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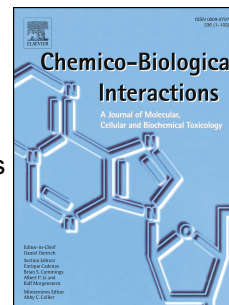
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Emerging therapeutic potential of the iridoid molecule, Asperuloside: A snapshot of its underlying molecular mechanisms

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Abstract

Over the years, the attention of researchers in the field of modern drug discovery and development has become further intense on the identification of active compounds from plant sources and traditional remedies, as they exhibit higher therapeutic efficacies and improved toxicological profiles. Among the large diversity of plant extracts that have been discovered and explored for their potential therapeutic benefits, asperuloside, an iridoid glycoside, has been proven to provide promising effects as a therapeutic agent for several diseases. Although, this potent substance exists in several genera, it is primarily found in plants belonging to the genus *Eucommia*. Recent decades have seen a surge in the research on Asperuloside, making it one of the most studied natural products in the field of medicine and pharmacology. In this review, we have attempted to study the various reported mechanisms of asperuloside that form the basis of its wide spectrum of pharmacological activities.

Keywords: Asperuloside; anti-obesity; anti-inflammatory; anti-cancer; anti-bacterial; laxative

List of abbreviations:

Abbreviations	Expanded forms
ASP	Asperuloside
ELE	<i>Eucommia</i> green leaf extract
HFD	High fat diet
MeOH	Methanol
RT-PCR	Real time polymerase chain reaction
mRNA	Messenger RNA
UCP1	Uncoupling protein 1
LPS	Lipopolysaccharide
NO	Nitric oxide
TNF- α	Tumour necrosis factor- α
IL-6	Interleukin-6
NF- κ B	Nuclear factor kappa-B
MAPK	Mitogen-activated protein kinases
PGE ₂	Prostaglandin E ₂
iNOS	Inducible nitric oxide synthase
COX-2	Cyclooxygenase-2
Erk1/2	Extracellular signal-regulated protein kinases 1/2
JNK	c-Jun N-terminal kinase
I κ B- α	kappa-B-alpha
IKKs	I κ B-kinases
RelA (p65)	REL-associated protein
RelB	Transcription factor RelB
ALI	Acute lung injury
ELISA	Enzyme-linked immunosorbent assay
IL-1 β	Interleukin 1-beta
CAM	Chorioallantoic membrane
VEGF	Vascular endothelium growth factors
VEGFR-2	VEGF-receptor-2
MTC	Active methylene chloride
SRB	Sulforhodamine B
CKZ	Chuankezhi
CIK	Cytokine-induced killer cells
IFN- γ	Interferon-gamma
CFs	Clastogenic factors
CHO	Chinese hamster ovary
AMR	Antimicrobial resistance
STC	Slow transit constipation
TXA ₂	Thromboxane A ₂
PGF ₂ α	Prostaglandin F-2-alpha
SAR	Structure activity relationship
WHO	The World Health Organisation

1. Introduction

Plants and herbs have long been utilized for medicinal purposes as early as 60,000 years ago since the middle Paleolithic age. The World Health Organization (WHO) estimates that over 80% of the population in developing nations resort to traditional herbs for healing various forms of sicknesses (1). Over the years, many researchers have proven that herb derived remedies contribute to favorable health outcomes, attributed to their wide range of pharmacological effects and better toxicological profiles (2-13). Subsequently, plant-derived medicines have become novel candidates in the field of modern drug discovery and development.

Various classes of natural compounds with biological activities have been explored; one of them being iridoids, which are metabolites commonly found in plants and animals. Iridoids are known to represent a large and expanding group of cyclopenta-(c)-pyranmonoterpenoids, whereby, they can be found in large amounts in dicotyledonous plant families and sympetalous plant families, such as the *Apocynaceae*, *Verbenaceae*, *Loganiaceae*, and *Rubiaceae* (14,15). Iridoids are characterized by a six-membered ring with an oxygen bound to a cyclopentane ring in their chemical structure (Figure 1). In plants, iridoids are mostly bound to glucose, and therefore, they are mostly referred to as iridoid glycosides (15). Asperuloside (ASP) (Figure 2) is an example of a natural compound classified under the category of iridoid glycosides. It was isolated in the year 1848 from several plants belonging to the *Rubiaceae* family (16). ASP has a molecular formula of $C_{18}H_{22}O_{11}$ and it is a member of O-glycosyl compounds in terms of its chemical structure, in which a sugar group is bonded through one carbon to another group via an O-glycosidic bond. ASP is highly soluble in water and is a weakly acidic compound (17). In the recent years, ASP

has gained much interest among researchers. Numerous studies have reported on various medicinal properties of ASP, namely, in the treatment of obesity, inflammatory diseases, cancer, and bacterial infections. ASP has also been reported to be used as a laxative (7-15).

2. *Anti-obesity*

Obesity, widely recognized as the largest and fastest growing health issue globally, is characterized by excessive fat accumulation leading to a body mass index of more than 30 kg/m², attributed to the imbalance between energy intake and expenditure (18). According to the World Health Organization, worldwide obesity has nearly tripled since 1975. In the year 2016, it was estimated that there were approximately 1.9 billion adults in the worldwide population who were overweight, of which over 650 million were obese (19). Obesity is usually accompanied by comorbidities such as type 2 diabetes, hypertension, myocardial infarction, stroke and other health risks. Today, obesity remains as one of the world's leading cause of mortality, as well as a huge disease burden due to the lack of effective remedies (5,20). Hence, researchers are in the search of new, effective, plant-derived anti-obesity remedies in a bid to improve the wellbeing of obese patients. One of the natural compounds that have shown promising potential in the control of obesity is ASP, whereby, several studies have successfully demonstrated that ASP has the ability to suppress clinical markers of obesity.

A study conducted by Hirata *et al.*, in 2011 demonstrated the potential anti-obesity effects of ASP. In the study, *Eucommia* green leaf extract (ELE) was divided into five fractions using high porous polystyrene gel. The fractions containing isolated geniposidic acid, ASP and chlorogenic acid respectively, were examined for their anti-obesity effect. Prior to the study, a 40% high-fat diet (HFD) was fed to mice in order to develop a metabolic syndrome-like clinical model. The

test substances were then chronically administered to the mice, followed by evaluation of anti-obesity effects by observing parameters such as body weight, white adipose tissue weight, plasma triglyceride levels, total cholesterol levels, and free fatty acid levels after four weeks. It was found that the 30% MeOH fraction of ELE, which contained the higher amount of ASP than other fractions, had significant inhibitory effects on the observation parameters. These effects were also comparable with the positive control. In short, these results have proved that chronic administration of ASP from *Eucommia* leaves greatly suppressed the elevated obesity parameters in the experimental mice, thereby, suggesting that ASP has remarkable anti-obesity properties (21).

