BIOLOGICAL ACTIVITIES OF KAEMPFERITRIN- A SHORT REVIEW

Rodrigo Rafael Ramos-Hernández[1,2] , Alberto Sánchez-Medina[3] , Ivette Bravo-Espinoza[1,2] ,
Fernando Rafael Ramos-Morales[3], Miguel Ángel Domínguez-Ortíz[4], Cynthia Fernández-Pomares [1], Gonzalo Emiliano Aranda-Abreu[1] and María Elena Hernández-Aguilar[1*]

[1]Laboratorio de Neuroendocrinología y [2]Posgrado en Investigaciones Cerebrales, Centro de Investigaciones Cerebrales de la Universidad Veracruzana, Dr. Luis Castelazo Ayala S/N, C.P. 91190, Xalapa, Veracruz, México

[3] Unidad de Servicios de Apoyo en Resolución Analítica de la Universidad Veracruzana, Luis Castelazo Ayala s/n Col. Industrial Animas 91190, Xalapa, Veracruz, México

[4]Laboratorio de Productos Naturales, Instituto de Ciencias

Básicas, Universidad Veracruzana, Dr. Luis Castelazo Ayala S/N, C.P. 91190 Xalapa, Veracruz, México

*elenahernandez@uv.mx

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, its 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 knows 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-⬚-L-rhamnoside (afzelin), kaempferol 7-O-⬚-rhamnoside, 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, concentratrion of kaempfertrin depends on the environment conditions where the plant grows. For example, *Bahuinia 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, Pseudomona aeruginosa, Salmonella typhi, Candida albicans, Shighella flexinerii, Salmonella typhimurium* and *Acinetobacter calcoaceticus* in dosis 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 *Criptococcus 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 PKF. 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

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 $IC_{50}= 84.0 \pm 7.8$ 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 kampeferitrin 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 tin order to evaluated the real effect of kaempferitrin on GLUT4 [71].

Kaempferitrin and cancer

Cancer is a disease characterized for 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 ($IC_{50}= 4.5$ 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 kampferitrin 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 HePG-2 cell lines. The results found that the four complexes affected the performance of DNA TOPO I. However, the cupper 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. Chronical 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, tumorigenesis, rheumatoid arthritis, psoriasis, arthritis and inflammatory bowel disease [76]. Using a spectrophotometric method, kaempferitrin was evaluated as an inhibitor of MPO ($IC_{50}= 15.8$ nM). It has been found that the inhibitory effect of kaempferitrin depends on its chemical structure; for example, flavonoids wich have a catechol derivate on B-ring exhibited a better inhibitory effect on MPO [51]. At a dose of 100 mg/kg, kanferritin 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-1 (IL-1) 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

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 immunostimulanting effect in *in vitro* conditions, and the reason is because of its high content of Kaempferitrin. Also, at a dosis 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 dosis 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 presynaptic 5-HT_{1A} 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, 4o. 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 antineoplastic drugs, radiation, and attack by oxygen free radical species. The protective effect of kaempferitrin on the LLC-PKi cell line, which has similar characteristics of the proximal tubular cells of the kidney, was evaluated. Kaempferirtin showed the ability to reduce the released of LDH when it was cocultured with the LLC-PKi 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 ovaractemized 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

References

1.-Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. Journal of natural products 2016; 79(3), 629-661.

2.-Yang XW, Zhang JY, Xu W, Li J, Zhang, W Q. The biotransformation of kaempferitrin by human intestinal flora. Acta Pharmaceutica Sinica 2005; 40(8):717-721.

3.-Rho HS., Ahn SM, Lee BC, Kim MK., Ghimeray, AK, Jin CW, Cho DH. Changes in flavonoid content and tyrosinase inhibitory activity in kenaf leaf extract after far-infrared treatment. Bioorganic & medicinal chemistry letters 2010; 20(24), 7534-7536.

4.-Jin CW, Ghimeray AK, Wang L, Xu ML, Piao, JP, Cho DH. Far infrared assisted kenaf leaf tea preparation and its effect on phenolic compounds, antioxidant and ACE inhibitory activity. Journal of Medicinal Plants Research 2013; 7(17), 1121-1128.

