td>
<td>[30]</td>
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<tr>
<td>Ficus carica</td>
<td>Leaves</td>
<td>Ethanol and water.</td>
<td>[31]</td>
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<td>Ficus septica Burm</td>
<td>Leaves</td>
<td>Ethanol 70%</td>
<td>[32]</td>
</tr>
<tr>
<td>Fritillaria thunbergii</td>
<td>Flowers</td>
<td>Ethanol 75 % Water.</td>
<td>[33]</td>
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<td>Geranium thunbergii</td>
<td>Aerials parts</td>
<td>Ethanol (soluble fraction in ethyl acetate)</td>
<td>[34]</td>
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<tr>
<td>Heritiera littoralis D</td>
<td>Leaves</td>
<td>Hot ethanol 98%</td>
<td>[35]</td>
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<td>Hibiscus cannabinus L</td>
<td>Leaves</td>
<td>Ethanol Water</td>
<td>[3,4]</td>
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<td>Ixora coccinea</td>
<td>Root</td>
<td>Ethanol</td>
<td>[36]</td>
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<td>Justicia spicigera</td>
<td>Leaves</td>
<td>Ethanol</td>
<td>[37-39,5]</td>
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<td>Leonurus heterophyllus</td>
<td>Fruit</td>
<td>Ethanol</td>
<td>[40]</td>
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<td>Lespedeza virgata</td>
<td>Aerials parts</td>
<td>Ethanol</td>
<td>[41]</td>
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<td>Ligustrum neilgherense var. obovata C.B.Cl.</td>
<td>Leaves</td>
<td>Methanol</td>
<td>[42]</td>
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<td>Lotus corniculatus</td>
<td>Aerials parts</td>
<td>Ethanol 96% Ethyl acetate soluble fraction</td>
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<td>Matricaria chamomilla</td>
<td>Flower</td>
<td>Hydroalcoholic</td>
<td>[45]</td>
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<td>Notonia grandiflora</td>
<td>Leaves</td>
<td>Ethanol</td>
<td>[46]</td>
</tr>
<tr>
<td>Onychium contiguum</td>
<td>Root</td>
<td>Ethanol</td>
<td>[47]</td>
</tr>
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<td>Oryza sativa (fermented with Annulohypoxylon boveri var. Microspora fungus)</td>
<td>Seed</td>
<td>Ethanol</td>
<td>[7]</td>
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<tr>
<td>Peumus boldus</td>
<td>Leaves</td>
<td>Hot water</td>
<td>[48]</td>
</tr>
<tr>
<td>Podocarpium podocarpum</td>
<td>Whole plant</td>
<td>Ethanol 80%</td>
<td>[49]</td>
</tr>
<tr>
<td>Prunus spinosa</td>
<td>Flowers</td>
<td>Methanol (ethyl acetate soluble fraction)</td>
<td>[50]</td>
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<td>Plant Name</td>
<td>Part Used</td>
<td>Extraction Method</td>
<td>Reference</td>
</tr>
<tr>
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<td>------------</td>
<td>------------------------------------------</td>
<td>------------</td>
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<tr>
<td><em>Pterogyne nitens</em></td>
<td>Fruit</td>
<td>Ethanol</td>
<td>[51,52]</td>
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<td><em>Sedum dendroideum</em></td>
<td>Leaves</td>
<td>Aqueous extraction; Juice obtained with a food processor.</td>
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<td><em>Siraitia grosvenori</em></td>
<td>Leaves</td>
<td>Ethyl acetate and n-butanol/water</td>
<td>[55]</td>
</tr>
<tr>
<td><em>Tagetes erecta</em></td>
<td>Stem and leaves</td>
<td>Ethanol</td>
<td>[56]</td>
</tr>
<tr>
<td><em>Tilia americana var. Mexicana</em></td>
<td>Inflorescences</td>
<td>Hot water</td>
<td>[57]</td>
</tr>
<tr>
<td><em>Tilia argentea</em></td>
<td>Leaves</td>
<td>Ethanol 80%</td>
<td>[58]</td>
</tr>
<tr>
<td><em>Uncaria guianensis</em></td>
<td>Leaves and branches</td>
<td>Ethanol</td>
<td>[59,60]</td>
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<tr>
<td><em>Viola lactiflora</em></td>
<td>Aerials parts</td>
<td>Methanol</td>
<td>[61]</td>
</tr>
<tr>
<td><em>Weigela subsesilis</em></td>
<td>Leaves</td>
<td>Ethanol and n-BuOH</td>
<td>[62]</td>
</tr>
</tbody>
</table>
Appearance = Crystalline white solid
MF = C_{27}H_{30}O_{14}
MW = 578.5 g/mol

**Figure 1.** Chemical structure and physical characteristics of kaempferitin.

Kaempferol  
α-rhamnoisorobine  
Afzelin

**Figure 2.** Kaempferitin derivatives from intestinal metabolism and infrared exposition of kaempferitin.