In addition, Fujikawa *et al.*, had also carried out a similar study in 2012 to examine the potential anti-obesity and anti-metabolic syndrome effects of ASP, as well as their mechanisms. A metabolic syndrome rat model was developed initially by feeding the mice with a 35 % HFD. The rat model was then chronically administered with ASP. The effects were studied in six rats over a period of three months in five different groups, namely HFD-Control, HFD-5% ELE, HFD-0.03% ASP, HFD-0.1% ASP, and HFD-0.3% ASP. The observed effects were then compared with the positive control, ELE. The results showed that ASP inhibited the increase in body weight, visceral fat weight, circulating levels of glucose, insulin and lipids, as well as food intake. Besides, ASP also stimulated the increase of plasma adiponectin levels in the HFD rats. All observed effects were comparable to those of standard ELE, with an exception of the influence on plasma glucose level. Real time polymerase chain reaction (RT-PCR) studies conducted, have also demonstrated that ASP, like ELE, stimulates anti-obesity and anti-metabolic syndrome activities in HFD rats across multiple organs, where it regulates the mRNA

expression of several enzymes in the metabolic pathways such as (i) diminished isocitrate dehydrogenase 3 α and fatty acid synthase levels in the white adipose tissue; (ii) elevated acyl-CoA dehydrogenase and carnitine palmitoyltransferase levels in the liver; and (iii) elevated citrate synthase, succinyl CoA synthase and succinate dehydrogenase levels in the skeletal muscle. However, unlike ELE, chronic ASP administration led to a remarkable increase in the expression of *uncoupling protein 1* (UCP1) in the brown adipose tissue of HFD rats. *UCP1* is a gene associated with obesity and is involved in thermogenesis, regulation of energy expenditure, as well as in the protection against oxidative stress (Figure 3) (22). Hence, these results suggest that ASP may be a promising candidate as an anti-obesity remedy (23,24).

3. *Anti-inflammatory*

Inflammation occurs as a natural defence response of the body against infections, triggering the recruitment of macrophages to the inflammatory sites and they function to stimulate intracellular cascades of cytokines and chemokines through the modulation of various signalling pathways. The most common inflammatory trigger, lipopolysaccharide (LPS), could result in elevated levels of inflammatory mediators and cytokines, such as nitric oxide (NO), tumour necrosis factor- α (TNF- α) and interleukin-6 (IL-6). In the inflammatory process triggered by LPS, nuclear factor kappa-B (NF- κ B) and mitogen-activated protein kinases (MAPK) are the two main signalling pathways, subsequently inducing the gene expression of pro-inflammatory cytokines, once these pathways are activated (5,25,26). Therefore, drug discovery researchers are in favour of bioactive compounds with substantial activity against NF- κ B and MAPK pathways, as they are said to be the ideal therapeutic agents in the treatment of a variety of inflammatory diseases (25).

One of the several studies that had demonstrated the anti-inflammatory effect of ASP was conducted by He *et al.*, in 2018. A traditional Asian herb from the *Rubiaceae* family, *Hedyotis diffusa* (*H. diffusa*) Willd, was utilized in this particular study. This herb is being widely used as a traditional Chinese remedy for the treatment of inflammatory diseases such as arthritis, nephritis, and bronchitis. The researchers investigated the underlying mechanisms of the anti-inflammatory activity shown by *H. diffusa* using the isolated iridoids from the plant on LPS-induced RAW 264.7 cells. In the cells specifically treated by ASP, the level of inflammatory mediators, NO and prostaglandin E₂ (PGE₂), as well as the inflammatory cytokine IL-6 were remarkably reduced. As inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) are the key enzymes catalysing the productions of NO and PGE₂ respectively, they are believed to be the mediators playing pivotal roles for a variety of inflammatory responses. Therefore, the protein and mRNA expression of iNOS and COX-2 were then further measured to investigate the underlying mechanism of the observed findings (27). The results had demonstrated that ASP significantly inhibited the mRNA transcript levels of iNOS and COX-2 (28,29). Typically, the inflammatory effects of NO are exerted through the stimulation of iNOS and COX-2. As a result of iNOS stimulation, prostaglandins are released and NOs are further activated, which then initiates the transcription of downstream inflammatory genes, namely the pro-inflammatory cytokines *IL-1 β* , *IL-6* and *TNF- α* (Figure 4) (27). Besides, it was also found that ASP treatment downregulated the phosphorylation of p38, extracellular signal-regulated protein kinases 1/2 (Erk1/2), and c-Jun N-terminal kinase (JNK), which are the major signalling cassettes in the MAPK pathway (30). Being one of the most important signalling pathways in inflammatory responses, the MAPK pathway is essential for the expression of several inflammatory mediator

genes, such as *COX-2*, *iNOS*, *IL-1 β* and *TNF- α* . Therefore, the downregulation of p38, JNK and Erk1/2 is said to attenuate the activity of COX-2, which subsequently suppresses inflammatory responses (27–30).

At the same time, the cells pre-treated with ASP demonstrated significant reduction of LPS-induced inhibition of kappa-B-alpha ($I\kappa B-\alpha$) phosphorylation and degradation via the NF- κB pathway which is further elaborated in the same study by He *et al.* It is well known that NF- κB signifies a large family of inducible transcription factors that regulate multiple genes that are involved in various processes of inflammatory responses. Besides, NF- κB also plays a vital role in regulating the activation, differentiation, as well as effector function of inflammatory T cells in the human body. Subsequently, dysregulated NF- κB often leads to the pathogenic processes of multiple inflammatory diseases (31). Generally, the regulation and activation of the NF- κB is modulated via two main signalling pathways, namely the canonical pathway and the alternative pathway. In the canonical pathway, inflammatory stimuli promotes the phosphorylation of $I\kappa B-\alpha$ at its two serine sites, via activation of $I\kappa B$ -kinases (IKKs) (32). As a result of proteasomal degradation of $I\kappa B-\alpha$ by IKKs, NF- κB protein dimers such as RelA (p65), RelB, NF- $\kappa B1$ (p105/p50) and NF- $\kappa B2$ (p100/52) are released, which will then induce the expression of pro-inflammatory cytokines. In other words, the masking of the nuclear localization signals of NF- κB by $I\kappa B-\alpha$ will result in an inactive state of NF- κB protein dimers in the cytoplasm (Figure 5) (32–35). Therefore, any compound that suppresses $I\kappa B-\alpha$ degradation may exhibit promising effect in inhibiting inflammatory cascades. Hence, these results have demonstrated that ASP exhibits potential anti-inflammatory property via its modulatory effects on both NF- κB and MAPK pathways in a concentration-dependent manner (25).