5.-Alonso AJ, Ortiz E, Domínguez F, Arana AV., Carmen VM, Chávez ZM, et al. Antitumor and immunomodulatory effects of Justicia spicigera Schltdl (Acanthaceae). Journal of Ethnopharmacology 2012; 141-888–894.

6.-Pinheiro TS, Johansson LA, Pizzolatti MG, Biavatti MW. Comparative assessment of kaempferitrin from medicinal extracts of Bauhinia forficata Link. Journal of pharmaceutical and biomedical analysis, 2006; 41(2), 431-436.

7.-Cheng MJ, Wu MD, Hsieh SY, Su YS, Chen IS,Yuan GF. Secondary metabolites from the endophytic fungus Annulohypoxylon boveri var. microspora BCRC 34012. Chemistry of Natural Compounds 2011; 47(4), 536-540.

8.-Yasue M, Yosisige K, Yuh ML, Jinsaku S. Studies on the Constituents of Acanthopanax sciadophylloides FRANCH. et SAV. I. :Isolation of Cyclitolis and Flavonoid Glycosides. Journal of the Pharmaceutical Society of Japan 1968; 88(6), 738.

9.-Lindberg J, Nabbie F, Milliot J, Smith J, Tettamanzi R, Peethambaran B. 14-3-3λ Affects Production of a Sinapoyl Derivative in Lignin Biosynthesis during Drought Stress in Arabidopsis Thaliana. Universal Journal of Plant Science 2014; 2(4), 77-85.

10.-Zhao H, Wang J, Xie C, Song S, Bai G, Luo, G. Study on Fingerprints of Different Organs of Arabidopsis thaliana by Using UPLC/ESI-Q-TOF MS. Asian Journal of Chemistry 2013; 25(6), 3023.

11.-Imperato F. Two new kaempferol 3, 7-diglycosides and kaempferitrin in the fernAsplenium trichomanes. Cellular and Molecular Life Sciences 1979; 35(9), 1134-1135.

12.-Sayago CT, Camargo VB, Barbosa F, F, Gularte C, Pereira G, Miotto S et al. Chemical composition and in vitro antioxidant activity of hydro-ethanolic extracts from Bauhinia forficata subsp. pruinosa and B. variegata. Acta Biologica Hungarica 2013; 64(1), 21-33.

13.-Menezes FD, Minto AB, Ruela HS, Kuster, RM, Sheridan H, Frankish N. Hypoglycemic activity of two Brazilian Bauhinia species: Bauhinia forficata L. and Bauhinia monandra Kurz. Revista Brasileira de Farmacognosia 2007, 17(1), 8-13.

14.-Marques GS, Leão WF, Lyra MA, Peixoto M S, Monteiro R, P Rolim, et al. Comparative evaluation of UV/VIS and HPLC analytical methodologies applied for quantification of flavonoids from leaves of Bauhinia forficata. Revista Brasileira de Farmacognosia 2013; 23(1), 51-57.

15.-Da Cunha AM, Menon S, Menon R, R Couto, AG Bürger, C Biavatti et al. Hypoglycemic activity of dried extracts of Bauhinia forficata Link. Phytomedicine 2010; 17(1), 37-41.

16.-Silva KD, Weber BM, Nair LS, Yunes R, Monache FD. Notes-Phytochemical and Pharmacognostic Investigation of Bauhinia forficata Link (Leguminosae). Zeitschrift fur Naturforschung C-Journal of Biosciences 2000; 55(5-6), 478-480.

17.-Tatsimo SJ, Tamokou JD, Havyarimana, L Csupor D, Forgo P, Hohmann , Tane P. Antimicrobial and antioxidant activity of kaempferol rhamnoside derivatives from Bryophyllum pinnatum. BMC research notes 2012; 5(1), 158.

18.-Chaturvedula VSP, Prakash I. The aroma, taste, color and bioactive constituents of tea. Journal of Medicinal Plants Research 2011; 5(11), 2110-2124.

19.-Yang F, Su YF, Bi YP, Xu J, Zhu ZQ, Tu GZ, Gao XM. Helvetica Chimica Acta 2010 ; 93(3), 536-541.