Another similar study had been conducted by Qiu *et al.*, in 2015, which aimed to investigate the anti-inflammatory effects ASP isolated from a traditional Chinese herbal medicine, *Herba Paederiae*, as well as its underlying mechanisms. The study evaluated the inflammatory responses in LPS-stimulated RAW 264.7 cells and LPS-induced acute lung injury (ALI) in an experimental rat model, whereby, the levels of pro-inflammatory cytokines were measured using enzyme-linked immunosorbent assay (ELISA) and Western blotting. For both *in vitro* and *in vivo* studies, the researchers found that ASP reduces the expressions of TNF- α , IL-6, and interleukin 1-beta (IL-1 β). In addition, it was discovered that ASP treatment in LPS-induced ALI rat model resulted in reduction of lung wet-to-dry weight, histological alterations, and myeloperoxidase activity, signifying that ASP is effective as an anti-inflammatory agent. Whereas, for the Western blot analysis, it was revealed that ASP has a significant effect in the regulation of I κ B- α , Erk1/2, JNK and p38 kinases in LPS-stimulated cells. All these findings demonstrates that ASP has substantial anti-inflammatory effect, in which its dose was directly correlated to the suppression of pro-inflammatory pathways, namely the NF- κ B and MAPK pathways (36–38).

4. *Anti-cancer*

Cancer is a disease that is caused by the outgrowth of clonal population of cells from body tissues. Carcinogenesis, a complex process referring to the development of cancer, can also be said to be a stepwise development functionally grouped into three different stages: (i) initiation, whereby the cancerous cells undergo genomic changes; (ii) promotion, whereby the development of tumour is accelerated by the survival and clonal expansion of the initiated cancer cells; and (iii) progression, whereby the cancer cells exhibit substantial increase in their size and

occurrence of metastasis (39). Currently, the anti-cancer therapeutics available in the market are highly toxic to the tumour cells. However, they also bring harmful effects to the other normal cells of the body at the same time. Therefore, research interest has been diverted to the discovery of novel anticancer therapeutics using naturally derived compounds as they are said to have fewer adverse effects on the human body (40,41). Several research findings have reported ASP to be a potential candidate as an effective alternative to current anti-cancer therapies. Generally, anti-cancer effects can be modulated through several targets that act to promote apoptosis, inhibit angiogenesis, as well as suppress proliferation and metastasis (5,42).

Cancer is regarded as a life-threatening disease due to its ability to spread to other adjacent and distant organs in the body system. In this case, growth of vascular network is essential for the spread of cancerous cells. This physiological process of growth and development of new blood vessels is known as angiogenesis. As this process has a vital role in ensuring the formation and expansion of blood capillaries, as well as supplying nutrition and oxygen to the cancerous body cells, compounds that exhibit inhibitory effects on angiogenesis may be considered as a promising approach in the development of novel anti-cancer therapies (43–45). A recent study by Camero *et al.*, in 2018 investigated the potential anti-angiogenic effects of *Galium genus*, a plant belonging to the *Rubiaceae* family. The plant is known to produce multiple classes of secondary metabolites, which include iridoids, triterpenes, anthraquinones, and saponins. ASP was reported to be one of the isolated iridoids that has the highest anti-angiogenic effect among the rest of the metabolites, where it exhibited a dose-dependent manner on inhibiting the formation of microvessels in the chick embryo chorioallantoic membrane (CAM) model. This is evidenced by microscopic images of the CAM treated using ASP, which demonstrated a significant 67% reduction of blood vessel branch points, which is opposed to the control eggs that showed

presence of clear vascular network converging towards the embryo. Moreover, ASP also has a high anti-angiogenic property of 62% in respect to retinoic acid, a known compound that has important roles in cancer treatment, which was used as the standard in this particular study. In a nutshell, these findings proved that the anti-angiogenic property of ASP is beneficial to the treatment of cancer via the prevention of inflammation and neoplastic cell growth (45). However, the exact mechanisms behind such property of ASP are yet to be fully elucidated. Generally, anti-angiogenic effects are modulated through multiple pathways. The key signalling pathway that modulates proliferation of endothelial cells is the vascular endothelium growth factors (VEGF) and their receptors, whereby, downregulation of VEGF-dependent signalling system results in several anti-angiogenic effects such as (i) prevention of VEGF-A binding to its receptors; (ii) blockade of VEGF-receptor-2 (VEGFR-2); and (iii) inhibited kinase activity of VEGFR-2 leading to blocked growth factor signalling. Other pathways include the signalling systems DII4/Notch, PDGFB/PDFGR β , as well as angiopoietins-1/2 (Ang1/Ang2) and their receptors (46–48).

Another *in-vitro* study conducted by Artanti *et al.*, in 2015 using the plant *Hedyotis corymbosa*, also from the *Rubiaceae* family, was found to suppress human breast cancer YMB-1, human leukemia HL60, and human oral cancer KB cell lines. In this research, the cytotoxicity property was investigated using (i) ethanol extract from the whole plant; (ii) the active methylene chloride (MTC) fraction; and (iii) a lead compound extracted via further purification of the MTC fraction. In this case, ASP was the lead compound isolated. Utilizing the Sulforhodamine B (SRB) method, the half-maximal inhibitory concentrations (IC₅₀) of (i), (ii), and (iii) were determined against YMB-1, HL60 and KB cell lines. The results showed that ASP isolated from *H.*

corymbosa plant has cytotoxic activity against YMB-1, HL60 and KB cell lines with IC₅₀ values of 0.7, 11.0, and 104.2 µg/mL respectively. Therefore, it was proven that ASP can be a potential anti-cancer compound that has comparable cytotoxic activity with antimycin A₃, which is a control used in this study with known cytotoxicity to human breast cancer and human leukemia cell lines (49). Nevertheless, further studies should be conducted to unveil the mechanisms underlying the cytotoxic activity of ASP on these human cell lines in order to provide a new direction for its future use in clinical practice.

On the other hand, poor cellular immunity has a great impact on cancer prognosis as it contributes to the proliferation and metastasis of cancer tissues. Therefore, immunotherapy had become a new approach in the treatment of cancers (50). Chuankezhi (CKZ) is a new Chinese medicine that is prepared from the extracts of *Morinda officinalis* and *Epimedium*. It is known that *M. officinalis* (Rubiaceae) comprises the dry roots of plants. CKZ contains several active compounds, one of which is the iridoids, which consists of ASP as one of the components in CKZ. A study was conducted by Zhao *et al.*, in 2013, which aimed to determine the immunoregulatory effect of CKZ on cytokine-induced killer (CIK) cells. Many existing studies had found that activated CIK cells can exert anti-tumour activity by lysing tumours and preventing tumour growth in the treatment of breast cancer, renal cancer, lung cancer and hepatocellular carcinoma (50–54). In this study, CIK cells were generated by culturing peripheral blood monocytes isolated from healthy donors with interferon-gamma (IFN-γ) and interleukin-2. Utilizing cytotoxicity assay, the anti-tumour effects of CIK cells were measured on the 14th day after different concentrations of CKZ were added. Besides, hepatocellular carcinoma mice model was also used in this study. The researchers demonstrated that CKZ treatment elevated the

percentage of CD3⁺ and CD56⁺ CIK cells subtypes, which subsequently promoted the secretion of IFN- γ and TNF- α , enhancing the function of immune-effector cells via induction of inflammatory cytokines production. In agreement, the results from the *in vivo* study revealed the suppression of tumour growth, most likely attributed to an increase in the number of circulating immune-killer cells. The apoptosis of CIK cells was also suppressed by CKZ. Overall, CKZ, which contains ASP, has promising anti-tumour activity via enhancement of CIK cells, which suggests that immunotherapy may contribute to improved treatment outcomes in cancer patients (50). However, these results may not be fully indicative that the anti-tumour effects induced by CKZ is attributed to the presence of ASP, as the observed anti-tumour effects may be contributed by other bioactive compounds in CKZ. Hence, further studies are warranted to establish a clear link between ASP and its possible anti-tumour property.

Furthermore, chemoprevention is regarded as another approach in the reduction in human cancer incidences via inhibition of carcinogenesis. Clastogens are mutagenic agents present in the plasma that cause disruption and breaking of the chromosomes. The damaging effects of clastogenic factors (CFs) are mediated by superoxide, whereby CFs are produced via superoxide and they further stimulate the production of superoxide by monocytes and neutrophils. Eventually, this leads to a clastogenic process that is self-sustained and long-lasting, which may surpass the capability of the body's DNA repair system, leading to cancer (55,56). Among the various plant derived compounds that have been explored, ASP was found to possess anticlastogenic activity. According to a study by Nakamura *et al.*, 'Tochu' tea, a popular beverage in Japan, extracted from *Eucommia ulmoides* leaves, was investigated for its suppressing effect on chromosome aberrations in Chinese hamster ovary (CHO) K1 cells and male CD-1

mice. A total of 17 components, including ASP, were purified from the aqueous extract of 'Tochu' tea leaves which were soluble in dimethyl sulfoxide. The clastogenicity of 'Tochu' tea and its components were then evaluated for up to 50% toxicity and anti-clastogenicity was evaluated at the concentrations at which they did not cause mitotic index depression by themselves. The results revealed that ASP extracted from 'Tochu' tea had bio-anticlastogenic activity, whereby, the process of mutation fixation is suppressed after the genes were damaged. This was attributed to the presence of an α -unsaturated carbonyl group in its structure which reacts with enzyme thiol groups via Michael adduct formation, leading to high-order structural changes in the DNA (57,58). The exact anticlastogenic mechanisms of ASP in respect to its structure-activity relationship (SAR) are yet to be elucidated; nevertheless, it was proposed that it is related to the promotion of DNA re-joining process in which DNA polymerase-beta acts (57).

5. *Anti-bacterial*

The widespread incidences of antimicrobial resistance (AMR) are posing a serious global health concern, whereby, antimicrobials are no longer effective in curing bacterial infections. Besides, medical researchers are unable to keep up with the pace of antimicrobial drug discovery to combat the emerging resistant pathogens. Therefore, researchers are now searching for novel antimicrobial compounds that exhibit broad spectrum action against both Gram-positive and Gram-negative bacteria with minimal adverse effects to the human body (59,60). For this purpose, a variety of plant-derived compounds have been explored for their anti-bacterial property, among which ASP had demonstrated antimicrobial effects against multiple bacteria strains, making it a potential candidate in combating AMR.

Morinda citrifolia, known as the Noni plant, is a popular traditional medicine that have been widely used as a dietary supplement and to cure a broad range of illnesses, as it contains phytochemicals that have multiple therapeutic effects (61,62). A variety of compounds have been identified in the Noni plant, whereby in a study by Leach *et al.*, *L*-asperuloside was proven to show anti-bacterial effect against several infectious strains, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus morganii*, *Bacillus subtilis*, *Escherichia coli*, *Shigella* and *Salmonella* (63). In agreement, Bushnell *et al.*, have also reported that *L*-asperuloside exhibited moderate antibacterial properties against infectious bacteria strains such as *Pseudomonas aeruginosa*, *Micrococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus morganii*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella*, and *Shigella* (3). On the other hand, a study by Bhardwaj *et al.*, evaluated the antimicrobial activity of *M. citrifolia* gel for the elimination of bacteria in root canal treatment. It was discovered that *L*-asperuloside contributed to 86.02% microbial inhibition at depths of 200 and 400 μm (64). In a nutshell, ASP can potentially be utilized for the treatment of infection-related illnesses, in which its antimicrobial effect is theoreticized by various mechanisms, such as (i) its antioxidative property which counteracts free-radicals and metabolites; (ii) enhanced activity of cortisol that promotes blood circulation, removing bacterial toxins from the body; (iii) promotion of humoral and cell-mediated immune function, enhancing antigenic surveillance; and (iv) blocking NF- κ B pathway and mitogenic responses in infected cells (65).

6. *Laxatives*

Constipation, a condition characterized by uncomfortable and infrequent bowel movement, is often associated with decreased mental health and social functioning. As the condition is rarely attributed to a life-threatening disease, empirical therapy is the first-line management option

recommended by current guidelines. As a result, more than half of the treated patients are unsatisfied with their treatment, most likely due to limited efficacy of drugs that do not target the underlying pathophysiology, alongside the association with adverse effects (66). Therefore, alternative compounds are needed to be explored for the development of novel therapeutic agents for constipation, including plant-derived compounds.