20.-Mototake K, Shimokoriyama M. A flavone glycoside of Celastrus orbiculata. Acta Phytochimica 1949; 15, 229-321.

21.-Edgar P, Marcia A, Maria EL, Sanzana, E Vega M, González M. Preliminary studies on antioxidant and anti-cataract activities of Cheilanthes glauca (Cav.) Mett. through various in vitro models. Electronic Journal of Food and Plants Chemistry 2007; 2 (1) 1-8.

22.-Gohara AA, Elmazar MA. Isolation of hypotensive flavonoids from Chenopodium species growing in Egypt. Phytother. Res. 1997; 11: 564–567.

23.-Amaro LJM, Delgado MP. Flavonoids from the leaves of Chromolaena subscandens. Journal of natural products 1993; 56(4), 610-612.

24.-Hsieh TJ, Hsieh SF, Chen CY. Chemical constituents from the stems of Cinnamomum insulari-montanum. Chemistry of natural compounds 2010; 46(1), 99-100.

25.-Fang SH, Rao YK, Tzeng YM. Inhibitory effects of flavonol glycosides from Cinnamomum osmophloeum on inflammatory mediators in LPS/IFN-γ-activated murine macrophages. Bioorganic & medicinal chemistry 2005; 13(7), 2381-2388.

26.-Huang F, Cui H, Yu ZC, Shen ZB. Chemical Constituents of Dicliptera chinensis [J]. Chinese Journal of Experimental Traditional Medical Formulae 2012, 1, 024.

27.-Zurabishvili TS, Kemertelidze EP. Flavonoids of Dorycnium intermedium. Chemistry of Natural Compounds 1974; 10(2), 265-265.

28.-Chi C, Fu YW, Jiang B, Zhang QZ, Wang G X. In vivo anthelmintic effect of flavonol rhamnosides from Dryopteris crassirhizoma against Dactylogyrus intermedius in goldfish (Carassius auratus). Parasitology research 2013; 112(12), 4097-4104.

29.-Lee TM, Der Marderosian AH. Studies on the constituents of dwarf ginseng. Phytotherapy Research 1988; 2(4), 165-169.

30.-Bi-huang H, Li-dong Z, Yong-long L. New Tetrasaccharide Flavonol Glycoside from Epimedium acuminatum. J. Nat. Prod 1992; 55 (5), pp 672–675.

31.-Olufemi BE, Olusegun OV. Antibacterial properties of ethanolic extract of Ficus carica on microorganisms isolated from pepper Capsicum frutescens. Res WebPub 2013; 1, 7-15.

32.-Wu PL, Rao KV, Su CH, Kuoh CS, Wu TS. Phenanthroindolizidine alkaloids and their cytotoxicity from the leaves of Ficus septica. Heterocycles 2002; 57(12), 2401-2408.

33.-Peng W, Han T, Liu QC, Qin LP. Chemical constituents of the flower of Fritillaria thunbergii. Chemistry of Natural Compounds 2012; 1-2.

34.-Choi SJ, Kim JK, Jang JM, Lim SS. Inhibitory effect of the phenolic compounds from Geranium thunbergii on rat lens aldose reductase and galactitol formation. Korean Journal of Medicinal Crop Science 2012; 20(4), 222-230.

35.-Tian Y, Wu J, Zhang S. Flavonoids from leaves of Heritiera littoralis D. Journal of Chinese Pharmaceutical Sciences 2004; 13, 214-216.

36.-Joshi AB, Surlikar PM, Bhobe M. Ixora coccinea Linn: Phytochemical investigation. International Journal of Research in Pharmacy and Chemistry 2013; 3, 691.

37.-Euler KL, Alam M. Isolation of Kaempferitrin from Justicia spicigera. Journal of Natural Products 1982; 45(2), 220-221.

38.-Domínguez XA, Achenbach H, González C, Ferré-D'Amare AR. Estudio químico del muitle (Justicia spicigera). Revista Latinoamericana de Química 1990; 21, 142-143.

39.-Ortiz R, Cabañas A, Arana VE, Alonso AJ, Zapata BR, Salazar LA, et al. Antidiabetic effects of Justicia spicigera Schltdl (Acanthaceae). Journal of ethnopharmacology 2012; 143(2), 455-462.