Galium aparine, or cleaver is an example of traditional herb that was studied for its laxative property. ASP is present as one of the valuable constituents in cleaver, and researchers have found that ASP has mild laxative action. This can be attributed to the ability of ASP to convert into prostanoid intermediates in the body (67,68). A study by Cong *et al.*, in 2007 was conducted to evaluate the mechanism underlying slow transit constipation (STC), which is a type of constipation characterized by reduced motility within the large intestine. The results suggested that lowered basal motility in STC patients was generally caused by low levels of thromboxane A₂ (TXA₂) and prostaglandin F-2-alpha (PGF₂α), leading to reduced muscle contraction and elevated levels of prostaglandin E₂ (PGE₂) which induced relaxation (69). Therefore, it was believed that ASP can play an essential role in the promotion of bowel movement through modulating the level of prostaglandin analogues in the body and subsequently, treating constipation. Besides that, there were studies that suggested SAR may also play a role in the purgative action of ASP, whereby presence of a free hydroxyl group at C-11 remarkably inhibited purgative activity; and the presence of hydroxyl/-oxy group at C-6 presented delayed onset of action (70). Nonetheless, the studies conducted on the purgative effects of ASP are limited and may not be sufficient to support its use as a medicinal laxative. Further studies may

be warranted to clarify the potential of ASP as a laxative and to uncover its underlying mechanism.

Conclusion

The popularity of plant extracts and molecules as medicinal natural products is increasing, and it is widely used for the overall maintenance of good health. Based on the evidences and results from the previous studies conducted on asperuloside from multiple plant sources, it is with no doubt justified that asperuloside has many therapeutic benefits that are believed to be modulated through various signalling pathways. Nevertheless, the results in both *in vivo* and *in vitro* studies are not significant enough to be used on human subjects due to lack of pre-clinical and clinical trials. Therefore, in paving a new direction in the field of drug discovery and development, more extensive studies are required to be performed in terms of the pharmacokinetic properties, as well as the identification of quality, safety, risk of toxicity and exact mechanism of action underlying the observed pharmacological properties of asperuloside. Furthermore, well-planned and systematically executed clinical studies are essential to evaluate the practicability of asperuloside for therapeutic use in humans.

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Figure Ligands:

Figure 1: Chemical structure of iridoids.

Figure 2(a): The chemical structure of asperuloside (C₁₈H₂₂O₁₁).

Figure 2(b): The three-dimensional chemical structure of asperuloside (C₁₈H₂₂O₁₁). The atoms are represented as: hydrogen (white), carbon (grey), oxygen (red).

Figure 3: An illustration of the location and function of UCP1 in the mitochondrial respiratory chain (MRC) in brown adipose tissue. UCP1 is located on the mitochondrial inner membrane and functions to uncouple oxidative mechanism from the synthesis of ATP, leading to dissipation of energy via heat release (22).

Figure 4: An illustration of role of nitric oxide (NO), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) and their effects on inflammatory responses.

Figure 5: An illustration of the role of inhibitor of kappa-B-alpha (I κ B- α) in NF- κ B signalling and its effect on inflammation. The binding of inflammatory cytokines to receptors generates signals that activates I κ B kinases (IKKs), which subsequently induces phosphorylation and proteosomal degradation of I κ B- α . The resulting free NF- κ B protein dimers such as RelA and NF- κ B1 will then translocate into the nucleus and activate transcription of the target genes that are responsible for the encoding of pro-inflammatory cytokines (34,35).

Figure 6: Summary of the proposed fundamental mechanisms of asperuloside. Further studies are warranted to elucidate the exact mechanisms responsible for its various therapeutic properties.

Table 1: A summary of the effect of asperuloside and its proposed mechanism of action on various types of cancers.

Type of cancer	Effect of asperuloside	Proposed mechanism of action	Reference
Breast cancer	<ul style="list-style-type: none"> - Inhibits the formation of new cancer cells. - Restores breast tumour-induced osteolysis. 	<ul style="list-style-type: none"> - Asperuloside inhibits metastasis and angiogenesis through modulation of signaling pathways such as VEGF, DII4, Notch and Ang1/Ang2, thereby destroying new cancer cells. - Immunomodulation of T cells, MDSC population and anti-tumour cytokine IL-12. 	(49,71)
Leukemia	Inhibits further growth and destroys cancer cells.	<ul style="list-style-type: none"> - Asperuloside displays cytotoxic activity through modulation of the VEGF signaling system. 	(49)
Liver cancer	Suppresses the growth of tumour.	<ul style="list-style-type: none"> - Asperuloside promotes secretion of IFN-γ and TNF-α, thereby enhancing the function of immune-effector cells. - Asperuloside enhances the effect of cytokine-induced killer (CIK) cells. 	(50)
Colorectal cancer	Inhibits the growth and formation of cancer cells.	<ul style="list-style-type: none"> - Asperuloside increases depolarization of the mitochondrial membrane, inducing apoptosis. - Asperuloside elevates caspase activities and ROS generation, promoting apoptotic cell death. 	(72)

Table 2: A summary of the major therapeutic properties of asperuloside documented in various studies.

Property	Findings	Type of study	Source(s) of asperuloside	Reference
Anti-obesity	Significant inhibitory effects of ASP on body weight, white adipose tissue weight, plasma triglyceride levels, total cholesterol levels, and free fatty acid levels.	<i>In-vivo</i> on 40% high-fat diet model mice	<i>Eucommia</i> leaves	(21)
	<ul style="list-style-type: none"> - ASP inhibits increase in body, visceral fat weight, circulating levels of glucose, insulin and lipids, as well as food intake. - Stimulated the increase of plasma adiponectin levels. 	<i>In-vivo</i> on 35% high-fat diet model mice	<i>Eucommia</i> leaves	(23)
	<ul style="list-style-type: none"> - Significant reduction of body weight with no changes in food intake. - MC4R protein expression significantly elevated in the nucleus accumbens of model mice. 	<i>In-vivo</i> on high-fat diet C57B/6 mice	N/A	(73)
	Increases the production of adenosine 5'-triphosphate in white adipose tissue and increases utilization of ketone bodies and glucose in the skeletal muscle.	<i>In-vivo</i> on obese female Wistar rats induced by ovariectomy; and high-fat diet mice	<i>Eucommia</i> leaves	(74)
	Observed significant reduction in body weight, body mass index, as well as fat tissues.	<i>In-vivo</i> on obese female Wistar rats	<i>Eucommia</i> leaves	(75)