40.-Cai W, Kangjun Z, Zhigang Z, Chang W, Wei X. Optimization of the conditions for Fructus Leonuri flavonoid extraction and research on its hypoglycemic effect. In Human Health and Biomedical Engineering (HHBE), 2011 International Conference on (pp. 1144-1147) IEEE.

41.-Chen Y, Deng HZ, Liang L. Contents of flavonoids in the extract of Lespedeza virgata. Nan fang yi ke da xue xue bao. Journal of Southern Medical University 2008; 28(5), 858-860.

42.-Joshi BS, Gawad DH. Flavones of Pajanelia multijuga P. DC. and Ligustrum neilgherense var. obovata CB Cl. In Proceedings of the Indian Academy of Sciences-Section A 1977; Vol. 86, No. 1, pp. 41-44.

43.-Dalmarco JB, Dalmarco EM, Koelzer J, Pizzolatti MG, Fröde TS. Isolation and identification of bioactive compounds responsible for the anti-bacterial efficacy of Lotus corniculatus var. São Gabriel. International Journal of Green Pharmacy 2010; (IJGP), 4(2).

44.-Koelzer J, Pereira DA, Dalmarco JB, Pizzolatti MG, Fröde TS. Evaluation of the anti-inflammatory efficacy of Lotus corniculatus. Food chemistry 2009; 117(3), 444-450.

45.-Al Bahtiti NH. Chemical analysis and biological activity of Jordanian chamomile extracts. Advance Journal of Food Science and Technology 2012; 4(1), 22-25.

46.-Rao DV, Rao EV. Phytochemical investigations on the leaves of Notonia grandiflora. Planta medica 1972; 22(06), 205-208.

47.-Wu TS, Chen MT, Kuoh CS, Yang JJ .Contstituents of Formosan Folk Medicines X. Chemical Constituents of the Rhizoma of Onychium Contiguum (Wall.) Hope. Jnl Chinese Chemical Soc 1981; 28:63–64.

48.-Fale P, Amaral F, Madeira PA, Silva MS, Florêncio MH, Frazã FN, et al. Acetylcholinesterase inhibition, antioxidant activity and toxicity of Peumus boldus water

extracts on HeLa and Caco-2 cell lines. Food and chemical toxicology 2012; 50(8), 2656-2662.

49.-Ye Q, Ma XQ, Hu CL, Lin B, Xu LS, Zheng CJ, et al. Antiosteoporotic activity and constituents of Podocarpium podocarpum. Phytomedicine 2015; 22(1), 94-102.

50.-Olszewska M, Wolbiś M. Further flavonoids from the flowers of Prunus spinosa L. Acta poloniae pharmaceutica 2002; 59(2), 133-137.

51.-Regasini LO, Vellosa JC, Silva DH, Furlan M, de Oliveira OM, Khalil M, et al. Flavonols from Pterogyne nitens and their evaluation as myeloperoxidase inhibitors. Phytochemistry 2008; 69(8), 1739-1744.

52.-De Souza A, Vendramini, RC, Brunetti IL, Regasini LO, Siqueira DH, Bolzani VDS, et al. Alcohol extract of Pterogyne nitens leaves fails to reduce severity of streptozotocin-induced diabetes in rats. Journal of Medicinal Plants Research 2010; 4(9), 802.

53.-Silva D, Casanova LM, Marcondes MC, Espindola JM, Paixão LP, Melo, et al. Antidiabetic activity of Sedum dendroideum: metabolic enzymes as putative targets for the bioactive flavonoid kaempferitrin. IUBMB life 2014; 66(5), 361-370.

54.-De Melo GO, David C, Malvar B, Frederico A, FA, Pires PA, Côrtes WS, et al. Phytochemical and pharmacological study of Sedum dendroideum leaf juice. Journal of Ethnopharmacology 2005; 102 217–220.

55.-Liang Y, Wei L, Zhu Z, Pan Y, Wang H, Liu, P. Isolation and purification of kaempferol-3, 7-O-α-L-dirhamnopyranoside from Siraitia grosvenori leaves by high-speed counter-current chromatograph and its free radical scavenging activity. Separation Science and Technology 2011;46(9), 1528-1533.

56.-Zhang Y, Zhang TT. Studies on the chemical constituents from the stem and leaves of Tagetes erecta. Zhong yao cai Zhongyaocai. Journal of Chinese medicinal materials 2011; 33(9), 1412-1414.

57.-Martínez AL, González ME, Aguirre-Hernández E, Moreno J, Soto M, López MJ. Antinociceptive activity of Tilia americana var. mexicana inflorescences and quercetin in the formalin test and in an arthritic pain model in rats. Neuropharmacology 2009, 56(2), 564-571.

58.-Toker G, Esra K, Merve M, Yesilada E. Flavonoids with antinociceptive and anti-inflammatory activities from the leaves of Tilia argentea (silver linden). Journal of Ethnopharmacology 2004; 95 393–397.

59.-Valente LM, Bizarri CH, Liechocki S, et al. Kaempferitrin Uncaria guianensis da (Rubiaceae) e seu potencial como um marcador químico para a espécie. Journal of the Brazilian Chemical Society 2009; 20(6), 1041-1045.

60.-Barboza RS, Mazzei JL, Valente LM, Siani AC. Optimized kaempferitrin isolation from Uncaria guianensis leaves by solid-phase extraction. Journal of Liquid Chromatography & Related Technologies 2015; 38(4), 532-542.

61.-Kiu C, Yook CS. Flavonol-glykoside aus Herba Viola lactiflora. Kor.J.Pharmacog. 12(3):147-148 1981.

62.-Won HM, Kwon YS, Lee JH, Kim CM. Chemical constituents of the leaves of Weigela subsesilis. Korean Journal of Pharmacognosy, 2004.

63.-Babb R, Pirofski LA. Help is on the way: monoclonal antibody therapy for multi-drug resistant bacteria. Virulence 2017; 1-4.

64.-Tamokou JD, Kuiate JR, Tene M, Nwemeguela TJK, Tane P. The Antimicrobial activities of extract and compounds isolated from Brillantaisia lamium. Iran J Med Sci 2011; 36:24-31.

65.-Mohammad AH, Shigefumi M, Kotaro M, Kakeya H, Sasaki E, Tomono K, et al. In vitro and in vivo activities of SCH5659 against Cryptococcus neoformans. J Antimicrob Chemother 1999; 44:827-829.

66.-Ndendoung JT, Tane P, Csupor D, Forgo P, Hohmann J. Kaempferol rhamnosides from Bryophyllum pinnatum, a medicinal plant used against infectious diseases and as analgesic in Mbouda, Cameroon. Planta Medica 2010; 76(12), P437.

67.-Ebersole JL, Huang C. (2010). U.S. Patent Application 12/959,163.

68.-Jorge AP, Horst H, Sousa ED, Pizzolatti M. G, Silva FRM. Insulinomimetic effects of kaempferitrin on glycaemia and on 14 C-glucose uptake in rat soleus muscle. Chemico-biological interactions 2004; 149(2), 89-96.

69.-De Sousa E, Zanatta L, Seifriz I, Creczynski-Pasa TB, Pizzolatti MG, Szpoganicz B, et al. Hypoglycemic Effect and Antioxidant Potential of Kaempferol-3, 7-O-(α)-dirhamnoside from Bauhinia f orficata Leaves. Journal of Natural Products 2004; 67(5), 829-832.

70.-Tzeng YM, Chen K, Rao YK, Lee MJ. Kaempferitrin activates the insulin signaling pathway and stimulates secretion of adiponectin in 3T3-L1 adipocytes. European journal of pharmacology 2009;607(1), 27-34

71.-Prasad CV, Mohan SS, Banerji, A Gopalakrishnapillai A. Kaempferitrin inhibits GLUT4 translocation and glucose uptake in 3T3-L1 adipocytes. Biochemical and biophysical research communications 2009; 380(1), 39-43.

72.- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144(5), 646-674.