		induced by ovariectomy		
Anti-inflammatory	Remarkable reduction of inflammatory mediators such as nitric oxide and prostaglandin E ₂ , and inflammatory cytokines such as interleukin-6.	<i>In-vitro</i> on LPS-induced RAW 264.7 cells	<i>H. diffusa</i>	(25)
	<ul style="list-style-type: none"> - Suppressed expression of IL-1β, TNF-α and IL-6. - Reduction of lung wet-to-dry weight, histological alterations, and myeloperoxidase activity. 	<i>In-vitro</i> on LPS-induced RAW 264.7 cells and <i>in-vivo</i> on LPS-induced acute lung injury mice model	<i>Herba Paederiae</i>	(36)
	Inhibited the release of TNF- α with IC ₅₀ value of 0.52 μ g/mL in the treatment of rheumatoid arthritis.	<i>In-vitro</i> on cultured mouse peritoneal macrophages	<i>Lasianthus acuminatissimus</i>	(76)
	Suppressed plasma levels of inflammatory cytokine TNF- α , producing anti-inflammatory and anti-colitis effects.	<i>In-vivo</i> on chronic dextran sulfate sodium-induced colitis mice model	<i>Eucommia ulmoides</i>	(77)
Anti-cancer	Demonstrated dose-dependent manner on the inhibition of microvessels formation.	<i>In-vivo</i> on chick embryo chorioallantoic membrane model	<i>Galium genus</i>	(45)
	Demonstrated cytotoxicity against human YMB-1 (breast cancer), HL60 (leukemia) and KB (oral cancer)	<i>In-vitro</i> on human cancer cell lines	<i>Hedyotis corymbosa</i>	(49)

	cell lines.			
	<ul style="list-style-type: none"> - Elevated percentage of cytokine-induced killer cells subtypes CD3+ and CD56+. - Promoted the secretion of IFN-γ and TNF-α. - Enhanced function of immune-effector cells. - Suppression of tumour growth. - Suppressed apoptosis of cytokine-induced killer cells. 	<i>In-vitro</i> on cytokine-induced killer cells and <i>in-vivo</i> on hepatocellular carcinoma mice model	<i>Morinda officinalis</i> and <i>Epimedium M. officinalis</i>	(50)
	Demonstrated bio-antitumor activity, whereby the process of mutation fixation is suppressed after the genes are damaged.	<i>In-vitro</i> on Chinese hamster ovary (CHO) K1 cells and <i>in-vivo</i> on male CD-1 mice	<i>Eucommia ulmoides</i> leaves	(57)
	<ul style="list-style-type: none"> - Inhibited proliferation of HT-29 cells for 24 hours. - Increased mitochondrial membrane depolarization. - Increased ROS generation and caspase activities. - Increased number of cells in sub-G1 peak in dose-dependent manner. 	<i>In-vitro</i> on HT-29 human adenocarcinoma cells	<i>Oldenlandia diffusa</i>	(72)
Anti-bacterial	Effective against <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Proteus morgaii</i> , <i>Bacillus subtilis</i> , <i>Escherichia coli</i> , <i>Shigella</i> and <i>Salmonella</i> .	<i>In-vitro</i> against Gram-positive and Gram-negative bacteria	<i>Morinda citrifolia</i>	(63)
	Effective against <i>Pseudomonas aeruginosa</i> , <i>Micrococcus pyrogenes</i> , <i>Escherichia coli</i> ,	<i>In-vitro</i> against Gram-positive and Gram-	<i>Morinda citrifolia</i>	(3)

	<i>Pseudomonas aeruginosa, Proteus morgaii, Staphylococcus aureus, Bacillus subtilis, Salmonella, and Shigella.</i>	negative bacteria		
	Exhibited 86.02% microbial inhibition in the root canal at both 200 and 400 μm .	<i>In-vitro</i> using dentin shavings collected at 200 and 400 μm	<i>Morinda citrifolia</i>	(64)

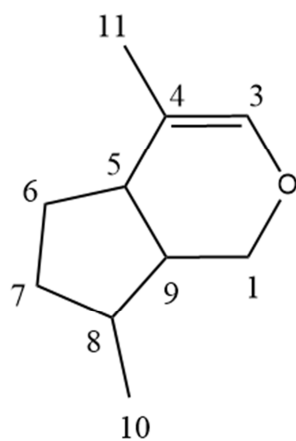


Figure 1: Chemical structure of iridoids.

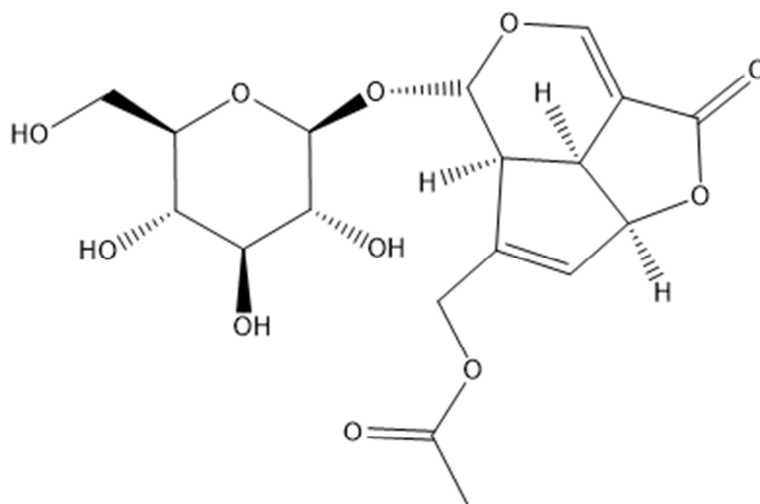


Figure 2(a): The chemical structure of asperuloside ($C_{18}H_{22}O_{11}$).

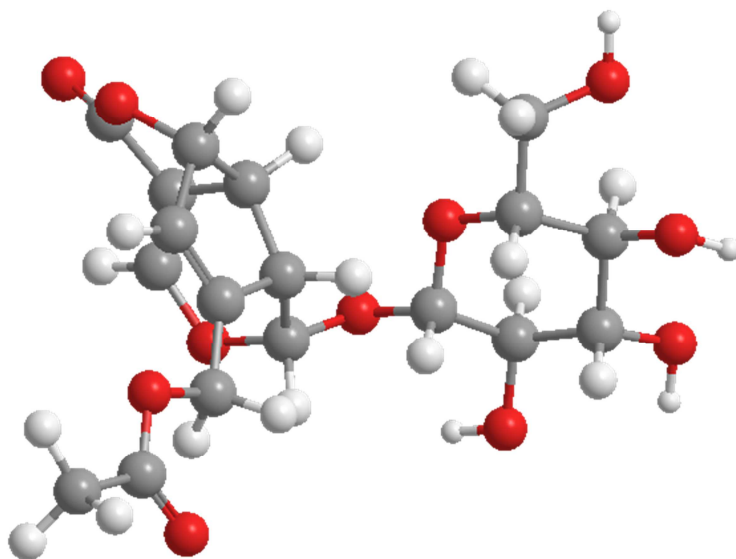


Figure 2(b): The three-dimensional chemical structure of asperuloside ($C_{18}H_{22}O_{11}$). The atoms are represented as: hydrogen (white), carbon (grey), oxygen (red).