73.-Alonso AJ, Ortiz E, García A, Ruiz G, Núñez JM, González SI, Cerbón, MA, et al. Kaempferitrin induces apoptosis via intrinsic pathway in HeLa cells and exerts antitumor effects. Journal of ethnopharmacology, 145(2), 476-489.

74.-Ouyang XL, Zhu ZR, Pan YM, Wang HS. Metal Ion Complexes of Kaempferitrin as DNA Topoisomerase I Inhibitors and Its' Cytotoxicities. Asian Journal of Chemistry 2015; 27(2), 503.

75.-García A, Guillamón E, Villares A, Rostagno MA, Martínez JA. Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. Inflammation Research 2009; 58(9), 537-552.

76.-Paul AT, Gohil VM, Bhutani KK. Modulating TNF-α signaling with natural products. Drug discovery today 2006; 11(15), 725-732.

77.-Koelzer J, Pereira DA, Dalmarco JB, Pizzolatti MG, Fröde TS. Evaluation of the anti-inflammatory efficacy of Lotus corniculatus. Food chemistry 2009; 117(3), 444-450.

78.-Fang SH, Rao YK, Tzeng YM. Inhibitory effects of flavonol glycosides from Cinnamomum osmophloeum on inflammatory mediators in LPS/IFN-γ-activated murine macrophages. Bioorganic & medicinal chemistry 2005; 13(7), 2381-2388.

79.-Juárez MC, Alonso AJ, García A. Kaempferitrin induces immunostimulatory effects in vitro. Journal of ethnopharmacology 2013; 148(1), 337-340.

80.-Cassani J, Dorantes AM, Novales LM, Real GA, Estrada RR. Anti-Depressant-Like Effect of Kaempferitrin Isolated from Justicia spicigera Schltdl (Acanthaceae) in Two Behavior Models in Mice: Evidence for the Involvement of the Serotonergic System. Molecules 2014; 19(12), 21442-21461.

81.-Medel JS, Ramos FR, Correa J, Sánchez, JS, Saavedra MV, Velásquez M. Participación de los canales de calcio dependientes de voltaje en el desarrollo de la epilepsia. Acta bioquímica clínica latinoamericana 2010; 44(3), 329-335.

82.-González ME, Domínguez F, Pérez G, Aguillón M, Martínez VD, Almazán AS, et al. Justicia

spicigera Schltdl. and kaempferitrin as potential anticonvulsant natural products. Biomedicine & Pharmacotherapy2017; 92, 240-248.

83.-Yokozawa T, Dong E, Kawai Y, Gemba M, Shimizu M. Protective effects of some flavonoids on the renal cellular membrane. Experimental and Toxicologic Pathology 199; 51(1), 9-14.

84.-Glaser DL, Kaplan FS. Osteoporosis: definition and clinical presentation. Spine 1997; 22(24), 12S-16S.

85.-Ma XQ, Han T, Zhang X Wu JZ, Rahman K, Qin LP, et al. Kaempferitrin prevents bone lost in ovariectomized rats. Phytomedicine 2015; 22(13), 1159-1162.

Table 1. Plant species where kaempferitrin has been isolated.

Plant specie	Part of the plant used	Extract	Reference
Acanthopanax sciadophylloides	Leaves	Methanol	[8]
Arabidopsis thaliana	Leaves	Methanol: Acetone 1:1	[9-10]
Asplenium trichomanes	Fronds	Ethanol 95 %	[11]
B. forficata subsp. *pruinosa*	Leaves	Water and ethanol	[12]
Bahuinia forficata	Leaves	Water/ethanol: Water/ methanol/ aqueous	[6], [13-16]
Bryophyllum pinnatum	Whole plant	Ethyl acetate	[17]
Camellia sinensis	Leaves	Water	[18]

Cardamine leucantha	Aerials parts	Ethanol	[19]
Celastrus orbiculatus	*Leaves*	Boiling water	[20]
Cheilanthes glauca	Stem	Methanol: Water	[21]
Chenopodium murale	Aerials parts	Ethanol 70%	[22]
Chromolaena subscandens	Leaves	Methanol	[23]
Cinnamomum insulari-montanum Hayata	Stem	Methanol	[24]
Cinnamomum osmophloeum	Leaves	Hot water Methanol	[25]
Dicliptera chinensis	Leaves	EtOAc-soluble portion of 95% ethanol extract	[26]
Dorycnium intermedium Ldb.	Aerials parts	Methanol 80%	[27]
Dryopteris crassirhizoma	Root	Methanol	[28]

Dwarf Ginseng	Leaves	Methanol	[29]
Epimedium acuminatum	Aerials parts	EtOH, *n*-BuOH soluble portion.	[30]
Ficus carica	Leaves	Ethanol and water.	[31]
Ficus septica Burm	Leaves	Ethanol 70%	[32]
Fritillaria thunbergii	Flowers	Ethanol 75 % Water.	[33]
Geranium thunbergii	Aerials parts	Ethanol (soluble fraction in ethyl acetate)	[34]
Heritiera littoralis D	Leaves	Hot ethanol 98%	[35]
Hibiscus cannabinus L	Leaves	Ethanol Water	[3,4]
Ixora coccinea	Root	Ethanol	[36]
Justicia spicigera	Leaves	Ethanol	[37-39,5]
Leonurus heterophyllus	Fruit	Ethanol	[40]
Lespedeza virgata	Aerials parts	Ethanol	[41]
Ligustrum neilgherense var. obovata C.B.Cl.	Leaves	Methanol	[42]
Lotus corniculatus	Aerials parts	Ethanol 96% Ethyl acetate soluble fraction	[43,44]
Matricaria chamomilla	Flower	Hydroalcoholic	[45]
Notonia grandiflora	Leaves	Ethanol	[46]
Onychium contiguum	Root	Ethanol	[47]
Oryza sativa (fermented with *Annulohypoxylon boveri* var. *Microspora* fungus)	Seed	Ethanol	[7]
Peumus boldus	Leaves	Hot water	[48]
Podocarpium podocarpum	Whole plant	Ethanol 80%	[49]
Prunus spinosa	Flowers	Methanol (ethyl acetate soluble fraction)	[50]

|			
Pterogyne nitens	Fruit	Ethanol	[51,52]
Sedum dendroideum	Leaves	Aqueous extraction Juice obtained with a food processor.	[53,54]
Siraitia grosvenori	Leaves	Ethyl acetate and n-butanol/water	[55]
Tagetes erecta	Stem and leaves	Ethanol	[56]
Tilia americana var. Mexicana	Inflorescences	Hot water	[57]
Tilia argentea	Leaves	Ethanol 80%	[58]
Uncaria guianensis	Leaves and branches	Ethanol	[59,60]
Viola lactiflora	Aerials parts	Methanol	[61]
Weigela subsesillis	Leaves	Ethanol and n-BuOH	[62]
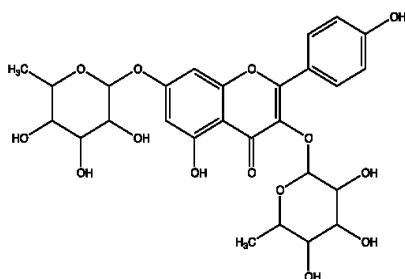
Appearance =Crystalline white solid
MF=$C_{27}H_{30}O_{14}$
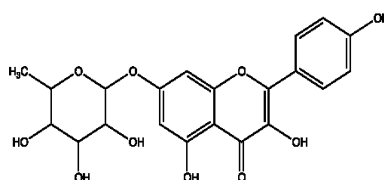MW= 578.5 g/mol

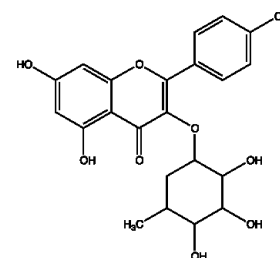**Figure 1.** Chemical structure and physical characteristics of kaempferitrin.
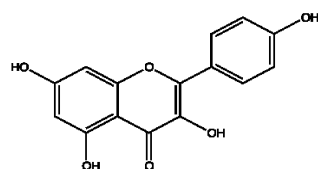
Kaempferol α-rhamnoisorobine   Afzelin

Figure 2. Kaempferitrin derivatives from intestinal metabolism and infrared exposition of
kaempferitrin.