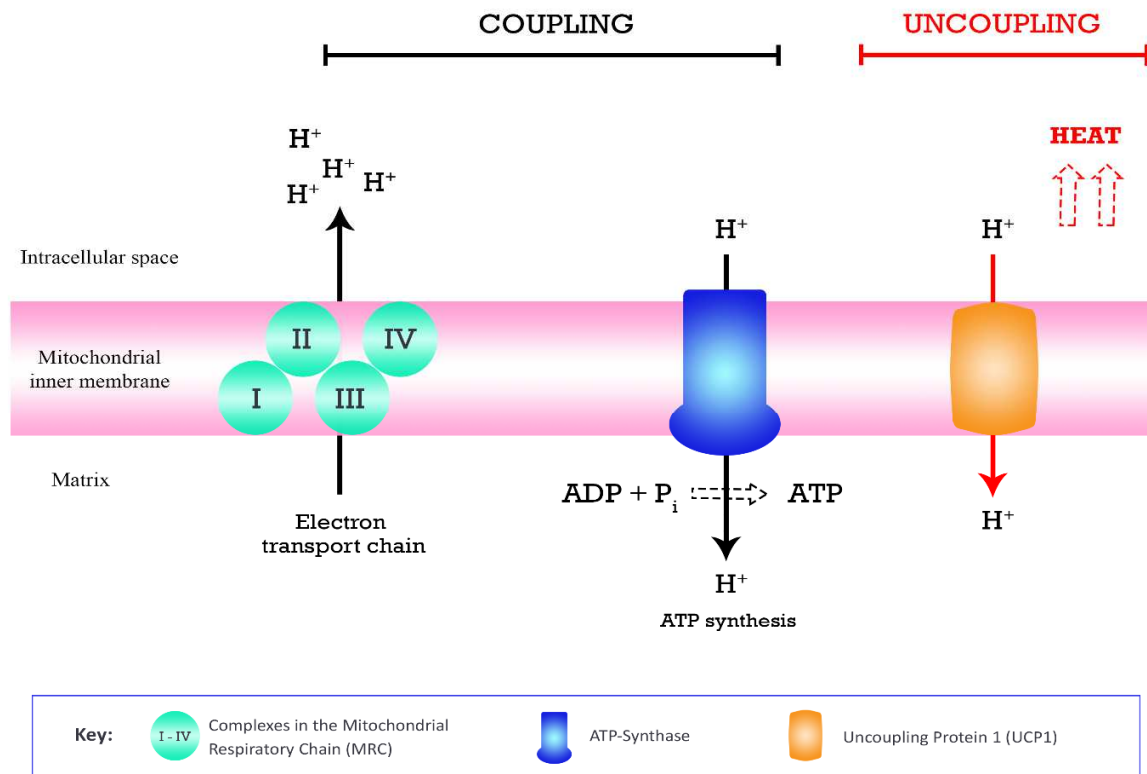


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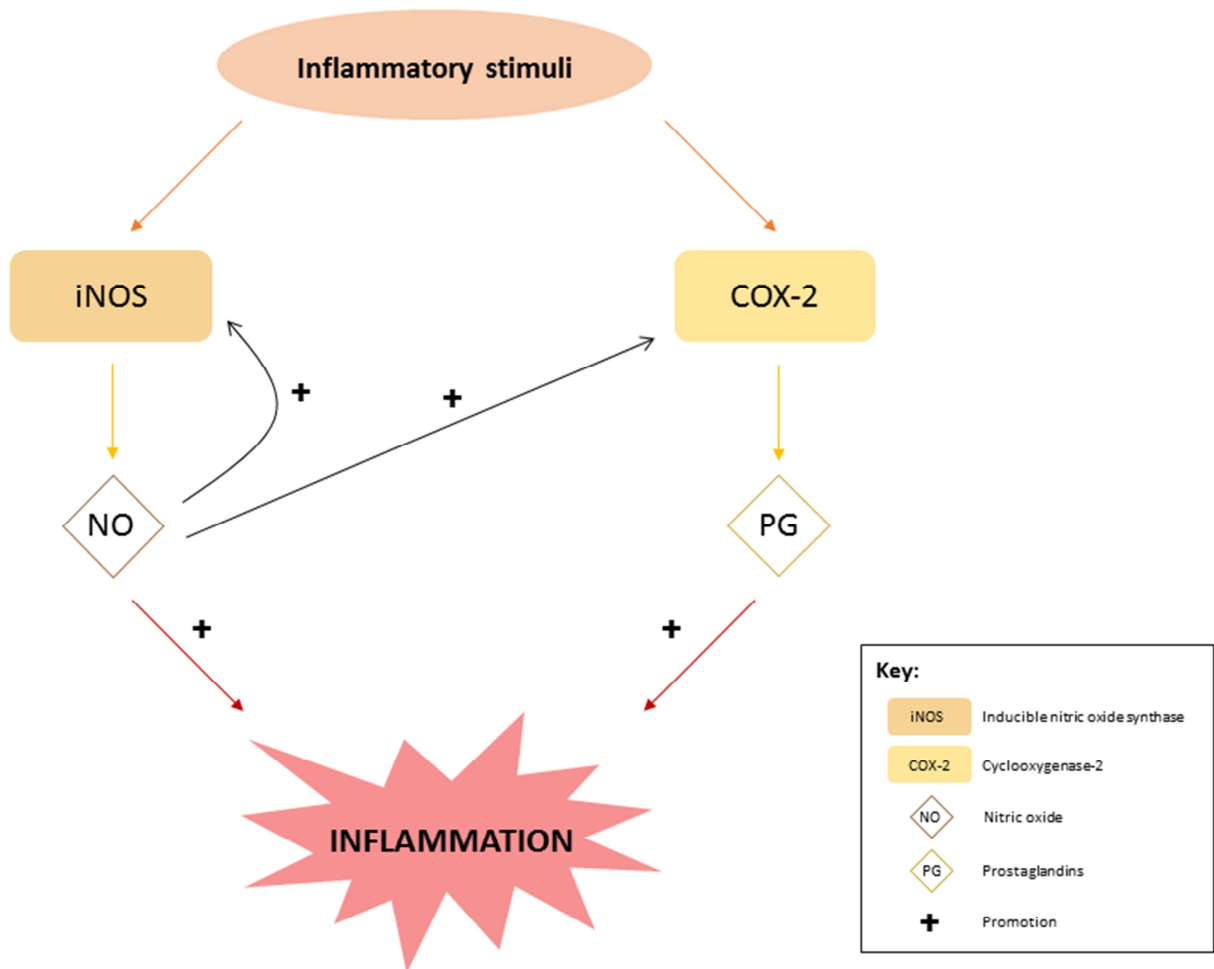


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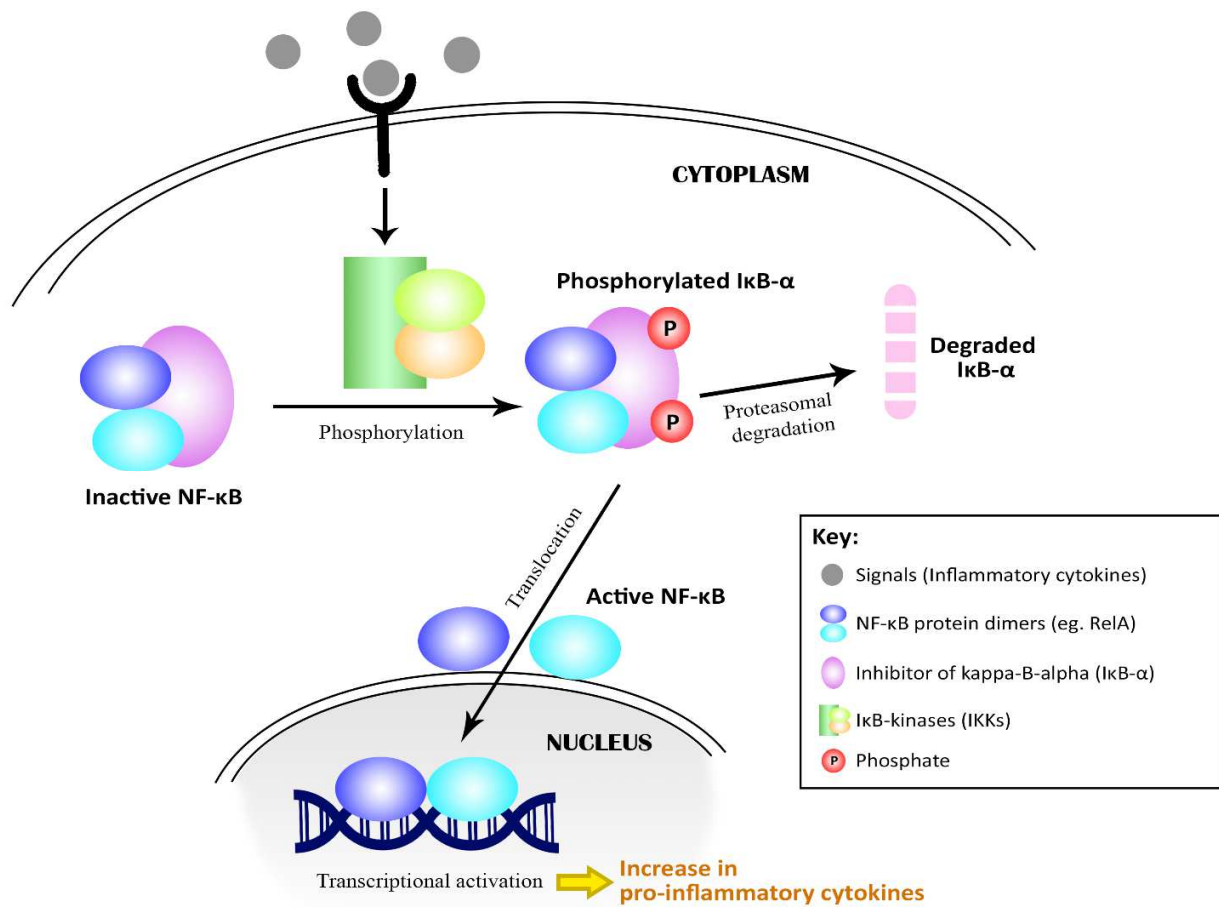


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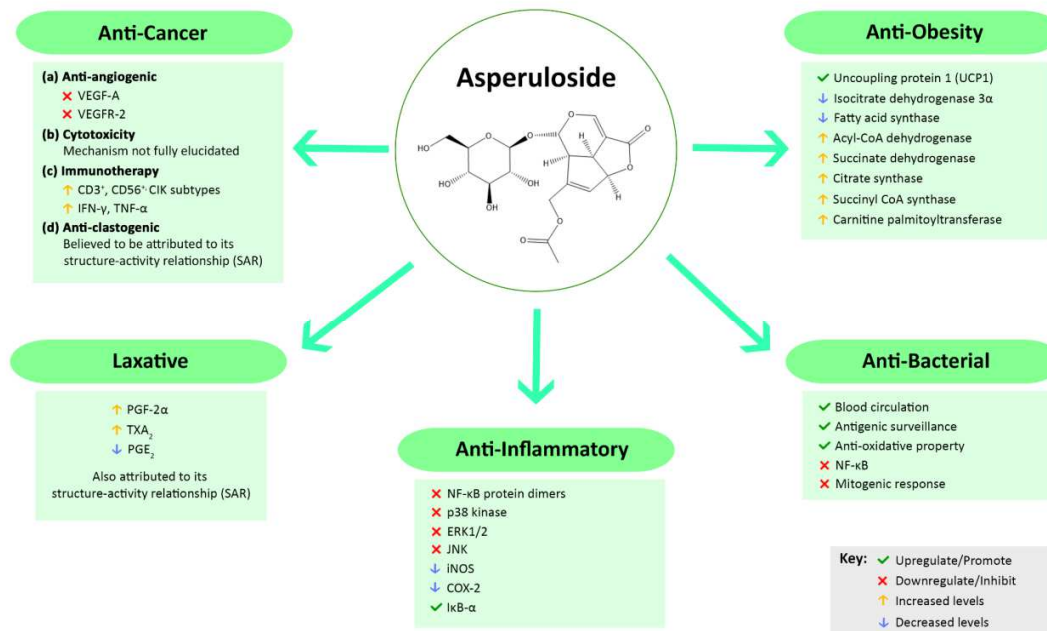


Figure 6: Summary of the proposed fundamental mechanisms of asperuloside. Further studies are warranted to elucidate the exact mechanisms responsible for its various therapeutic properties.

Highlights

- Asperuloside have potent medicinal properties including antiobesity and anti-inflammatory
- Asperuloside inhibited the increase in body weight and visceral fat weight
- The anti-inflammatory mechanism of Asperuloside was demonstrated in LPS-induced RAW 264.7 cells.
- The anticancer activity of Asperuloside was comparable with antimycin A₃
- Asperuloside also demonstrated moderate antibacterial effects on several known microbes

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: